Species-rich Nardus stricta grasslands host a higher vascular plant diversity on calcareous than on siliceous bedrock

Species-rich *Nardus stricta* grasslands host a higher vascular plant diversity on calcareous than on siliceous bedrock

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Abstract

***Background*:** Species-rich *Nardus stricta* grasslands are a priority habitat for conservation in Europe. They typically occur on siliceous substrates and less frequently are found on calcareous bedrock.

***Aims*:** The present paper aimed to identify the environmental factors (i.e. bedrock type, topographic, and climatic factors) that are related with community diversity and to assess if differences in plant diversity between *N. stricta* communities on calcareous and siliceous bedrock occur. We hypothesised that *Nardus* grasslands on calcareous bedrock hosted a higher vascular plant diversity than those on siliceous bedrock.

***Methods*:** Based on 579 vegetation surveys carried out in the south-western Alps, we assessed vascular plant diversity (species richness, Shannon diversity, and Pielou's equitability index) of species-rich *Nardus* grasslands and compared it between *N. stricta* communities on calcareous and siliceous bedrock.

***Results*:** Elevation was identified as the main factor related to species composition, while species diversity was mostly related to mean annual precipitation and bedrock type. Species richness, Shannon diversity, and Pielou's equitability index were higher within the communities on calcareous rather than on siliceous bedrock and a total of 89 and 34 indicator species were detected, respectively.

***Conclusions*:** Based on our results, we suggest to protect primarily, as a habitat of priority interest, *N. stricta* grasslands on calcareous substrates for the higher vascular plant diversity hosted.

Keywords: calcareous substrate; indicator species; Pielou's equitability index; Shannon diversity index; siliceous substrate; species richness

Introduction

Due to their high vascular plant diversity, species-rich *Nardus stricta* grasslands are recognised by the European Habitat Directive (92/43/EEC) as a habitat of priority interest. Specifically, they are defined as 'Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (and submountain areas, in continental Europe)' (habitat code 6230*). *Nardus* grasslands are widespread in Europe, occurring over about 1918 km^2 within Natura 2000 sites in 24 countries (Galvánek and Janák 2008). They occur across wide elevation and moisture gradients, from Atlantic lowlands up to the mountain areas of continental Europe, such as the Alps, Apennines, Carpathians, and Pyrenees (Galvánek and Janák 2008; Gennai et al. 2014). As a consequence of the wide spectrum of ecological conditions in which they are found, *N. stricta* grasslands encompass a large variety of different communities. The Italian Interpretation Manual of Directive 92/43/EEC (Biondi et al. 2009) associates habitat 6230* to the following phytosociological alliances occurring in the montane belt: *Violion caninae*, *Nardo-Agrostion tenuis*, and *Ranunculo-Nardion*, belonging to the order *Nardetalia strictae* (*Nardetea strictae* class). Moreover, the habitat includes the *Nardion strictae* (*Caricetea curvulae* class) communities located in the sub-alpine belt, while *N. stricta* communities in the alpine belt (i.e. the ones developed above the tree line) are attributed to habitat 6150 ('Siliceous alpine and boreal grasslands').

Even if the Directive 92/43/EEC expressly indicates that species-rich *Nardus* grasslands occur on siliceous substrates, without any reference to the calcareous ones, these communities do occur also on calcareous bedrock, where precipitation has leached calcium from the top soil (Galvánek and Janák 2008; Ellenberg 2009; Biondi et al. 2012). Such conditions have been reported in Austria (Lüth et al. 2011), France (Bensettiti et al. 2005), Slovakia (Stanová and Valachovič 2002), Spain (Sebastià 2004), and Italy, both in the Alps and in the northern Apennines (Gennai et al. 2014). In general, calcareous bedrock can harbour a high number of plant species (Wohlgemuth 1998, 2002; Ewald 2003; Marini et al. 2008); however, the vascular

plant composition of *Nardus* grasslands on calcareous and siliceous bedrocks has never been compared. Based on a large dataset collected over a broad area of the south-western Alps, representative of different environmental gradients, the present paper tests the hypothesis that *Nardus* grasslands on calcareous bedrock host a higher vascular plant diversity than those on siliceous bedrock by (1) identifying which environmental factors (i.e. bedrock type, topographic and climatic factors) mainly affect *Nardus* grassland plant diversity and (2) comparing plant diversity between *N. stricta* communities on calcareous and siliceous bedrocks.

Material and methods

Study area

The study was carried out on the *N. stricta*-dominated communities of the Piedmont region, western Italian Alps (Figure 1). In this broad area of the Alps, 92 different grassland types cover more than 187,000 ha, of which 12% is dominated by *N. stricta* (Argenti and Lombardi 2012; Cavallero et al. 2007). The grasslands are mostly used as summer pastures (Cavallero et al. 2007). Total annual precipitation ranges from continental regimes with 760 mm in the western part of the study area to sub-oceanic regimes with 2400 mm in the northern part (i.e. the 'Insubric district'). Generally, precipitation decreases from the external to the internal sectors of the valleys and follows a bimodal distribution, with spring and autumn peaks (Biancotti et al. 1998). Soils are developed on siliceous (about 70%; mainly gneiss and granite) and calcareous bedrock (ca. 30%; mainly calc-schists, dolomite, and limestones) (Regione Piemonte 2006).

Vegetation and environmental data

Vegetation data. During the period 2001–2007, 3888 25-m transects were recorded for vascular plant composition by using the point-quadrat method (Daget and Poissonet 1971) 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 (i.e. the 'vegetation plot', having a 50-m^2 area) was also recorded. The frequency of occurrence of each plant species, which is an estimate of species canopy cover, was converted to percentage cover (Pittarello et al. 2016). Moreover, the species relative abundance (SRA) was determined in each transect to detect the proportion of different species according to the following equation (1) :

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

where $\mathrm{SRA_i}$ and f_i are the species relative abundance and the frequency of occurrence of the species *i*, respectively (Daget and Poissonet 1971). To all occasional plant species found within vegetation plots and not along the linear transect a SRA value of 0.3 was attributed (Vacchiano et al. 2016). The phytosociological optimum was associated to each plant species according to Aeschimann et al. (2004).

Within this dataset, 579 samples were assigned to habitat 6230* following a two-step selection. First, we selected *Nardus*-dominated grasslands when *N. stricta* percentage cover was higher than 25%, according to the threshold used by Illyés et al. (2007). Second, to exclude the *Nardus*-dominated grasslands ascribable to habitat 6150, we retained only those surveys located at the montane and sub-alpine belts, according to the Italian Interpretation Manual of Directive 92/43/EEC (Biondi et al. 2009) (Figure 1a).

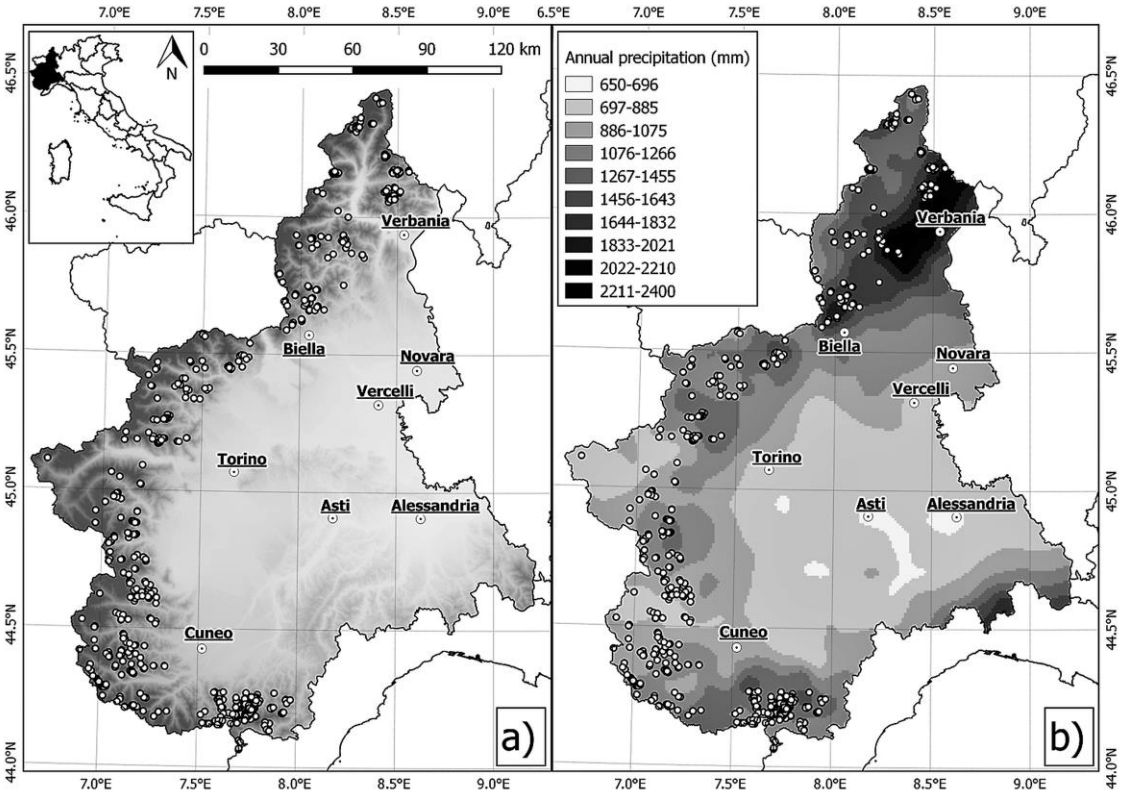

Figure 1. (a) Distribution of *Nardus* grasslands belonging to habitat 6230* in the Alpine chain of Piedmont region (represented on Digital Terrain Model). (b) Mean total annual precipitation map of Piedmont region.

We attributed the samples to the alpine or sub-alpine/montane belts depending on whether their elevation was higher or lower than the interpolated tree line limit computed for the latitude at which the survey was carried out. Since the elevation limit between the sub-alpine and alpine belt (i.e. the tree line limit) linearly changes with latitude (Ozenda 1985), we set the tree line limit with a linear interpolation from the southern zone of Piedmont (tree line at 2300 m a.s.l. – 43.5° latitude) to the northern one (2000 m a.s.l. – 46.5° latitude), which corresponds to elevation and latitude limits set by Ozenda (1985).

We computed species richness, Shannon diversity (hereafter ‘H’’), and Pielou’s equitability index (hereafter ‘J’ = H’/H’$_{max}$’, Pielou 1975), based on SRA values for each species in each sample. Floristic nomenclature followed Pignatti et al. (1982).

Environmental data. For each sample we computed topographic and climatic variables and mean R Landolt indicator value for soil reaction (Landolt et al. 2010), which is a proxy for soil pH (Orlandi et al. 2016). The R value was calculated by averaging species values weighted by their SRA (Ravetto Enri et al. 2016). Topographic variables (elevation, slope, and aspect) were determined from a 50-m resolution digital elevation model (Piemonte CSI 2005). Aspect was transformed into southness (southness = 180 - |aspect - 180|) to avoid circular variable issues (Chang et al. 2004). A 5-km resolution raster grid derived from long-term datasets of 521 weather stations spread over the Piedmont region (Biancotti et al. 1998) supplied total mean annual precipitation (Figure 1b). To provide a general estimate of large-scale climatic patterns, we used Gams' continentality index, calculated as the arctan of the ratio between precipitation (P) and elevation (A) (Ozenda 1985).

Based on geological maps (Regione Piemonte 2006), samples were attributed to calcareous or siliceous bedrock types. Spatial analyses were carried out with the software Quantum GIS (Quantum GIS 2016, http://qgis.osgeo.org).

Data analysis

Multivariate relations between environmental variables and vegetation composition were evaluated by a canonical correspondence analysis (CCA), using CANOCO 4.5 (ter Braak and Smilauer 2012). Being R Landolt indicator value computed from vegetation composition, it was not included in the CCA to avoid a mathematical dependence with the ordination scores (Wildi 2016). Statistical significance of canonical axes was assessed by using the Monte Carlo test (499 permutations). The species percentage cover data were log(x+1)-transformed before analysis and rare species (i.e. species with a low frequency) were down-weighted to reduce their effect on the other plant species distribution in the ordination diagram (Šmilauer and Lepš 2003). Pearson's correlation was computed between topographic and climatic variables and the first and second axes of CCA.

Relationships among species richness, H', and J' and environmental data were modelled by fitting Generalised Linear Models (GLMs, Zuur et al. 2009). Species richness, H', and J'

were used as dependent variables, whereas environmental data as explanatory variables. Since species richness was a count overdispersed variable, a negative binomial distribution was specified (overdispersion was tested with the *qcc* R package, according to Scrucca 2004). Gamma distribution was set for Shannon diversity index, being it a not-normally distributed continuous variable with positive values (normality was tested with Shapiro-Wilk test), while J' was modelled with a Beta distribution, as it assumes values in the standard unit interval (0,1) (Cribari-Neto and Zeileis 2010). Highly collinear predictors ($r > |0.70|$) were excluded after a correlation analysis of environmental data. Since Gams' continentality index was positively correlated with elevation ($r = 0.80$) and negatively correlated with total annual precipitation ($r = - 0.91$), it was excluded from subsequent analyses. To analyse the size of each effect by comparing model parameter estimates (β-coefficients), explanatory variables were standardised (Z-scores). Generalised Linear Models were carried out using the glmm ADMB package (Fournier et al. 2012) in the R v. 3.2.3 environment (R Development Core Team 2015).

To assess differences in species richness, H', and J' between calcareous and siliceous bedrock surveys, Mann–Whitney U tests were performed with the 'wilcox.test' R function, as assumptions for parametric t-tests were not met even after the transformation of variables.

An Indicator Species Analysis was used to identify specific plant species associated to calcareous and siliceous bedrocks. The analysis was performed following the original Indicator Value (IndVal) function of Dufrêne and Legendre (1997) by using the 'multipatt' function of the 'indicspecies' package of R (De Cáceres and Legendre 2009). The IndVal is an index to measure the association between a species and a group (calcareous or siliceous bedrock); it is higher for species occurring in only one group (specificity) and with a high-abundance rate in all the samples belonging to that group (fidelity). The statistical significance of the association of a species with a group was obtained by 999 permutations. Differences between samples on calcareous and siliceous bedrock in their proportion of indicator species associated to the phytosociological classes typical of acidic (*Juncetea trifidi* and *N. strictae*) or calcicole swards (*Elyno-Seslerietea variae*) were assessed with a χ^2-test on a contingency table.

Results

We recorded a total of 540 plant species. The samples had a high variability in species richness, H', J', and environmental conditions (Table 1), underlying the wide ecological spectrum in which they occur. In particular, the mean Landolt soil reaction value (R) varied between 1.29 and 3.04, a range typical of vegetation communities growing from extremely to weakly acidic soils. Moreover, 374 samples out of 579 (64.6%) belonged to siliceous bedrock and 205 (35.4%) were recorded on calcareous bedrock.

The total variance explained by axes 1 and 2 of CCA amounted to 80.7% (Monte-Carlo test: F = 11.02, P = 0.002). The CCA ordination diagram (Figure 2a) showed a predominant spatial gradient along axis 1, explaining 56.6% of the total variance. Along this axis, vegetation composition was mainly affected by elevation (Pearson's correlation coefficient R_P = -0.96***), but also Gams' continentality index and precipitation were important factors (R_P = 0.85** and 0.59***, respectively). The communities occurring at the highest elevations were dominated by species having their phytosociological optimum within the classes *J. trifidi* (e.g. *Alopecurus gerardi* Vill., *Luzula spicata* (L.) DC, and *Ranunculus pyrenaeus* L.*)* and *Elyno-Seslerietea variae* (e.g. *Festuca* gr. *ovina*, *Festuca* gr. *violacea*, and *Potentilla crantzii* (Crantz) Beck). At lower elevations and with higher amount of precipitation, species having their phytosociological optimum within the class *N. strictae* prevailed (e.g. *Carex pilulifera* L., *Carex pallescens* L., and *Potentilla erecta* (L.) Rauschel). Eutrophic species, e.g. *Dactylis glomerata* L., *Cerastium holosteoides* Fries, and *Galium mollugo* L., were associated with lower elevations. Aspect and slope were less important factors to explain differences in vegetation composition, as they were less correlated to the first and second axes of the CCA. Vegetation composition did not distinctly separate calcareous and siliceous bedrock samples, due to the overlapping of calcareous bedrock surveys within the multivariate ecological space of siliceous bedrock surveys (Figure 2b).

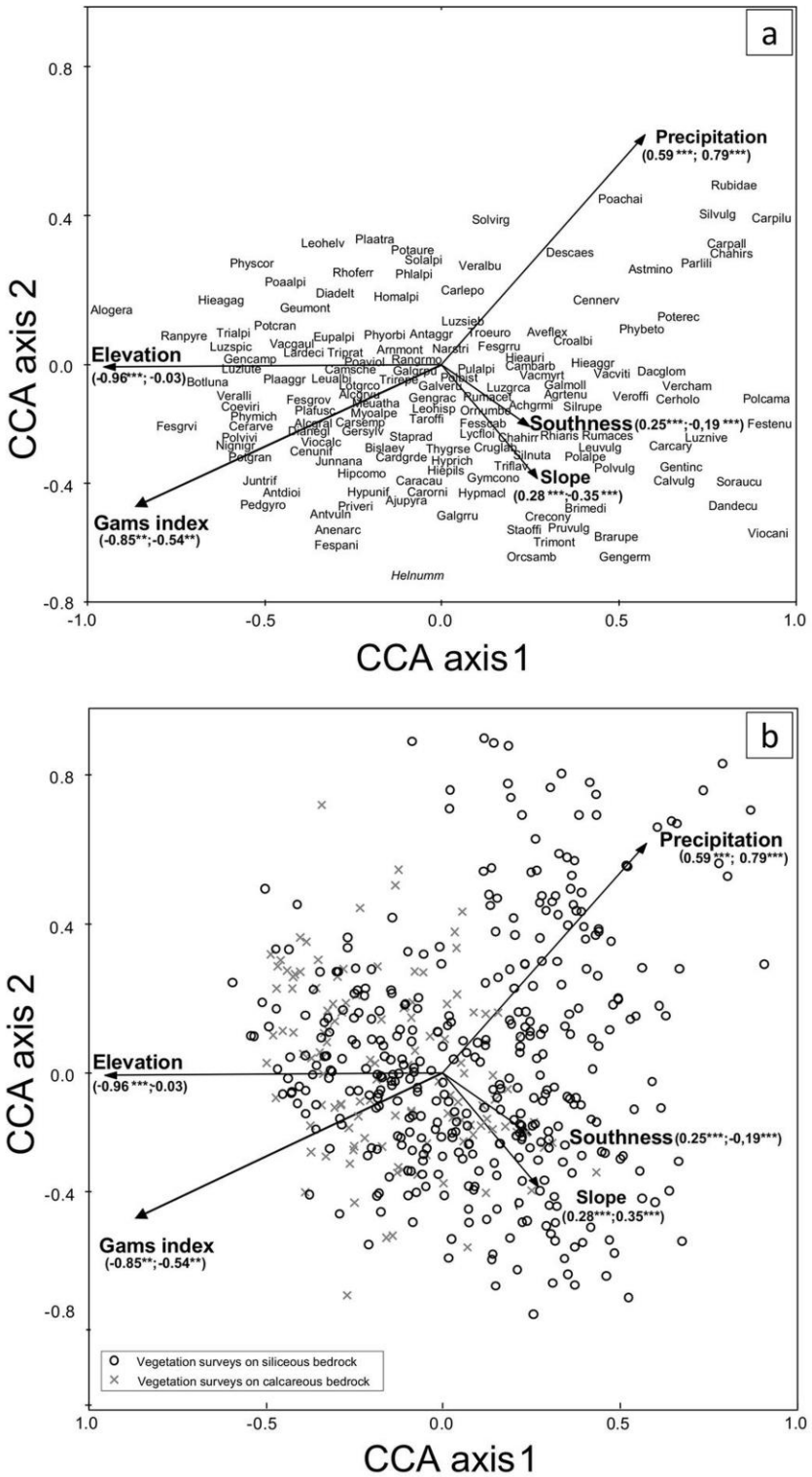

Figure 2. (a) species-environmental variable biplot ordination diagram of the Canonical Correspondence Analysis (CCA). Only species occurring in more than 5% of 579 vegetation surveys are represented. Species codes and their attribution to the phytosociological optimal

class are reported in the Appendix. Pearson's linear correlation coefficient (R_P) and significance between each ecological variable and the first and second axes are reported within brackets, respectively. (b) vegetation survey-environmental variable biplot ordination diagram of the CCA.

According to the standardised beta coefficients of GLMs, total annual precipitation and bedrock type were the two most influential factors affecting species richness, H', and J' (Table 2). Increasing annual precipitation was associated with lower values of species richness, H' and J', which instead increased on calcareous bedrock. Species richness and H' were higher at lower elevations, whereas J' was not influenced by elevation. Vascular plant diversity was higher on steeper slopes.

Samples on calcareous bedrock were characterised by a higher number of plant species (seven species more, on average), H', and J', with a more even distribution of species percentage covers, than those on siliceous bedrock (Table 3).

The indicator species analysis identified 34 species significantly associated with samples on siliceous bedrock and 89 with the calcareous bedrock (Appendix). The main difference between these two groups was related to the proportion of calcicole species, i.e. species having the phytosociological optimum in the *Elyno-Seslerietea* class, that were exclusive of *Nardus* grasslands on calcareous bedrock (Table 4).

Discussion

The high variability in topographic and climatic variables and bedrock type markedly affected species richness, H', and J' of the *Nardus* grasslands. Elevation explained most of *N. stricta* community composition and negatively influenced both species richness and H', as highlighted by literature on other plant communities (Moser et al. 2005; Nogués-Bravo et al. 2008). However, since the proportion of species with different percentage cover was rather stable at

different elevations, it did not affect the evenness of vegetation community. Moreover, elevation was not amongst the most important variables influencing plant diversity, which was instead mostly affected by mean annual precipitation and bedrock type. All the biodiversity parameters showed the highest values within the locations with lower precipitation, typically associated to the inner-alpine valleys of the Alpine chain, as also the negative relationship between precipitation and Gams' continentality index confirmed. A higher water availability, associated with frequent and abundant rainfall typical of sub-oceanic areas, determines optimal conditions for the growth of *N. stricta*, which results in an increase in its dense litter layer and competitiveness against other plant species (Chadwick 1960). Moreover, high precipitation dissolves carbonate rocks progressively, with leaching resulting in top-soil acidification (Gigon and Rorison, 1972; Partel 2002).

The positive relationship between calcareous bedrock and the diversity indexes indicates that their weakly acidic soils can host a greater number of plant species, which, according to J', were also more equally distributed in terms of their percentage cover. This relationship has never been demonstrated on acidic to weakly acidic *N. stricta*-dominated grasslands, although several studies assessed higher species richness in vegetation communities with higher soil pH (Michalet et al. 2002; Marini et al. 2008). The higher number of plant species, H', and number of indicator species in the *N. stricta* communities located on calcareous bedrock with respect to those on siliceous one may be related to the species pool effect (Zobel et al. 1998). Indeed, acidophilous species suffered more repeated extinction, by absence of refugia, than calcicole species (Ewald 2003) as during the Quaternary period calcareous substrates were widely available over large areas and for a long time, resulting in the adaptation of many species to higher pH soils (Partel 2002). Furthermore, grasslands on calcareous bedrock are probably also characterised by a larger number of microhabitats (Körner 2003) and can host a high number of plant species with different ecological needs due to their wider ecological spectrum. Indeed, under these conditions, a mosaic of calcareous rocks and acidified soil patches frequently occurs, resulting in a co-occurrence of different soil conditions at various spatial

scales, able to support the co-existence of both calcicole and acidophilous plant species (Legros et al. 1987). Some calcicole species, such as *Onobrychis montana* DC. and *Helictotrichon sedenense* (Clarion) Holub, can also occur together with acidophilous plant species in sites where the most superficial layers of the soil have been leached and acidified, as their deep root systems allow them to reach the calcareous substrata located in the subsoil (Landolt et al. 2010). Therefore, although the proportion of acidophilous plant species (i.e. species with the phytosociological optimum within the *J. trifidi* and *N. strictae* classes) between calcareous and siliceous bedrock samples was similar, the proportion of calcicole plant species was higher in samples on calcareous bedrock. Another possible factor likely to affect the vegetation composition of species-rich *Nardus* grasslands on calcareous substrates is the presence of loess deposits in the soil, as suggested by Béguin and Pochon (1971) for the Jura Mountains. However, to our knowledge, no specific data on the presence of loess deposits are available for Piedmont alpine region. For this reason, we would advise additional research on the soil features of *N. stricta* grasslands.

Conclusions

Even though the European Habitat Directive (92/43/EEC) defines as ‘species-rich’ the *N. stricta* communities on siliceous substrates, we highlighted that the *N. stricta* communities on calcareous bedrock, with acidic-to-neutral substrates where calcium has been leached in the top soil, host a higher vascular plant diversity compared to those on the siliceous one. Based on our results, we suggest that conservation actions should primarily focus on *N. stricta* habitats on calcareous substrates for the higher vascular plant diversity they host and to change the title of habitat 6230* ‘Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (and submountain areas, in continental Europe)’ to take communities of calcareous substrates into consideration.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix

Indicator species analysis for *Nardus* grasslands belonging to habitat 6230* located on siliceous and calcareous bedrocks within the Piedmont region. The Indicator value is given for each species together with its significance after 999 permutations (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Specificity and fidelity values as well as the phytosociological optimum class of each species are presented.

Tables

Table 1. Minimum, maximum, mean values ± standard deviation of mean (SD), and coefficient of variation (CV%) of the biodiversity indexes and environmental variables within *Nardus* grasslands belonging to habitat 6230* of the Piedmont region, north-western Italy.

	min	**max**	**mean**	**±**	**SD**	**CV%**
Biodiversity indexes						
Shannon diversity index (H')	0.9	5.1	3.6	±	0.68	18.62
Species richness	9.0	64.0	33.9	±	11.81	34.84
Pielou's equitability index (J')	0.26	0.90	0.73		0.10	13.70
Topographic variables						
Elevation (m)	1035.0	2229.0	1833.9	±	250.56	13.66
Slope (%)	0.7	95.8	34.2	±	16.38	47.89
Southness (°)	0.6	180.0	113.2	±	45.47	40.18
Climatic variables						
Precipitation (mm)	762.9	2298.4	1338.9	±	291.81	21.79
Gams' continentality index	27.8	70.2	53.9	±	8.20	15.21
Landolt value for soil reaction (R)	1.3	3.0	2.1	±	0.29	13.50

Table 2. Results of Generalised Linear Models (GLMs) showing the effects of topographic (elevation, slope, southness), climatic variables (total annual precipitation) and bedrock type on species richness, Shannon diversity (H'), and Pielou's equitability (J') indexes of *Nardus* grasslands belonging to habitat 6230* of Piedmont region.

	Species richness			**H'**			**J'**		
	Stand. β[1]	SE[2]	*P*-value	Stand. β	SE	*P*-value	Stand. β	SE	*P*-value
Intercept	3.44	0.02	***[3]	1.26	0.01	***	0.95	0.02	***
Elevation	-0.10	0.02	***	-0.04	0.01	***	-0.01	0.02	n.s.
Slope	0.07	0.01	***	0.03	0.01	***	0.04	0.02	*
Southness	-0.01	0.01	n.s.[4]	-0.01	0.01	n.s.	-0.03	0.02	n.s.
Total annual precipitation	-0.19	0.02	***	-0.09	0.01	***	-0.10	0.02	***
Calcareous bedrock[5]	0.17	0.03	***	0.08	0.02	***	0.09	0.04	*

[1]Stand β indicates that each coefficient of the variables (β) has been standardized

[2]SE is of standardized coefficients (β).

[3]* = $P < 0.05$; *** = $P < 0.001$

[4]n.s. not significant

[5]Siliceous bedrock was used as the reference category

Table 3. Mean values and SE for species richness, Shannon diversity index (H'), and Pielou's equitability index (J') of *Nardus* grasslands belonging to habitat 6230* on siliceous and calcareous bedrocks (Mann-Whitney U-test).

	Siliceous bedrock			**Calcareous bedrock**			***P*-value**
	mean	±	SE	mean	±	SE	
Species richness	31.4	±	0.57	38.4	±	0.84	***
H'	3.5	±	0.04	3.9	±	0.04	***
J'	0.72		0.005	0.75		0.006	***

***$P < 0.001$

Table 4. Proportion (in bold type) and number (within brackets) of indicator plant species having the phytosociological optimum within acidic (*Juncetea trifidi* and *Nardetea strictae* classes) and calcicole swards (*Elyno-Seslerietea variae* class) with the results of the χ^2-test on a contingency table.

	Siliceous bedrock	**Calcareous bedrock**	χ^2	**P-*value***
Acidic swards				
Juncetea trifidi	**14.7 %** (5/34)	**20.2 %** (18/89)	0.2	n.s.
Nardetea strictae	**11.8 %** (4/34)	**7.9 %** (7/89)	0.11	n.s.
Calcicole swards				
Elyno-Seslerietea variae	**0.0 %** (0/34)	**27 %** (24/89)	10.03	***

n.s. = not significant; *** = P < 0.001