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Plant diversity and pastoral value in alpine pastures are maximized at different nutrient indicator values

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Abstract

In alpine environments, very low and very high amounts of soil nutrients are generally associated to the lowest plant diversity and forage quality levels. Both soil nutrient content and forage quality and productivity of a site can be inferred from plant species lists, by attributing each species a nutrient indicator value (N value) and a quality value, and computing respectively average N Value and Pastoral Value (PV) at site scale. We used a wide dataset of vegetation surveys carried out in the pastures of Western Italian Alps to 1) evaluate if N values, PV, and plant diversity (species richness and Shannon diversity index) change along an elevation gradient, from montane/sub-alpine pastures (i.e. the ones located below treeline) to alpine pastures (above treeline), 2) analyze the relationships between N value and plant diversity indexes and between N value and PV, and 3) evaluate whether the N values associated to the highest plant diversity and PV differ.

Plant diversity, PV, and N values were higher in the pastures located at lower elevation. Plant diversity and PV showed a unimodal relation with N values, both in the montane/sub-alpine and alpine belts. Plant diversity indexes peaked at intermediate N indicator values, confirming the Intermediate Disturbance Hypothesis, while PV peaked at higher N values, where higher nutrient availability in the soil increased plant species productivity, growth rate, leaf turnover and nutrient concentration, digestibility, and palatability. The overall shape of the curves as well as the N values at which plant diversity and PV values peaked did not considerably change from montane/sub-alpine to alpine pastures. These results suggest that an extensive pastoral management is recommended when plant diversity conservation is the main goal. Conversely, a more intensive management can produce an overall enhancement of forage quality/productivity of alpine pastures, but only if restricted under certain critical N values.

Keywords. Biodiversity conservation, Forage quality, Generalized Additive Models (GAM), Grazing management, Hump-shaped curves, Landolt indicator values

Abbreviations. PV = Pastoral Value, N Landolt = Landolt indicator value for soil nutrient content (N), H’= Shannon diversity Index

Nomenclature. Pignatti 1982

1. Introduction

Pastoral management is one of the most important drivers of soil and plant nutrient concentration in alpine pastures, due to the removal and accumulation of nutrients that livestock exert by grazing and deposing dung and urine, respectively (Jewell et al., 2007; Lonati et al., 2015). The concentration of soil nutrients, mainly nitrogen and phosphorous, affects plant diversity and forage yield and quality as well (Güsewell et al., 2012; Gardarin et al., 2014). In alpine environments, very low and very high amounts of soil nutrients are generally associated to the lowest plant diversity and forage quality levels; low amounts of nutrients favor the dominance of few oligotrophic plant species in the sward, whereas very high amounts promote the dominance by a few nitrophilous plants. In both cases, these plant species are generally characterized by low nutritive value or high levels of toxic compounds (Aerts and Chapin, 1999; Iussig et al., 2015; Orlandi et al., 2016). For these reasons, identifying and maintaining adequate levels of nutrient concentration in the soil is a major management goal when targeting plant diversity conservation and forage yield and quality.

Soil nutrient content can be measured directly by chemical analyses or through vegetation-derived ecological indicators, such as nutrient (N) indicator values, which have the advantage to be cost-effective, since they are calculated from plant species lists (Hintermann et al., 2000). The N indicator values were originally proposed by Ellenberg (1974) for Central Europe and by Landolt (1977) for Swiss flora. Recently, they have been updated and extended to whole alpine flora by Landolt et. al. (2010), so that they are now available for each plant species growing in the Alps. Such indicator values rely on the knowledge and extensive field experience of botanists and ecologists, so to correctly characterize the condition of a site by means of ecological indicator values, a consideration of as many as possible plant species growing at that site is recommended (Landolt et. al., 2010). The N indicator values can properly characterize an area (Tölgyesi et al., 2014) and they are well correlated to the supply of several nutrients (e.g. nitrogen, phosphorous, and potassium) and to the potential biomass production of the site (Diekmann, 2003). For these reasons, their application has strongly increased in the literature since year 2000 (Wildi, 2016).

Another synthetic index derived from vegetation surveys is the Pastoral Value (PV), which summarizes forage yield, quality, and palatability for livestock (Daget and Poissonet, 1969). Since it is calculated from sward botanical composition, the PV is more constant and less influenced by temporal fluctuations than other forage parameters, such as aboveground biomass, organic matter digestibility, or crude protein content (Daget and Poissonet, 1969). Therefore, especially in pastures characterized by a high cover of perennial species, it can provide a reliable estimate of the grassland carrying capacity, which has been defined by Allen et al. (2011) as the maximum livestock stocking rate achieving a target level of animal performance, in a specified grazing system, that can be applied over a defined time without deterioration of the grazing land. The average annual carrying capacity of a particular alpine grassland can thus be calculated by multiplying its grazable area with PV and with altitudinal and slope coefficients, as defined by Cavallero et al. (2007). Moreover, the PV is directly related to forage energy and alpha-linolenic acid content (Daget and Poissonet, 1969; Ravetto Enri et al., 2017). Because of its reliability and simplicity of computation, PV has been widely used, e.g. in south-western Alps, (Probo et al., 2014, 2016; Pittarello et al., 2016a), in the Apennines (Cervasio et al., 2016), in Sardinia (Bagella et al., 2013; Bagella et al., 2017), in southern Italy (Fracchiolla et al., 2017), in central and eastern Pyrenees (Sebastià et al., 2008), in Romania (Sărăţeanu and Alexandru, 2011), and in central Chile (Ovalle et al., 1999).

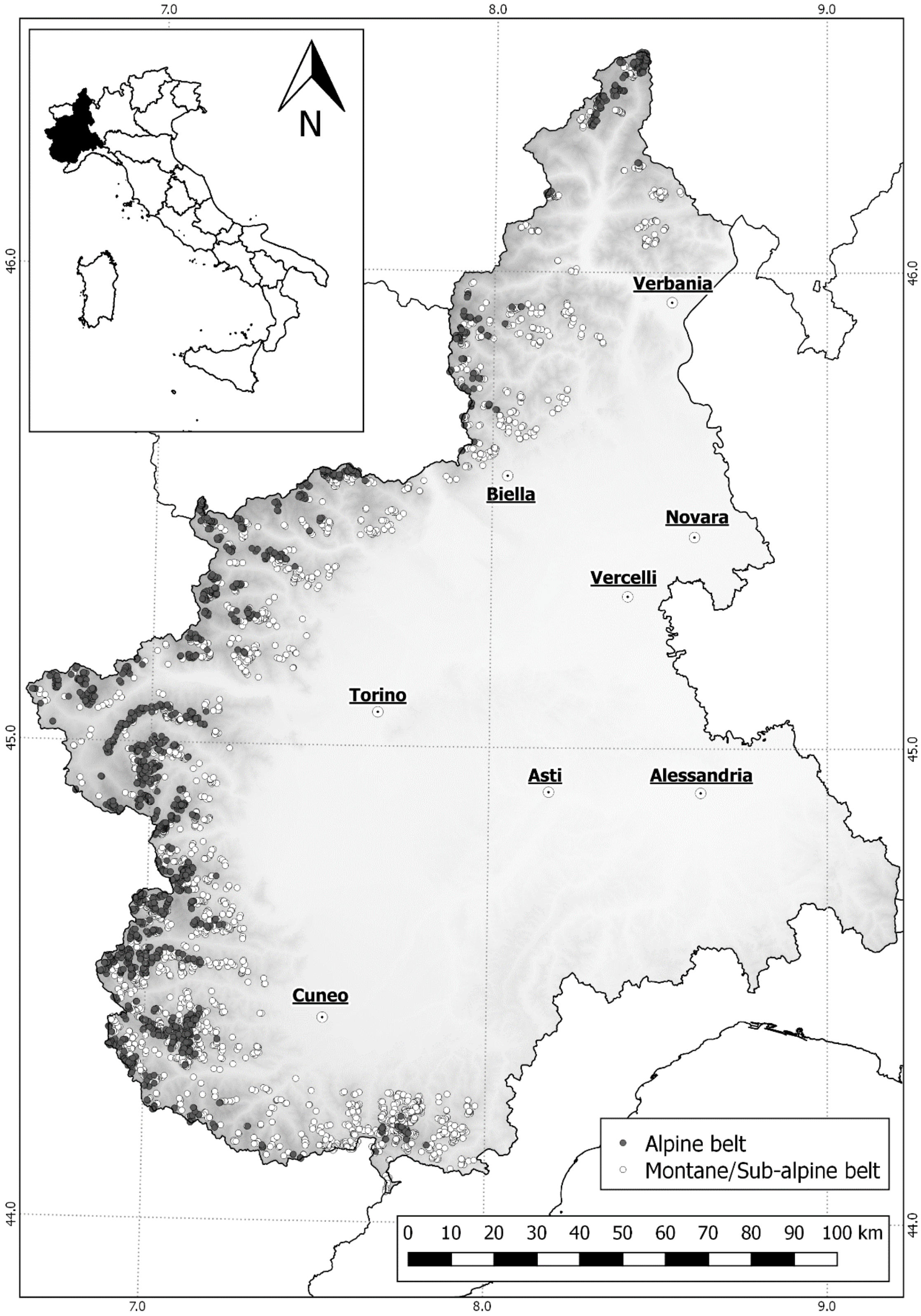
In mountain ecosystems a general decrease in plant diversity, N indicator, and forage values occur with increasing elevation, due to differences in growing season, temperature, precipitation, bedrock type, soil, nutrient contents, deposition, and mineralization rates (Körner, 2003; Güsewell et al., 2012). In this study we used a wide dataset of vegetation surveys carried out in the pastures of the Western Italian Alps to:1) evaluate if N indicator, PV, and plant diversity indexes (species richness and Shannon diversity) change along an elevation gradient, from montane/sub-alpine pastures (i.e. the ones located below treeline) to alpine pastures (i.e. the ones located above treeline), 2) analyze the relationships between N value and plant diversity indexes and N value and PV, and 3) evaluate whether the N values associated to the highest plant diversity and PV differ.

2. Materials and Methods

2.1. Study area and vegetation surveys

Data were collected across the Western Italian Alps of Piedmont Region during the period 2001 – 2007. In that period, 3839 surveys were carried out to characterize the vegetation composition of alpine pastures, which are mainly grazed by domestic livestock during summertime (Cavallero et al., 2007) (Figure 1).

Figure 1. Distribution of 3839 vegetation surveys in the Western Italian Alps, represented on Digital Terrain Model. White circles represent the vegetation surveys located below the treeline (i.e. in the montane and sub-alpine belts), dark circles the ones located above (i.e. in the alpine belt).



Elevation ranged from 491 to 2901 m a.s.l.. Vegetation surveys were carried out within vegetation communities developed over a wide spectrum of soil nutrient content conditions as described in Cavallero et al. (2007), from oligotrophic (e.g. pastures dominated by *Carex sempervirens* Vill., *Nardus stricta* L., *Trifolium alpinum* L. and *Carex sempervirens, Festuca paniculata* (L.) Sch. et Th., and *Festuca ovina* s.l.) to nitrophilous vegetation communities (e.g. pastures dominated by *Chenopodium bonus-henricus* L, *Rumex alpinus* L., and *Urtica dioica* L.), through mesotrophic (e.g. pastures dominated by *Festuca rubra* s.l. and *Agrostis tenuis* Sbith. and *Festuca violacea* s.l.) and eutrophic (e.g. pastures dominated by *Alchemilla vulgaris* s.l., *Dactylis glomerata* L., and *Trisetum flavescens* (L.) Beauv.) vegetation communities.

Each survey was conducted along a 25-m linear transect in which botanical composition was determined using the vertical point-quadrat method (Daget and Poissonet, 1971). At every 50-cm interval along the transect, plant species touching a steel needle were identified and recorded (i.e. a total of 50 measurements). Since occasional 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 (vegetation plot) was also recorded (Pittarello et al., 2016b). Plant nomenclature followed Pignatti (1982).

The N Landolt indicator value (hereafter 'N Landolt'; Landolt et al., 2010) was attributed to each plant species recorded in vegetation surveys and to all occasional plant species within vegetation plots. An average N Landolt was calculated afterwards for each survey using species presence/absence data.

For each plant species recorded in the vegetation surveys, the frequency of occurrence (f_i = number of occurrences/50 points), which is an estimate of species canopy cover (Probo et al., 2013), was calculated. Species Relative Abundance (SRA_i) was computed at each transect and used to detect the proportion of different species according to the equation of Daget and Poissonet (1971):

$$\mathrm{SRA}_i = \frac{\mathrm{f}_i}{\sum_{i=1}^{n} \mathrm{f}_i} \cdot 100(\%)$$

A SRA value = 0.3 was attributed to all occasional plant species found within vegetation plot but not along linear transects (Vacchiano et al., 2016). To estimate PV, we attributed each species an Index of specific quality (ISQ) (Daget and Poissonet, 1971; Cavallero et al., 2007). The ISQ depends on the preference, morphology, structure, and productivity of the plant species and it ranges from 0 (low) to 5 (high) (Daget and Poissonet, 1971). The PV, which ranges from 0 to 100, was calculated as follows (Daget and Poissonet, 1971):

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

where ISQ_i is the ISQ value for the species *i* (Cavallero et al., 2007).

Plant diversity was expressed in terms of species richness and Shannon diversity index (H'). Shannon diversity index (H') was calculated for each vegetation transect according to the following equation:

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

The elevation of each vegetation survey was calculated from a Digital Terrain Model (50-m resolution) (CSI Piemonte 2005). Since the altitudinal limit between montane/sub-alpine and alpine belt can vary linearly with the latitude (Ozenda, 1985), the treeline limit was linearly interpolated from the southern zone (2300 m a.s.l. – 43.5° latitude) up to the northern zone (2000 m a.s.l. – 46.5° latitude) of Piedmont. Elevational and latitudinal limits were set according to Ozenda (1985). Vegetation surveys were attributed to the montane/sub-alpine or alpine belt depending on whether their elevation was lower or higher than the interpolated treeline limit computed for the latitude at which the survey was conducted. According to this method, 2196 vegetation surveys were located below the treeline and 1643 above it (Figure 1).

2.2.<u>Data analysis</u>

Mann-Whitney U-tests (Sokal and Rohlf, 1995) were used to assess whether N Landolt, PV, species richness, and H' differed between montane/sub-alpine and alpine pastures.

Generalized Linear Models (GLMs) and Generalized Additive Models (GAMs) were performed to analyze the relationships between N Landolt and PV, species richness, and H'. The models were performed separately for the vegetation surveys located in the montane/sub-alpine and alpine belts. The GLMs (Zuur et al., 2009) were fitted by using both the linear and quadratic term of N Landolt to check for non-linear relationships. For the GAMs, a cubic regression spline was used as smoothing function of N Landolt and the cross-validation was applied to estimate the optimal amount of smoothing, expressed as 'effective degree of freedom' (edf). This is a value ranging between 0 and infinity, and the higher the edf, the more non-linear is the smoothing spline (a GAM with edf = 1 is a straight line). The more complex pattern described by GAMs through non-parametric smoothers may give additional information in the graphical output compared to GLMs, as they allow to capture the shape of a relationship without choosing a specific parametric form (Crawley, 2007). Being PV and H' positive and continuous variables not normally distributed (the normality was tested using the Shapiro-Wilk test), a gamma distribution was used in the models. Since species richness was a count overdispersed variable, a negative binomial distribution was specified (overdispersion in the data was tested by the *qcc* R package; Scrucca, 2004). In case of a possible unimodal relationship, peak values were detected by the first derivative of GLMs.

Statistical analyses were performed using the software R 3.2.3 for Windows (R Core Team, 2015). Generalized Linear Models were performed using the "glm" and "glm.nb" functions of the "stats" package (R Core Team, 2015), whereas GAMs were run using the "gam" function of the "mgcv" package (Wood, 2011).

3. Results

A total of 1033 plant species was recorded in the vegetation surveys (the complete list of all plant species, with their respective N and ISQ values is provided in Appendix A). Mann-Whitney tests showed significant differences between N Landolt, PV, species richness, and H' of the pastures located in the montane/sub-alpine belt compared to the alpine belt ones (Table 1).

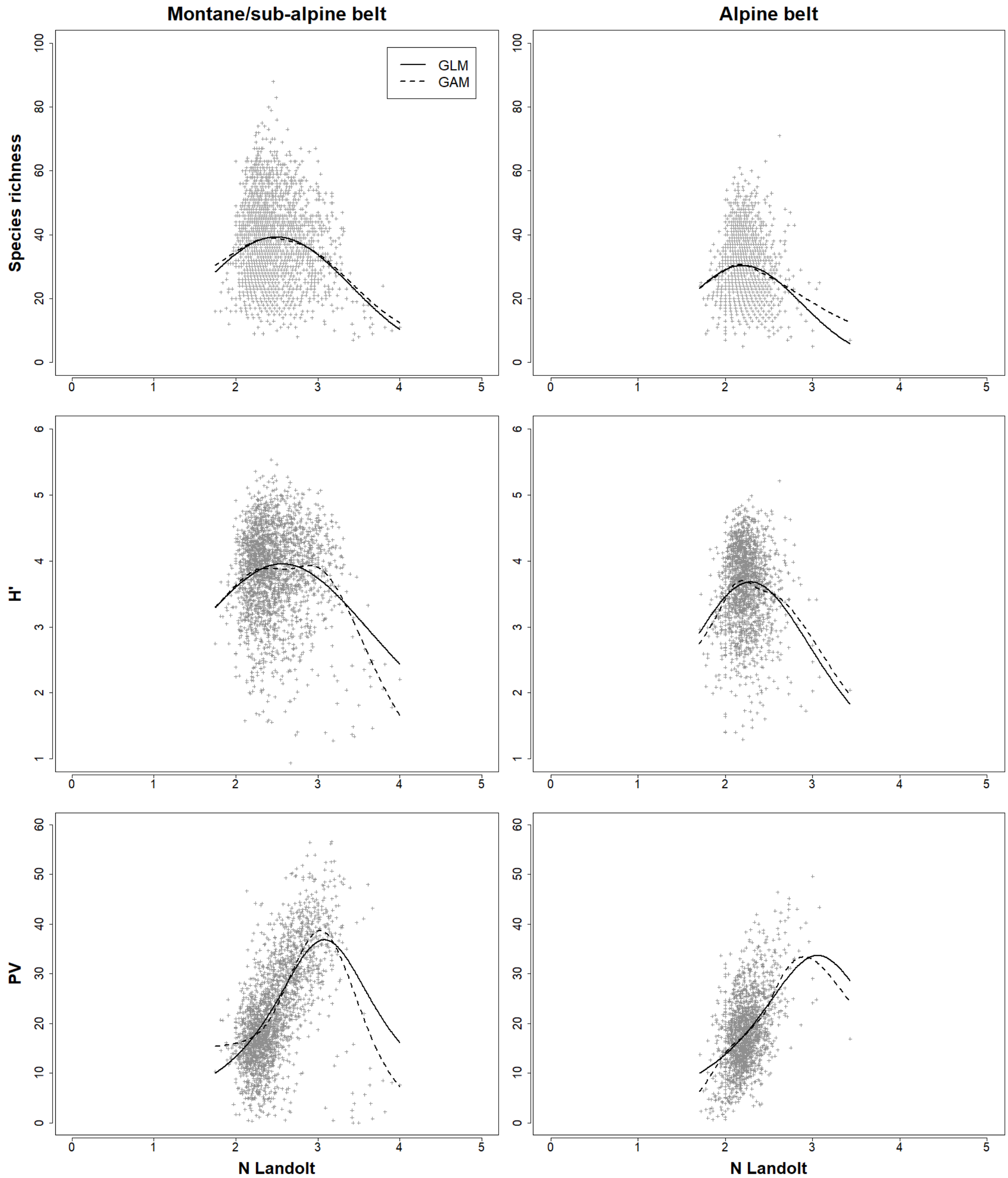
Table 1. Mean values and Standard Error (SE) for Landolt indicator value for soil nutrient content (N Landolt), forage pastoral value (PV), species richness, and Shannon diversity index (H') of montane/sub-alpine and alpine pastures.

	Montane/sub-alpine pastures	Alpine pastures	
	mean ± SE	mean ± SE	*P*-value
N Landolt	2.5 ± 0.01	2.2 ± 0.01	***
PV	22.5 ± 0.22	18.3 ± 0.18	***
species richness	37.3 ± 0.28	29.4 ± 0.25	***
H'	3.8 ± 0.01	3.6 ± 0.02	***

*** P < 0.001 (Mann-Whitney U-test)

With both GLMs and GAMs, a unimodal relationship of plant diversity indexes and PV with the N Landolt was detected, both in the montane/sub-alpine and alpine belts (Figure 2). A hump-shaped relation emerged due to the significance of the quadratic term in all GLMs (Appendix B) as well as of the smoothing function of N Landolt and the effective degree of freedom (edf), which was always greater than 1 in all GAMs (Appendix C). Moreover, the fitted values of both the GLMs and GAMs widely overlapped (Figure 2). The N Landolt to which each predictor peaked was similar between montane/sub-alpine and alpine belts: species richness peaked at N Landolt of 2.5 and 2.2, H' at 2.6 and 2.3, and PV at 3.1 and 3.1, respectively at montane/sub-alpine belt and at alpine belt.

Figure 2. Relationships between Landolt indicator value for soil nutrient content (N Landolt) and species richness, Shannon diversity index (H'), and pastoral value (PV) of montane/sub-alpine and alpine pastures. The solid lines represent the predicted values by the Generalized Linear Models (GLM) using both the linear and quadratic term of N Landolt. The dashed lines represent the predicted values by the Generalized Additive Models (GAM) using a cubic regression spline as smoothing function of N Landolt and the cross-validation to estimate the optimal amount of smoothing.



4. Discussion

The lower values of plant diversity, pastoral value, and soil nutrient content of alpine pastures if compared to montane/sub-alpine ones were consistent with the results obtained by other studies (Moser et al., 2005; Güsewell et al., 2012). The number of species functionally adapted to tolerate the stress imposed by extreme pedo-climatic conditions at high elevation (e.g. short growing season, low temperatures, and shallow soils) decreases with increasing altitude (Körner, 2003). Due to such environmental constraints, aboveground biomass is also generally lower than in montane/sub-alpine belts, which results in a lower ISQ of the species found at higher elevation and a lower PV of plant communities. Under these lower productivity conditions, pastures have lower carrying capacity and can be exploited less intensively, i.e. with lower stocking rates. Consequently, weaker organic fertilization by grazing animals and human activities contributes to determine a lower soil nutrient content if compared to montane/sub-alpine belt pastures. Indeed, N Landolt has been considered as a proxy of management intensity (Dietschi et al., 2007; Strebel and Bühler, 2015). The PV measured in these extensively managed alpine pastures was comparable with the PV assessed in other extensive semi-natural grassland ecosystems, such as Mediterranean (Bagella et al., 2013; Bagella et al., 2017; Fracchiolla et al., 2017) and Apennine grasslands (Cervasio et al., 2016). The same authors measured PV up to 60-70 only under more intensive management, i.e. after N and P fertilization (Bagella et al., 2017), ploughing and sowing of forage mixtures (Cervasio et al., 2016), or in permanent grasslands developed over former arable lands, where the contribution of sown legumes was still considerable (Fracchiolla et al., 2017).

Even though plant diversity and PV differed between montane/sub-alpine and alpine pastures, all these variables showed a unimodal relationship with N Landolt, both in the montane/sub-alpine and alpine belts. Gusewell et al. (2012) detected a “hump-shaped” curve between species richness and N Landolt only in sub-alpine and alpine grasslands, but they found species richness linearly and negatively related to N Landolt in Swiss lowland and montane grasslands. The different shape of this relationship at lower elevations might result from a narrower range of the different conditions analyzed compared to our study. Indeed, the greater the range in the N value predictors, the more probable is the development of “hump-shape” relationships (Guo and Berry, 1998; Espinar, 2006). Such a response was also assessed by other authors with the direct measurement of soil nitrogen content (Vermeer and Berendse, 1983; Janssens et al., 1998).

Species richness and H’ peaked at intermediate N Landolt level, while PV peaked at higher N Landolt levels. The highest level of plant diversity at intermediate levels of management intensity was found by several other studies (Olff and Ritchie, 1998; Dupre and Diekmann, 2001; Eek and Zobel, 2001; Dietschi et al., 2007; Orlandi et al., 2016), confirming the Intermediate Disturbance Hypothesis, which states that species richness peaks at intermediate levels of disturbance/management as a result of the co-existence of several species due to ecological niche overlaps (Grime, 1973; Connell, 1978; Marini et al., 2008).

In contrast, PV peaked at higher management intensity, where the higher nutrient availability in the soil increased plant species productivity (Mattson, 1980), growth rate, leaf turnover and nutrient concentration, digestibility, and palatability (Aerts, 1999). The PV had low values where the nutrient content in the soils was low, as plant species were characterized by lower ISQ because of tougher leaves with lower concentrations of nutrients, slower turnover rates, and higher concentration of secondary compounds, acting as defense against herbivories (Aerts and Chapin, 1999). A sharp decline in PV was also detected when soil nutrient content exceeded optimal levels, due to the dominance of a low number of nitrophilous species (e.g. *R. alpinus*) within plant communities (Zaller, 2004; Bohner, 2005). These species, which are competitive, fast growing, and highly efficient in the use of both above- and below-ground resources (Aerts, 1999; Bohner, 2005; Hejcman et al., 2012; Šilc and Gregori, 2016) are often characterized by prickles (e.g. *Carduus* and *Cirsium*) or high content of irritating (e.g. *U. dioica*) and/or toxic compounds (e.g. *C. bonus-henricus, R. alpinus, Veratrum album* L.) (Schaffner et al., 2001; Šilc and Gregori, 2016). These morphological and chemical attributes negatively affect their forage quality and palatability, and strongly lower their ISQ and the PV of the communities in which they develop (Roggero et al., 2002; Cavallero et al., 2007). At N Landolt values lower than the peak, i.e. when soil nutrient content was below optimal levels, the reduction in PV was much more pronounced than that in plant diversity. This result can be interpreted considering that some plant species, which were often dominant under nutrient poor conditions and thus characterized by low productivity, forage quality, and palatability (e.g. the mat-grass *Nardus stricta* L.), were often found in species-rich communities, such as *N. stricta* grasslands, which are also protected by the European Habitat Directive (92/43/CEE) because of their high plant diversity. Indeed, when nutrient availability in the soil is not sufficient to allow nitrophilous species become dominant, the number of plant species is generally high (Huston, 1979).

Interestingly, the overall shape of curves as well as N indicator values at which plant diversity and PV values peaked did not considerably change from montane/sub-alpine to alpine pastures. Consequently, even if different plant communities with diverse ecological needs and functional adaptations occurred along the explored elevation gradient, they showed similar inherent ecological relationships. This result underlines that pastoral management intensity produced similar gradients and responses in plant communities, regardless they were located in the lower or upper alpine belts.

5. Conclusions

Despite plant diversity and PV were lower in alpine than in montane/sub-alpine pastures and plant diversity peaked at lower N Landolt values than PV, they showed similar unimodal relationships with N indicator values along the elevational gradient analyzed. Management implications regarding the identification of specific and sustainable N Landolt thresholds, which are proxies of pastoral management intensity, can be derived from the current study: an intermediate intensity pastoral management, associated to intermediate stocking and fertilization rates, is recommended when biodiversity conservation is the main goal. Conversely, a more intensive management can produce an overall enhancement of forage quality/productivity of alpine pastures, but only if restricted under certain critical N values.

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7. References

Aerts, R., 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant–soil feedbacks. J. Exp. Bot. 50, 29–37. doi:10.1093/jexbot/50.330.29

Aerts, R., Chapin, F.S., 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. 30, 1–67. doi:10.1016/S0065-2504(08)60016-1

Allen, V.G., Batello, C., Berretta, E.J., Hodgson, J., Kothmann, M., Li, X., McIvor, J., Milne, J., Morris, C., Peeters, A., 2011. An international terminology for grazing lands and grazing animals. Grass forage Sci. 66, 2–28.

Bagella, S., Sitzia, M., Roggero, P. P. 2017. Soil fertilization contributes to mitigating forest fire hazard associated with *Cistus monspeliensis* L. (rock rose) shrublands. Int J. Wildland Fire 26: 156-166. doi: 10.1071/WF16114.

Bagella, S., Salis, L., Marrosu, G. M., Rossetti, I., Fanni, S., Caria, M. C., Roggero, P. P. 2013. Plant Ecol. 214: 621-631. doi:10.1007/s11258-013-0194-x

Bohner, A., 2005. *Rumicetum alpini* Beger 1922 – species composition, soil-chemical properties, and mineral element content. Wulfenia 12, 113–126.

Cavallero, A., Aceto, P., Gorlier, A., Lombardi, G., Lonati, M., Martinasso, B., Tagliatori, C., 2007. I tipi pastorali delle Alpi piemontesi. (Pasture types of the Piedmontese Alps). Alberto Perdisa Editore. p. 467, Bologna.

Cervasio, F., Argenti, A., Genghini, M., Ponzetta, M. P. 2016. Agronomic methods for mountain grassland habitat restoration for faunistic purposes in a protected area of the norhern Apennines (Italy). iForest 9: 490-496. doi: 10.3832/ifor1515-008.

Connell, J.H., 1978. Diversity in tropical rain forests and coral reefs. Science. 199, 1302–1310. doi:10.1126/science.199.4335.1302

Crawley, M.J., 2007. The R book. The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England.

Daget, P., Poissonet, J., 1971. A method of plant analysis of pastures. Ann. Agron. 22, 5–41.

Daget, P., Poissonet, J., 1969. Analyse phytologique des praries, Centre Nat. ed, Applications agronomiques. Montpellier - France.

Diekmann, M., 2003. Species indicator values as an important tool in applied plant ecology - a review. Basic Appl. Ecol. 4, 493–506. doi:10.1078/1439-1791-00185

Dietschi, S., Holderegger, R., Schmidt, S.G., Linder, P., 2007. Agri-environment incentive payments and plant species richness under different management intensities in mountain meadows of Switzerland. Acta Oecologica 31, 216–222. doi:10.1016/j.actao.2006.10.006

Dupre, C., Diekmann, M., 2001. Differences in species richness and life-history traits between grazed and abandoned grasslands in southern Sweden. Ecography. 24, 275–286. doi:10.1111/j.1600-0587.2001.tb00200.x

Eek, L., Zobel, K., 2001. Structure and diversity of a species-rich grassland community, treated with additional illumination, fertilization and mowing. Ecography. 24, 157–164. doi:10.1034/j.1600-0587.2001.240206.x

Ellenberg, H., 1974. Indicator values of vascular plants in central Europe. Scr. Geobot. 9.

Espinar, J.L., 2006. Sample size and the detection of a hump-shaped relationship between biomass and species richness in Mediterranean wetlands. J. Veg. Sci. 17, 227–232. doi:10.1658/1100-9233(2006)17[227:SSATDO]2.0.CO;2

Fracchiolla, M., Terzi, M., D’Amico, F. S., Tedone, L., Cazzato, E. 2017. Conservation and pastoral value of former arable lands in the agro-pastoral system of the Alta Murgia National Park (Southern Italy). Italian J. Agron. 12: 847. doi: 10.4081/ija.2017.847

Gardarin, A., Garnier, É., Carrère, P., Cruz, P., Andueza, D., Bonis, A., Colace, M.-P., Dumont, B., Duru, M., Farruggia, A., Gaucherand, S., Grigulis, K., Kernéïs, É., Lavorel, S., Louault, F., Loucougaray, G., Mesléard, F., Yavercovski, N., Kazakou, E., 2014. Plant trait-digestibility relationships across management and climate gradients in permanent grasslands. J. Appl. Ecol. 51, 1207–1217. doi:10.1111/1365-2664.12293

Grime, J.P., 1973. Competitive exclusion in herbaceous vegetation. Nature 242, 344–347. doi:10.1038/242344a0

Guo, Q., Berry, W.L., 1998. Species richness and biomass: dissection of the hump-shaped relationships. Ecology 79, 2555–2559.

Güsewell, S., Peter, M., Birrer, S., 2012. Altitude modifies species richness-nutrient indicator value relationships in a country-wide survey of grassland vegetation. Ecol. Indic. 20, 134–142. doi:10.1016/j.ecolind.2012.02.011

Hejcman, M., Strnad, L., Hejcmanova, P., Pavlu, V., 2012. Response of plant species composition, biomass production and biomass chemical properties to high N, P and K application rates in *Dactylis glomerata*- and *Festuca arundinacea*-dominated grassland. Grass Forage Sci. 67, 488–506. doi:10.1111/j.1365-2494.2012.00864.x

Hintermann, U., Weber, D., Zangger, A., 2000. Biodiversity monitoring in Switzerland. Schriftenr. für Landschaftspfl. und Naturschutz 62, 47–58.

Huston, M., 1979. A General Hypothesis of Species Diversity. Am. Nat. 113, 81–101. doi:10.1086/283366

Iussig, G., Lonati, M., Probo, M., Hodge, S., Lombardi, G., 2015. Plant species selection by goats foraging on montane semi-natural grasslands and grazable forestlands in the Italian Alps. Ital. J. Anim. Sci. 14, 484–494. doi:10.4081/ijas.2015.3907

Janssens, F., Paaters, A., Tallowin, J., Bakker, J., Bekker, R., Fillat, F., Oomes, M., 1998. Relationship between soil chemical factors and grassland diversity. Plant Soil 202, 69–78. doi:10.1023/A

Jewell, P.L., Käuferle, D., Güsewell, S., Berry, N.R., Kreuzer, M., Edwards, P.J., 2007. Redistribution of phosphorus by cattle on a traditional mountain pasture in the Alps. Agric. Ecosyst. Environ. 122, 377–386. doi:10.1016/j.agee.2007.02.012

Körner, C., 2003. Alpine plant life: functional plant ecology of high mountain ecosystems. Springer.

Landolt, E., 1977. Ökologische Zeigerwerte zur Schweizer Flora. Zürich.

Landolt, E., et. al, 2010. Flora indicativa:Ökologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen (Ecological Indicator Values and Biological Attributes of the Flora of Switzerland and the Alps). Haupt Verlag Ag, Bern; Stuttgart; Wien.

Lonati, M., Probo, M., Gorlier, A., Lombardi, G., 2015. Nitrogen fixation assessment in a legume-dominant alpine community: comparison of different reference species using the 15N isotope dilution technique. Alp. Bot. 125, 51–58. doi:10.1007/s00035-014-0143-x

Marini, L., Fontana, P., Scotton, M., Klimek, S., 2008. Vascular plant and Orthoptera diversity in relation to grassland management and landscape composition in the European Alps. J. Appl. Ecol. 45, 361–370. doi:10.1111/j.1365-2664.2007.01402.x

Mattson, W.J., 1980. Herbivory in relation to plant Nitrogen content. Annu. Rev. Ecol. Syst. 11, 119–161. doi:10.1146/annurev.es.11.110180.001003

Moser, D., Dullinger, S., Englisch, T., Niklfeld, H., Plutzar, C., Sauberer, N., Zechmeister, H.G., Grabherr, G., 2005. Environmental determinants of vascular plant species richness in the Austrian Alps. J. Biogeogr. 32, 1117–1127. doi:10.1111/j.1365-2699.2005.01265.x

Olff, H., Ritchie, M.E., 1998. Effects of herbivores on grassland plant diversity. Trends Ecol. Evol. 13, 261–265.

Orlandi, S., Probo, M., Sitzia, T., Trentanovi, G., Garbarino, M., Lombardi, G., Lonati, M., 2016. Environmental and land use determinants of grassland patch diversity in the western and eastern Alps under agro-pastoral abandonment. Biodivers. Conserv. 25, 275–293. doi:10.1007/s10531-016-1046-5

Ovalle, C., Aronson, J., Del Pozo, A., Avendano, J., 1999. Restoration and rehabilitation of mixed espinales in central Chile: 10-year report and appraisal. Arid Soil Res. Rehabil. 13, 369–381. doi:Doi 10.1080/089030699263258

Ozenda, P., 1985. La Vegetation de la Chaine Alpine dans l'Espace Montagnard Europeen, Masson. ed. Paris.

Pignatti, S., 1982. Flora d'Italia (Flora of Italy). Edagricole, Bologna.

Pittarello, M., Probo, M., Lonati, M., Lombardi, G., 2016a. Restoration of sub-alpine shrub-encroached grasslands through pastoral practices: Effects on vegetation structure and botanical composition. Appl. Veg. Sci. 19, 381–390. doi:10.1111/avsc.12222

Pittarello, M., Probo, M., Lonati, M., Bailey, D.W., Lombardi, G., 2016b. Effects of traditional salt placement and strategically placed mineral mix supplements on cattle distribution in the Western Italian Alps. Grass Forage Sci. 71, 529–539. doi:10.1111/gfs.12196

Probo, M., Pittarello, M., Lonati, M., Lombardi, G., 2016. Targeted grazing for the restoration of sub-alpine shrub-encroached grasslands. Ital. J. Agron. 11, 268. doi:10.4081/ija.2016.775

Probo, M., Lonati, M., Pittarello, M., Bailey, D.W., Garbarino, M., Gorlier, A., Lombardi, G., 2014. Implementation of a rotational grazing system with large paddocks changes the distribution of grazing cattle in the south-western Italian Alps. Rangel. J. 36, 445–458.

Probo, M., Massolo, A., Lonati, M., Bailey, D.W., Gorlier, A., Maurino, L., Lombardi, G., 2013. Use of mineral mix supplements to modify the grazing patterns by cattle for the restoration of sub-alpine and alpine shrub-encroached grasslands. Rangel. J. 35, 85–93. doi:10.1071/RJ12108

R Core Team., 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ravetto Enri, S., Renna, M., Probo, M., Lussiana, C., Battaglini, L.M., Lonati, M., Lombardi, G., 2017. Relationships between botanical and chemical composition of forages: a multivariate approach to grasslands in the Western Italian Alps. J. Sci. Food Agric. 97(4): 1252-1259. doi:10.1002/jsfa.7858

Roggero, P.P., Bagella, S., Farina, R., 2002. Un Archivio dati di Indici specifici per la valutazione integrata del valore pastorale. Riv. di Agron. 36, 149–156.

Sărăţeanu, V., Alexandru, M., 2011. Influence of *Prunus spinosa* L. shrub on the grassland vegetation in western Romania. J. Water L. Dev. 65–71. doi:10.2478/v10025-012-0006-z

Schaffner, U., Kleijn, D., Brown, V., Muller-Scharer, H., 2001. *Veratrum album* in montane grasslands: a model system for implementing biological control in land management practices for high biodiversity habitats. Biocontrol News Inf. 22, 19N--28N.

Scrucca, L., 2004. *qcc*: an R package for quality control charting and statistical process control. R news 4, 11–17.

Sebastià, M.T., de Bello, F., Puig, L., Taull, M., 2008. Grazing as a factor structuring grasslands in the Pyrenees. Appl. Veg. Sci. 11, 215–222. doi:10.3170/2008-7-18358

Šilc, U., Gregori, M., 2016. Control of alpine dock (*Rumex alpinus*) by non- chemical methods. Acta Biol. Slov. 1, 23–32.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: the principles and practice of statistics in biological research. W.H. Freeman. p. 887, New York, NY, USA.

Strebel, N., Bühler, C., 2015. Recent shifts in plant species suggest opposing land-use changes in alpine pastures. Alp. Bot. 125, 1–9. doi:10.1007/s00035-015-0145-3

Tölgyesi, C., Bátori, Z., Erdos, L., 2014. Using statistical tests on relative ecological indicator values to compare vegetation units - Different approaches and weighting methods. Ecol. Indic. 36, 441–446. doi:10.1016/j.ecolind.2013.09.002

Vacchiano, G., Meloni, F., Ferrarato, M., Freppaz, M., Chiaretta, G., Motta, R., Lonati, M., 2016. Frequent coppicing deteriorates the conservation status of black alder forests in the Po plain (northern Italy). For. Ecol. Manage. 382, 31–38. doi:10.1016/j.foreco.2016.10.009

Vermeer, A.J.G., Berendse, F., 1983. The relationship between nutrient availability, shoot biomass and species richness in grassland and wetland communities. Plant Ecol. 53, 121–126.

Wildi, O., 2016. Why mean indicator values are not biased. J. Veg. Sci. 27, 40–49. doi:10.1111/jvs.12336

Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. Roy. Stat. Soc. (B) 73(1):3-36

Zaller, J.G., 2004. Ecology and non-chemical control of *Rumex crispus* and *R. obtusifolius* (Polygonaceae): A review. Weed Res. 44, 414–432. doi:10.1111/j.1365-3180.2004.00416.x

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R, 2009th ed. Springer. p. 596, New York, NY, USA.

8. Appendices

Appendix B. Results of Generalized Linear Model (GLM) in which forage Pastoral Value (PV), species richness, and Shannon diversity index (H') were modeled with the linear term and the quadratic term of Landolt indicator value for soil nutrient content (N), in the alpine and montane/sub-alpine belt, respectively.

	Montane/Sub-alpine belt				Alpine belt			
	Estimate	SE	t/z	*P-value*	Estimate	SE	t/z	*P-value*
Species richness								
Family: Negative binomial								
Link: Log								
Intercept	0.00	0.387	-0.01	n.s.	-1.84	0.792	-2.32	*
N	2.95	0.298	9.878	***	4.78	0.690	6.921	***
N^2	-0.59	0.057	-10.4	***	-1.09	0.150	-7.23	***
H'								
Family: Gamma								
Link: Log								
Intercept	0.76	0.053	14.23	***	1.37	0.121	11.39	***
N	-0.39	0.041	-9.54	***	-0.96	0.106	-9.14	***
N^2	0.08	0.008	9.686	***	0.21	0.023	9.147	***
PV								
Family: Gamma								
Link: Log								
Intercept	0.41	0.017	24.42	***	0.39	0.038	10.21	***
N	-0.25	0.013	-19.8	***	-0.23	0.031	-7.42	***
N^2	0.04	0.002	17.36	***	0.04	0.006	5.861	***

***: P < 0.001
n.s.: not significant

Appendix C. Results of Generalized Additive Model (GAM) in which forage Pastoral Value (PV), species richness, and Shannon diversity index (H’) were modeled with a smoothing function of N Landolt, in the alpine and montane/sub-alpine belt, respectively. A cubic regression spline was used as smoothing function and the cross-validation was applied to estimate the optimal amount of smoothing.

	Montane/Sub-alpine belt				Alpine belt			
Species richness								
Family: Negative binomial								
Link: Log								
	Estimate	**SE**	**z**	***P-value***	**Estimate**	**SE**	**z**	***P-value***
Intercept	3.61	0.022	167.1	***	3.37	0.025	134.6	***
	edf	**Ref.df**	**Chi.sq**	***P-value***	**edf**	**Ref.df**	**Chi.sq**	***P-value***
s(N)	3.00	3.780	16.46	**	2.69	3.443	9.12	*
H'								
Family: Gamma								
Link: Log								
	Estimate	**SE**	**t**	***P-value***	**Estimate**	**SE**	**t**	***P-value***
Intercept	1.34	0.004	370.0	***	1.28	0.004	310.5	***
	edf	**Ref.df**	**F**	***P-value***	**edf**	**Ref.df**	**F**	***P-value***
s(N)	5.295	6.317	27.04	***	5.81	6.106	19.65	***
PV								
Family: Gamma								
Link: Log								
	Estimate	**SE**	**t**	***P-value***	**Estimate**	**SE**	**t**	***P-value***
Intercept	3.13	0.007	432.7	***	2.89	0.009	319.6	***
	edf	**Ref.df**	**F**	***P-value***	**edf**	**Ref.df**	**F**	***P-value***
s(N)	5.24	6.263	247.3	***	5.93	6.959	65.41	***

***: P < 0.001; **: 0.001< P < 0.0.1