

Article

Nutrient Analysis and Species Diversity of Alpine Grasslands: A Comparative Analysis of Less Studied Biodiversity Hotspots

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Abstract: The alpine grasslands of Kashmir Himalaya act as a treasure house of floristic biodiversity. They have remained largely unstudied because of their remoteness and inaccessibility. It is imperative to have quantitative studies of these areas to allow the long-term monitoring of flora in these fragile ecosystems. During the present study, nutrient analysis and species diversity of some alpine grasslands were investigated. Electroconductivity (EC) of the soils ranged between 0.12 and 0.33 (dSm⁻¹). With an increase in altitude and precipitation and a decrease in temperature, soil pH and available macro-nutrients (OC, N, P, K) show a considerable decrease. Sixty-six plant species belonging to twenty-nine families and fifty-one genera were reported with members predominantly from the Asteraceae, Rosaceae and Plantaginaceae families. Seven species were common to all study areas and Renyi diversity profiles showed that Kongwattan was the most diverse followed by Poshpathri and Yousmarg. The results of the Sorensen β diversity index showed a relatively lower dissimilarity index among the three studied alpine sites. In the majority of the growth forms, growth initiation was recorded in April, whereas senescence occurred in September. The highest bloom was seen in June–July. The plant species exhibited a greater variability in their phenophases under different environmental conditions and altitudinal gradients. Plants were more vigorous at lower altitudes and showed rapid response to the prevailing conditions. Stoloniferous forbs and tussock forming graminoids such as *Sibbaldia cuneata*, *Trifolium repens*, *Plantago major*, *Trifolium pratense*, *Poa compressa*, *Poa angustifolia*, and *Plantago lanceolata* showed a greater importance value index (IVI). The sedentary system of livestock rearing at Yousmarg resulted in the decreased density of the palatable species. This study allowed us to conclude that direct knowledge of soil nutrient composition and species diversity in alpine ecosystems can enhance conservation and ensure better management practices over a period of time.

Keywords: alpine grasslands; Kashmir Himalaya; species diversity; stoloniferous forbs; tussock graminoids; phenophase; livestock grazing



Citation: Wani, I.A.; Verma, S.; Gupta, R.; Ganaie, M.M.; Nigam, G.; Shafik, H.M.; Al-Misned, F.A. Nutrient Analysis and Species Diversity of Alpine Grasslands: A Comparative Analysis of Less Studied Biodiversity Hotspots. *Sustainability* **2022**, *14*, 887. <https://doi.org/10.3390/su14020887>

Academic Editors: Susana Santos and Alejandro Rescia

Received: 22 November 2021

Accepted: 21 December 2021

Published: 13 January 2022

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1. Introduction

The Himalaya is home to one of the most diverse and unique ecosystems on the planet [1]. Himalayan alpine plant communities are ecologically significant because they govern soil stability, play an important role in ecosystem functioning, and are important in cultural, ethical, and aesthetic aspects [2]. These regions show low productivity due

to unusual seasonal fluctuations with respect to abrupt changes in the wind velocity, low temperature, permafrost, extremely cold winters, and heavy snowstorms [3]. The flora of these fragile ecosystems responds to the severe climatic conditions by growing in sparse populations, showing reduced morphological characteristics, and developing a mosaic patch of different forms. Alpine plants show an early growth initiation with a short vegetative span ranging from several days to a few months [4]. Himalayan grasslands are important both ecologically and economically [5]. They harbor the world's largest freshwater sources. In India, the Himalaya extends from Jammu and Kashmir to north-eastern India through Sikkim, occupying an area of 236,000 Km². Kashmir Himalaya constitutes the northern most part of the Indian Himalayan region [6]. The importance of the Himalaya in Kashmir is substantiated by the fact that a sizeable population of Jammu and Kashmir directly or indirectly depend upon it. Jammu and Kashmir, the western extremity of the Himalayan Mountain chain, sustain a predominantly large number of alpine grasslands [7] which spread above the timberline and below the snow-covered mountain peaks of the Zaskar and Pir Panjal mountain ranges. Such grasslands are dominated by herbaceous plant communities that grow in tussocks [8,9].

Grasslands provide various ecological functions, including biodiversity conservation, control of physical and chemical fluxes in ecosystems, pollution mitigation, and landscape preservation [10–12]. Alpine grasslands consist of two management categories, meadows and pastures. These are highly valued for their great species diversity in comparison to forest and shrub vegetation [13–16]. Soil pH, bulk density, available nutrients and moisture, temperature, photoperiod, and soil organic carbon (SOC) are the major factors that determine the biodiversity of the alpine regions [17–19]. Soils which are acidic (pH < 7) show less plant diversification [20–22]. High soil organic carbon increases soil water-holding ability, thereby increasing water-retention capacity and sustaining soil fertility [23]. In grassland ecosystems, soil bulk density (SBD) affects the microbial activity, aeration, soil porosity, nutrient composition, and water holding capacity of soil which influences floristic diversity [24–26]. In addition, the floristic composition of alpine grasslands is also influenced by various anthropogenic activities, livestock management practices, and different levels of livestock density and/or the use of feed supplements [27].

For the past several decades, the Himalayan meadows have been heavily grazed [28–31]. The rise in the livestock population and the decrease in the grazing area has resulted in the overgrazing of these fragile ecosystems [32,33]. According to the reports of Malik [34], available grazing space in Kashmir's subalpine and alpine pastures dropped from 0.15 ha/animal in 1977 to 0.10 ha/animal in 1982 [35]. The area of these grasslands shows significant contraction and continued to decline thereafter [36]. Overgrazing compounded with trampling has resulted in the general degradation of community structure of these fragile ecosystems. Besides harboring the plant diversity with immense medicinal values, these meadows are major sources of forage to livestock and provide territory for a huge range of wild fauna [37]. These alterations have led to a change in the composition and overall growth patterns of the plant species inhabiting these ecosystems [38,39].

Understanding of the soil nutrient composition, biodiversity pattern, and phytosociological interactions becomes imperative in ecological studies and landscape conservation. From different plant community studies, it is evident that the floristic diversity of an area affects its ecosystem functions [40,41], particularly its stability and productivity [42]. In the north-western mountains of the Kashmir Himalaya, attempts to study the distribution pattern and community structure of alpine grasslands have been made by different researchers, however, they remain poorly understood due to their remoteness, inaccessibility, danger, and lack of local infrastructure [43,44]. In the present study, we made an integrated effort to (1) focus on the impact of altitude, temperature, and precipitation on the soil pH, nutrient composition, and bulk density of different alpine grasslands, (2) study the floristic composition and phenology of different alpine grasslands, (3) understand the variation in the phenology and growth characteristics of plant species at different study areas, and (4) to study the dominance pattern and impact of grazing on species composition at different

alpine grasslands. Statistical analysis was performed using different statistical analyses with R software.

2. Materials and Methods

2.1. Study Sites

The present study was carried out at the alpine regions of Yousmarg, Poshpathri and Kongwattan in Kashmir, India. Yousmarg lies in the Pir Panjal range of Kashmir Himalaya and is renowned for harboring a large number of alpine grasslands. The study was conducted in the upper reaches at 2831 m above mean sea level (m.a.s.l.). These areas remain free from snow from April to late October. In early growing months (April and May), the weather is cloudy and foggy, however, in June, July and August, it is clear with bright and longer durations of sunlight. The alpine grasslands of Poshpathri-Tral lie in the Zaskar mountainous range of Kashmir Himalaya. The grassland is located at 3103 m.a.s.l. The grassland of the Kongwattan lies in the Pir Panjal range of Kashmir Himalaya. It is located in the Kulgam district at an altitude of about 3347 m.a.s.l. The study was carried out on a gentle slope at all three sites. Poshpathri is situated on the southern slope of the mountain range, while Yousmarg and Kongwattan are on the northern slope. The meteorological information of all the sites during different months was provided by the Meteorological Department, Rambagh, Srinagar (J and K) and is given in Figure 1.

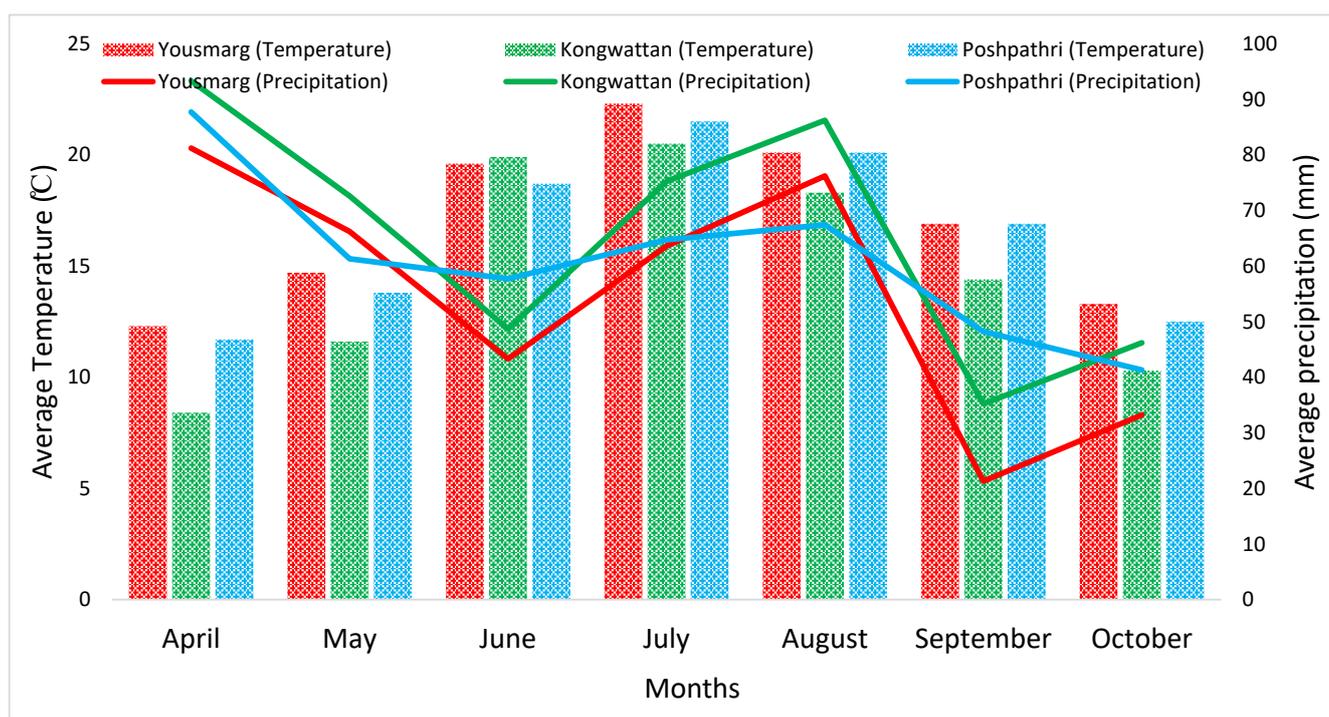


Figure 1. Average monthly temperature (°C) and precipitation (mm) at three grassland sites during the study period. Data is taken from the Meteorological Department at Rambagh Srinagar, J&K, India.

2.2. Soil, pH, Nutrient Analysis, and Bulk Density

Hydrogen ion concentration (pH) of the soil samples was determined in 1:2.5 soil:water ratio (w/v) with the help of a glass electrode pH meter [45]. Electrical conductivity was estimated in 1:2.5 soil:water suspension with EC meter [45]. The texture class of the soil was determined by the hydrometer method [46]. Organic carbon was estimated by the rapid titration method [47]. Available nitrogen was determined by using alkaline permanganate as per the modified Kjeldahl method [48]. Available phosphorus was extracted from the soil with 0.5 M NaHCO_3 (pH 8.5) and determined by the ammonium molybdate blue color method using a spectrophotometer [49]. A total of 1 N ammonium acetate was used as

an extractant and the available potassium content was determined by feeding the extract to a flame photometer [45]. Exchangeable Ca was analyzed using ammonium acetate extract through the EDTA method. Available micronutrients (Zn, Cu, Mn, and Fe) were analyzed through the DTPA extractable method [50]. The bulk density of the study areas was determined by the soil core sampling method [51].

2.3. Floristic Composition and Phenological Studies

Regular field visits were made from April to October at all three study sites to determine their floristic composition. Flora analysis was performed by placing random quadrats (1 m²), following Dad and Khan, [52]. Unknown plant species were identified with the help of regional floras of Dhar and Kachroo [53]. The phenology of the species was recorded throughout the study period from April to October. Data on each of the six phenophases viz. germination, vegetative growth, flowering, fruiting, seed maturation, and senescence were recorded [54,55]. The appearance of the first leaf in case of dicots was considered as initiation of germination [54,55], while in the case of graminoids seedlings upto 2 cm length were considered in the germination phase [55].

The impact of different environmental conditions (temperature and precipitation) and edaphic factors (altitude) was studied on the phenology (germination, vegetative phase, flowering, fruiting, seed maturation, and senescence) of the plant species.

2.4. Species Dominance Pattern

The quadrat method was used for the collection of such data following the method outlined by Curtis and Cottam [56]. Appropriate numbers of quadrats (1 m²) were laid randomly in the study area. Density and abundance were calculated on a per meter square basis. Frequency was determined by dividing the total number of quadrants in which species were present by the total number of quadrants laid, and multiplying the result by 100. The seasonal changes in the flora were studied through tiller analysis [57]. Other structural parameters of different grasslands were reported using the following formulae:

$$\text{Relative density} = \frac{\text{number of individuals of particular species}}{\text{total number of individuals in area}} \times 100$$

$$\text{Relative frequency} = \frac{\text{frequency value of particular species}}{\text{total of all frequency values for all species}} \times 100$$

$$\text{Area} = C^2/4\pi$$

$$\text{Relative area} = \text{Basal cover of particular species} / \text{Total basal area}$$

$$\text{IVI} = \text{Relative density} + \text{Relative frequency} + \text{Relative area}$$

2.5. Statistical Analysis

2.5.1. Ecological Indices

All analyses were conducted in R software v4.0.2 [58]. We used the Renyi diversity profile approach to calculate the plant species diversity of the three studied alpine sites using the vegan (v2.5-7) package [59]. Renyi diversity profiles are a function dependent parametric family of diversity indices that reflect sensitivity to rare and common species and display a graphical ordering of community diversity [60–62]. Renyi diversity profile values (H_α) were calculated from the frequencies of each contributing species and a scaling parameter (α) that ranges from zero to infinity [63] according to the following formula:

$$H_\alpha = \frac{1}{1 - \alpha} \ln \sum_i^S p_i^\alpha$$

where p_i is the proportion abundance of each species and α is a scaling parameter [47,49,51]. The values of the Renyi profile at the given scales of 0, 1, 2, and ∞ correspond to species richness (S), the Shannon diversity index (H'), the Simpson diversity index (D^{-1}) and the

Berger–Parker diversity index (d^{-1}), respectively [62,63]. According to the Renyi diversity profile, a community can be regarded as more diverse if all of its Renyi diversities are higher than that of the other community [60,62]. We calculated the diversity values in the current study at the α scale of 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, and infinity (∞) and plotted the diversity profiles for each site separately.

2.5.2. β -Diversity

To gain an in-depth understanding of composition change between the studied alpine sites, we calculated the turnover (i.e., species replacement between sites) and nestedness (i.e., species gain or loss between sites) components of beta diversity to study spatial patterns of turnover and nestedness-resultant dissimilarity among the three sites using a betapart package [64]. More specifically, it partitions the pairwise Sorenson dissimilarity between the two sites (β_{sor}) into two additive components which in turn account for species spatial turnover (β_{sim}) and nestedness-resultant dissimilarities (β_{sne}) [65].

2.5.3. Predictors of Phenological Stages

We evaluated the relative effects of altitude, temperature, and precipitation on different phenological events with the generalized linear mixed models (GLMMs) and a Poisson error distribution using the lme4package [66]. Model performance was evaluated with the help of the Akaike information criterion corrected for small sample sizes (AICc) and the marginal (mR^2) and conditional (cR^2) R^2 values, using the ‘dredge’ function in the Mu Min package [67] to select the simplest model with $\Delta AICc < 2$.

2.5.4. Pearson’s Correlation Coefficient

We also performed Pearson’s multiple correlation between community indices (i.e., plant species richness, diversity, and evenness measures) and environmental variables (temperature, precipitation, and altitude). The statistical significance associated with the resulting correlation was detected at the 5% level.

3. Results

3.1. Nutrient Analysis

The soil pH showed a considerable decrease with an increase in altitude and precipitation. pH at Yousmarg ranged between 7.11 and 8.3, while at Kongwattan the pH of the soil ranged between 6.3 and 6.5. Electroconductivity of the soil ranged from 0.18 ± 0.03 dSm⁻¹ (at Yousmarg) to 0.19 ± 0.04 (at Kongwattan). Soil organic carbon and macro soil nutrients (nitrogen, phosphorus, and potassium) showed a significant decrease with an increase in altitude and precipitation, and a decrease in the temperature. The highest organic carbon was reported at Yousmarg (2.33 ± 0.31) followed by Poshpathri (1.84 ± 0.29) and Kongwattan (1.45 ± 0.36). The concentration of the micronutrients (zinc, iron, calcium, and copper) did not show any significant change with respect to change in altitude, temperature, and precipitation. There were no significant statistical differences in the bulk density and electroconductivity of soils at different study areas. Bulk density ranged between 1.23 ± 0.9 (at Kongwattan) and 1.34 ± 1 Mgm³ (at Yousmarg). Detailed results are provided in Table 1.

3.2. Floristic Composition

During the entire study period, 23 plant species from 17 genera and 17 families were documented at Yousmarg Budgam, 32 plant species from 25 genera and 18 families were reported at Poshpathri Tral, and 36 plant species from 32 genera and 21 families were reported at Kongwattan Kulgam. Only 7 plant species were common at all the three study sites, while Yousmarg had 9 exclusive plant species, Tral had 15, and Kongwattan had 22. A total of 9 plant species were common between the Yousmarg and Poshpathri grasslands, 10 between Poshpathri and Kongwattan, and 5 common species were reported from the grasslands of Kongwattan and Yousmarg (Table 2).

Table 1. Soil pH, electroconductivity, nutrient analysis, and bulk density of soil at different alpine grasslands.

Study Area	pH	EC (dSm ⁻¹)	OC (%)	N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Ca (cmol _c kg ⁻¹)	BD (Mgm ³)
K	6.59 ± 0.39	0.19 ± 0.04	1.45 ± 0.36	192.51 ± 4.2	16.33 ± 0.9	129.18 ± 6.2	0.80 ± 0.18	19.9 ± 2	2.08 ± 0.16	7.49 ± 0.53	1.23 ± 0.9
P	7.01 ± 0.43	0.21 ± 0.03	1.84 ± 0.29	199.42 ± 6.1	19.75 ± 1.4	139.62 ± 4.8	0.83 ± 0.21	19.2 ± 1.9	1.98 ± 0.09	7.65 ± 0.44	1.29 ± 1.1
Y	7.6 ± 0.66	0.18 ± 0.03	2.33 ± 0.31	204.41 ± 6.6	22.31 ± 1.65	142.67 ± 5.70	0.82 ± 0.19	20.22 ± 2.3	2 ± 0.11	7.48 ± 0.49	1.34 ± 1

K: Kongwattan, P: Poshpathri, Y: Yousmarg, pH: Hydrogen ion concentration, EC: Electroconductivity, OC: Organic Carbon, N: Nitrogen, P: Phosphorus, K: Potassium, Zn: Zinc, Fe: Iron, Cu: Copper, Ca: Calcium, BD: Bulk Density.

Table 2. Floristic composition at different alpine grasslands of Kashmir Himalaya.

Plant Species	Family	Yousmarg	Poshpathri	Kongwattan
<i>Achillea millefolium</i> L. 1753	Asteraceae	A	A	P
<i>Ajuga parviflora</i> Benth 1830	Lamiaceae	A	A	P
<i>Alchemilla trollii</i> Rothm 1938	Rosaceae	P	A	A
<i>Arabis alpina</i> Krock. exSteud. 1840	Brassicaceae	A	P	A
<i>Arenaria stricta</i> Michx. 1803	Caryophyllaceae	A	P	A
<i>Astragalus propinquus</i> Schischk. 1933	Fabaceae	A	P	A
<i>Callianthemum pimpinelloides</i> (D. Don) Hook f. Thomson 1855	Ranunculaceae	A	A	P
<i>Capsella bursa-pastoris</i> (L.) Medik. 1792	Brassicaceae	A	P	A
<i>Cardamine impatiens</i> L. 1753	Brassicaceae	P	A	A
<i>Cerastium brachypetalum</i> Pers 1805	Caryophyllaceae	P	A	A
<i>Cerastium cerastoides</i> (L.) Britton 1894	Caryophyllaceae	P	A	A
<i>Cirsium arvense</i> (L.) Scop 1975	Asteraceae	A	A	P
<i>Cirsium falconeri</i> (Hook.f.) Petr	Asteraceae	A	P	A
<i>Cirsium wallichii</i> DC. 1838	Asteraceae	A	P	A
<i>Conyza bonariensis</i> (L.) Cronquist 1943	Asteraceae	A	A	P
<i>Corydalis diphylla</i> Wall. 1824	Papaveraceae	A	P	A
<i>Corydalis scouleri</i> Hook. 1829	Papaveraceae	A	P	A
<i>Crepis sancta</i> (L.) Bab. 1941	Asteraceae	A	A	P
<i>Cynoglossum wallichii</i> G. Don 1837	Boraginaceae	A	A	P
<i>Duchesnea indica</i> (Andrews) Focke 1888	Rosaceae	A	P	A
<i>Epilobium hirsutum</i> L. 1753	Onagraceae	A	A	P
<i>Euphorbia wallichii</i> Hook. F. 1887	Euphorbiaceae	A	A	P
<i>Fimbristylis dichotoma</i> (L.) Vahl 1805	Cyperaceae	A	A	P
<i>Fragaria nubicola</i> (Hook F.) Lindl. ex Lacaite 1916	Rosaceae	P	A	P
<i>Fragaria virginiana</i> Duchesne, Hist. Nat. Frais. 204. 1766.	Rosaceae	A	P	A
<i>Gallium aparine</i> L. 1753	Rubiaceae	P	A	P
<i>Gentiana carinata</i> Griseb. 1839	Gentianaceae	P	A	P
<i>Geranium himalayense</i> Klotzsch 1862	Geraniaceae	A	P	A
<i>Geum vernum</i> (Raf.) Torr. and A. Gray 1840	Rosaceae	P	P	A
<i>Hypericum perforatum</i> L. 1753	Hypericaceae	A	P	P
<i>Impatiens brachycentra</i> Kar and Kir. 1842	Balsaminaceae	P	A	A
<i>Iris hookeriana</i> R.C. Foster 1887	Iridaceae	P	P	P
<i>Ligularia amplexicaulis</i> DC. 1838	Asteraceae	A	A	P
<i>Mazus reptans</i> N.E.Br. 1914	Mazaceae	P	P	A
<i>Myosotis stricta</i> Link ex Roem and Schult 1819	Boraginaceae	A	A	P
<i>Nepeta cataria</i> L. 1753	Lamiaceae	A	A	P
<i>Oxalis acetosella</i> L. 1753	Oxalidaceae	A	P	A
<i>Oxalis corniculata</i> L. 1753	Oxalidaceae	A	P	A
<i>Plantago lanceolata</i> L. 1753	Plantaginaceae	P	P	A
<i>Plantago himalaica</i> Pilg C	Plantaginaceae	A	A	P
<i>Plantago major</i> L. 1753	Plantaginaceae	P	P	P
<i>Plantago ovata</i> Phil. 1895	Plantaginaceae	A	A	P
<i>Poa angustifolia</i> L. 1753	Poaceae	P	A	A
<i>Poa annua</i> L. 1753	Poaceae	A	A	P
<i>Poa compressa</i> L. 1753	Poaceae	P	P	P
<i>Podophyllum hexandrum</i> Royle 1834	Berberidaceae	A	A	P
<i>Polygonum alpinum</i> All. 1173	Polygonaceae	P	A	P
<i>Polygonum heterophyllum</i> Lindm 1912	Polygonaceae	A	A	P
<i>Potentilla atrosanguinea</i> Lodd., G. Lodd. and W. Lodd 1823	Rosaceae	A	P	A
<i>Primula denticulata</i> Wight 1853	Primulaceae	A	P	P
<i>Ranunculus hirtellus</i> Royle 1834	Ranunculaceae	P	P	P
<i>Rheum webbianum</i> Royle 1839	Polygonaceae	A	P	A
<i>Rumex acetosa</i> L. 1753	Polygonaceae	P	A	A
<i>Rumex nepalensis</i> Spreng. 1825	Polygonaceae	A	A	P
<i>Sambucus wightiana</i> Wall. ex Wight and Arn. 1834	Adoxaceae	A	A	P
<i>Sibbaldia cuneata</i> Hornem. ex Kuntze 1847	Rosaceae	P	P	P
<i>Taraxicum officinale</i> F.H. Wigg. 1780	Asteraceae	P	P	P
<i>Thlaspi cochleariforme</i> DC. Syst. Nat. 1821	Brassicaceae	A	P	A
<i>Thymus linearis</i> Benth. 1830	Lamiaceae	A	A	P
<i>Thymus serpyllum</i> L. 1753	Lamiaceae	A	P	A
<i>Trifolium pratense</i> L. 1753	Fabaceae	A	A	P
<i>Trifolium repens</i> L. 1753	Fabaceae	P	P	P
<i>Urtica dioica</i> L. 1753	Utricaceae	P	A	A
<i>Valeriana jatamansi</i> L. 1753	Valerianaceae	A	P	A
<i>Veronica serpyllifolia</i> L. 1753	Plantaginaceae	A	P	P
<i>Viola biflora</i> L. 1753	Violaceae	P	A	A

P: Present, A: Absent.

Environmental (temperature, precipitation) and edaphic (altitude) factors at different grasslands show a profound effect on the dominance pattern of the plant families. Asteraceae followed by Plantaginaceae and Polygonaceae were the dominant families with the highest genera and species at Kongwattan. Rosaceae followed by Asteraceae, Brassicaceae, and Fabaceae dominated the flora at Poshpathri, while Rosaceae followed by Polygonaceae, Plantaginaceae, Caryophylliaceae and Poaceae with a maximum number of genera and species dominated the flora of the alpine grassland at Yousmarg (Figure 2).

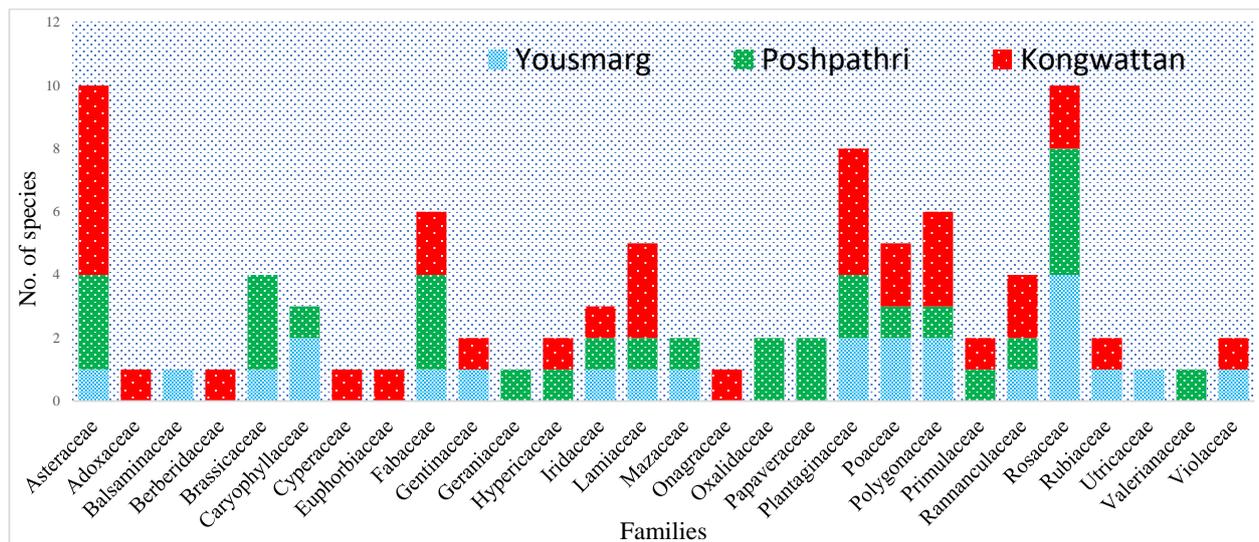


Figure 2. Contribution of various plant families at the different study sites.

3.3. Phenology

The phenological spectrum as analyzed for the various plant species documented during the survey in the alpine grasslands of Kashmir Himalaya revealed that all the plants exhibited a distinct phenological pattern corresponding to their response to the harsh climatic conditions of the alpine grasslands. In alpine conditions the climate is not favorable for plant growth throughout the year, thus the plants have got a fixed period of 7–8 months to complete their life cycle. Germination of most plant species started in April following the melting of the snow. The melting of the snow makes moisture available which is necessary for germination. This period also witnessed a temperature increase favoring plant germination. The highest percentage of germination was recorded in April, followed by May and June. The highest number of species in the vegetative phase was found in May followed by June and July. The flowering of the plant species was observed from May to August. The highest number of plants in the flowering phase was seen in July followed by June, May, and August. Fruiting started in June and ended in September. The highest percentage of plants in the fruiting phase was seen in August followed by July and September. The signs of seed set were seen from the month of July. The highest number of species in the seed maturation phase was observed in September followed by August. By the end of September almost all of the species entered their senescence phase. Though the majority of the plant species completed their life cycle by the end of September, some plant species were found to be in the final stage of their life cycle (senescence) in October (Supplementary Tables S1–S3).

3.4. Impact of Environmental and Edaphic Factors on the Phenology of Plant Species

The climate and edaphic factors provide a significant impact on the germination and senescence of the plant species, whereas flowering, fruiting, and seed maturation were least affected. Lower altitude and precipitation and higher temperature at Yousmarg resulted in the early germination of *Taraxicum officinale*, *Ranunculus hirtellus*, *Iris hookeriana*,

Sibbaldia cuneata, *Poa compressa*, *Plantago major*, and *Trifolium repens* as compared to the grassland sites at Poshpathri and Kongwattan where germination started in May. Similar phenological shifts were seen for plant species that were common between different study areas (Kongwattan and Poshpathri, Kongwattan and Yousmarg, Poshpathri and Yousmarg). Senescence of the plant species was initiated earlier at Kongwattan where the majority of the plant species entered their senescence phase in the month of September. Detailed results are provided in Figure 3.

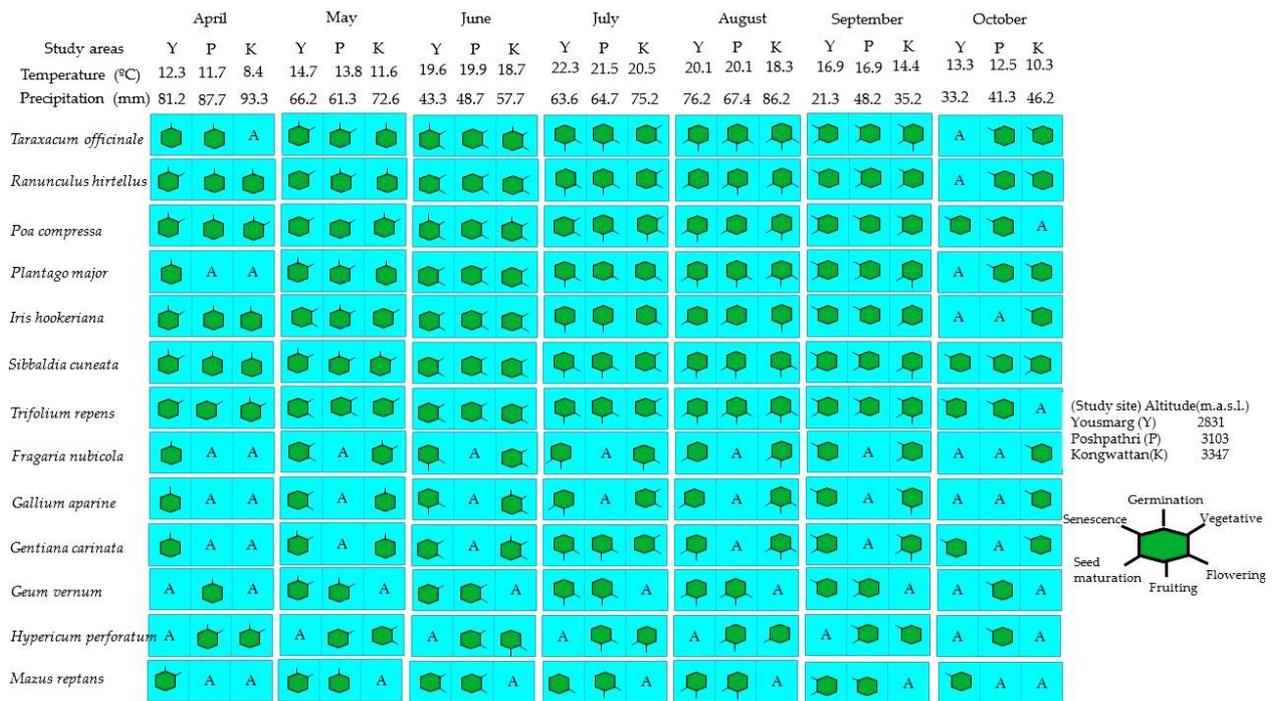


Figure 3. Impact of altitude, temperature, and precipitation on life history traits (phenophases) of different plant species. (A: Absent).

3.5. Environmental Correlates of Phenological Stages

The results of the generalized linear mixed models (GLMMs) are shown in Figure 4 and Table 3. The results indicate that temperature and elevation were the most influential environmental variables which significantly affected all of the studied phenological stages except vegetative growth and reaching mR^2 value from 0.34 to 0.94 (Figure 4; Table 3). In contrast, precipitation had a significant effect on germination, vegetative growth, seed maturation, and senescence only (Figure 4; Table 3).

3.6. Species Dominance Pattern

Density, frequency, and abundance were reported on a monthly basis from April to October to determine the effect of grazing, temperature, and precipitation on plant species. Relative density, relative frequency, cover, and relative cover was analyzed to determine the importance value index (IVI). A definite trend in the increase of temperature, favorable precipitation, least pressure of grazing, and a considerable increase in the density of plant species was reported from April to July at all three grasslands. However, the decrease in temperature and movement of shepherds to these areas to feed their livestock resulted in a drastic decrease in the density of plant species from July onwards. While analyzing the density of the plant species, it was calculated that *Trifolium repens*, *Poa angustifolia*, *Poa compressa*, *Sibbaldia cunenata*, *Mazus reptans*, *Trifolium pratense*, *Primula denticulata*, and *Myosotis stricta* showed the highest density, while *Impatiens brachycentra*, *Geum vernum*, *Viola biflora*, *Arabis alpina*, *Arenaria stricta*, *Crepis sancta*, and *Fimbristylis dichotoma* were the least dense plant species at their respective sites. The highest total

tiller density was reported at Kongwattan followed by Poshpathri and Yousmarg. Grazing intensity was highest at Yousmarg, while Kongwattan showed the lowest pressure of grazers and trampers. The grassland of Yousmarg is located at an altitude of below 3000 m.a.s.l. and receives a sedentary system of livestock rearing. To feed their livestock, shepherds and the Bakharwal tribe show early and prolonged movement to these regions. This results in greater anthropogenic stress causing the decreased diversity of palatable species and the increased density of non-palatable species, such as *C. falconeri*, *C. wallichii*, etc. Stoloniferous forbs and tussock forming graminoids were the dominant groups at these alpine grasslands. Based on importance value index (IVI), *Trifolium repens*, *Sibbaldia cuneata*, *Poa compressa*, *Plantago major*, *Plantago lanceolata*, *Trifolium pratense*, *Poa angustifolia*, *Primula denticulata*, and *Sambucus wightiana* dominated the flora composition on these alpine grasslands (Table 4). Plant species such as *Trifolium repens*, *Trifolium pratense*, *Sibbaldia cuneata*, *Plantago major*, *Poa compressa*, *Poa annua*, and *Plantago lanceolata* were reported to be the most frequent plant species with a frequency percentage greater than 50%. Plant species such as *Viola biflora*, *Thymus serpyllum*, *Rheum webbianum*, *Podophyllum hexandrum*, *Fimbristylis dichotoma*, *Epilobium hirsutum*, *Cardamine impatiens*, and *Capsella bursa-pastoris* were the least frequent plant species with a frequency percentage less than 5%.

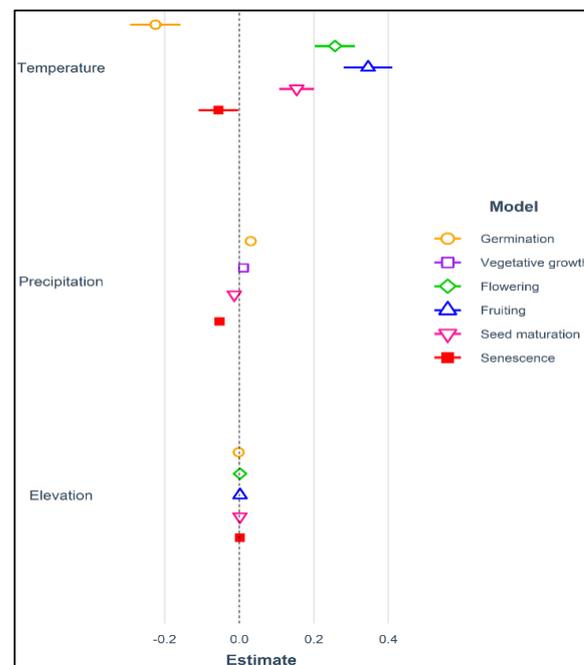


Figure 4. Coefficient estimates (slopes) derived from generalized linear mixed models (GLMMs) to evaluate the best environmental determinants of different phenological stages.

Table 3. Results of generalized linear mixed models (GLMMs) testing the effect of best performing environmental variables on different phenological stages. Numbers for variables show the z statistic of model significance. mR^2 and cR^2 indicate the fit of the models without (marginal) and with(conditional) random effect, respectively. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$.

	Temperature	Precipitation	Elevation	AIC	mR^2	cR^2
Germination	−0.93 ***	0.60 ***	−0.37 ***	147.56	0.88	0.88
Vegetative growth	-	0.22 **	-	357.20	0.34	0.34
Flowering	1.06 ***	-	0.47 *	161.08	0.83	0.90
Fruiting	1.42 ***	-	0.47 ***	126.09	0.94	0.94
Seed maturation	0.63 ***	−0.27 **	0.37 ***	304.39	0.80	0.80
Senescence	−0.23 *	−1.03 ***	0.33 *	211.75	0.85	0.90

Table 4. Relative density, relative frequency, relative area, and IVI of the plant species.

Plant Species	Yousmarg				Poshpathri				Kongwattan			
	RD	RF	RA	IVI	RD	RF	RA	IVI	RD	RF	RA	IVI
<i>A. millefolium</i>									1.40	3.50	0.38	5.29
<i>A. parviflora</i>									1.09	1.41	0.43	2.93
<i>A. trollii</i>	1.81	3.18	0.96	5.95								
<i>A. alpina</i>					0.50	2.26	0.09	2.86				
<i>A. stricta</i>					0.71	1.91	0.07	2.69				
<i>A. propinquus</i>					1.72	1.58	0.30	3.61				
<i>C. pimpinelloides</i>									0.31	0.77	0.07	1.15
<i>C. bursa-pastoris</i>					1.19	1.09	0.04	2.33				
<i>C. impatiens</i>	1.13	1.25	0.17	2.55								
<i>C. brachypetalum</i>	4.08	2.66	0.38	7.12								
<i>C. cerastoides</i>	2.47	3.02	0.35	5.84								
<i>C. arvense</i>									1.37	1.59	0.77	3.74
<i>C. falconeri</i>					0.97	0.89	1.07	2.94				
<i>C. wallichii</i>					0.86	0.79	0.82	2.47				
<i>C. bonariensis</i>									1.66	1.16	3.31	6.13
<i>C. diphylla</i>					2.60	2.39	0.19	5.20				
<i>C. scouleri</i>					2.26	2.08	0.09	4.43				
<i>Crepis sancta</i>									1.12	1.47	0.36	2.95
<i>C. wallichii</i>									2.42	2.10	4.52	9.05
<i>D. indica</i>					1.32	1.21	0.07	2.60				
<i>E. hirsutum</i>									1.59	1.59	0.31	3.49
<i>E. wallichi</i>									1.40	1.21	0.52	3.14
<i>F. dichotoma</i>									0.62	0.89	0.04	1.56
<i>F. nubicola</i>	6.30	7.48	0.55	14.33					2.51	3.28	0.21	6.00
<i>F. virginiana</i>					4.24	3.91	0.34	8.50				
<i>G. aparine</i>	1.13	2.06	0.43	3.62					2.99	0.60	0.21	3.80
<i>G. carinata</i>	2.37	2.70	0.33	5.4					3.93	1.55	0.22	5.71
<i>G. himalayense</i>					1.69	1.56	0.34	3.60				
<i>G. vernum</i>	0.99	2.42	0.09	3.5	2.30	2.12	0	4.43				
<i>H. perforatum</i>					3.70	3.41	0.72	7.84	2.51	0.81	0.25	3.58
<i>I. brachycentra</i>	0.23	0.85	0.01	1.09								
<i>I. hookeriana</i>	0.76	1.90	2.74	5.4	0.84	1.77	2.80	5.42	0.78	1.29	2.10	4.17

Table 4. Cont.

Plant Species	Yousmarg				Poshpathri				Kongwattan			
	RD	RF	RA	IVI	RD	RF	RA	IVI	RD	RF	RA	IVI
<i>L. amplexicaulis</i>									0.61	1.14	0.06	1.81
<i>M. reptans</i>	6.75	5.49	1.28	13.52	1.63	0.97	0.21	2.81	0	0	0	0
<i>M. stricta</i>									3.46	6.13	1.28	10.88
<i>N. cataria</i>									1.13	1.05	0.05	2.23
<i>Oxalis acetosella</i>					5.85	5.38	0.60	11.85				
<i>O. corniculata</i>					0.89	0.82	0.04	1.76				
<i>P. lanceolata</i>	1.42	6.66	33.79	41.87	5.30	4.88	28.9	39.08				
<i>P. himalaica</i>									3.93	4.35	11.01	19.29
<i>P. major</i>	7.31	6.48	20.68	34.47	2.18	6.46	29.90	38.55	3.63	6.18	25.77	35.59
<i>P. ovata</i>									0.69	0.99	9.39	11.07
<i>P. angustifolia</i>	16.73	9.08	2.12	27.93					0	0	0	0
<i>P. annua</i>	0	0	0	0					11.04	5.98	0.21	17.23
<i>P. compressa</i>	15.09	9.65	1.88	26.62	8.40	7.73	1.27	17.42	7.56	6.87	0.91	15.35
<i>P. hexandrum</i>	0	0	0	0					0.49	0.98	0.75	2.22
<i>P. alpinum</i>	1.62	2.85	0.20	4.67					1.57	1.13	0.09	2.80
<i>P. heterophyllum</i>									0.46	3.54	0.11	4.12
<i>P. atrosanguinea</i>					0.84	0.78	0.03	1.66				
<i>P. denticulata</i>					5.87	5.41	1.25	12.54	1.19	2.47	0.77	4.43
<i>R. hirtellus</i>	2.28	3.71	0.08	6.07	4.32	3.98	0.07	8.39	1.73	3.65	0.82	6.20
<i>R. webbianum</i>					1.61	1.49	24.86	27.96				
<i>R. acetosa</i>	0.67	1.74	21.44	23.85								
<i>R. nepalensis</i>									1.02	1.18	18.82	21.03
<i>S. wightiana</i>									0.15	1.84	10.54	12.54
<i>S. cuneata</i>	7.76	8.73	0.59	17.08	8.21	7.56	0.19	15.97	9.61	6.41	0.21	16.23
<i>T. officinale</i>	3.64	4.76	3.83	12.23	2.77	2.55	2.90	8.23	2.82	4.60	4.33	11.75
<i>T. cochleariforme</i>					3.34	3.07	0.30	6.71				
<i>T. linearis</i>									0.12	0.47	0.16	0.76
<i>T. serpyllum</i>					0.47	0.43	0.22	1.12				
<i>T. pratense</i>					9.13	8.40	0.80	18.33	11.64	6.72	1.08	19.44
<i>T. repens</i>	13.94	9.70	2.32	25.96	8.50	7.83	1.22	17.56	11.03	7.38	0.58	18.99
<i>U. dioica</i>	0.63	1.69	5.61	7.93								
<i>V. jatamansi</i>					3.98	3.66	0.41	8.06				
<i>V. serpyllifolia</i>					1.63	1.50	0.20	3.33	0.15	3.11	0.27	3.53
<i>V. biflora</i>	0.84	1.83	0.05	2.58					0.09	0.46	0.03	0.59

RD = Relative density, RF = Relative frequency, RA = Relative area, IVI = Importance value index.

3.7. Species Diversity

The Renyi diversity profiles of the three studied alpine sites are presented in Figure 5. The results showed that among all three sites Kongwattan is the most diverse followed by Poshpathri, and Yousmarg. In addition, this pattern of Kongwattan being the most diverse is consistent with all the α values used (Figure 5).

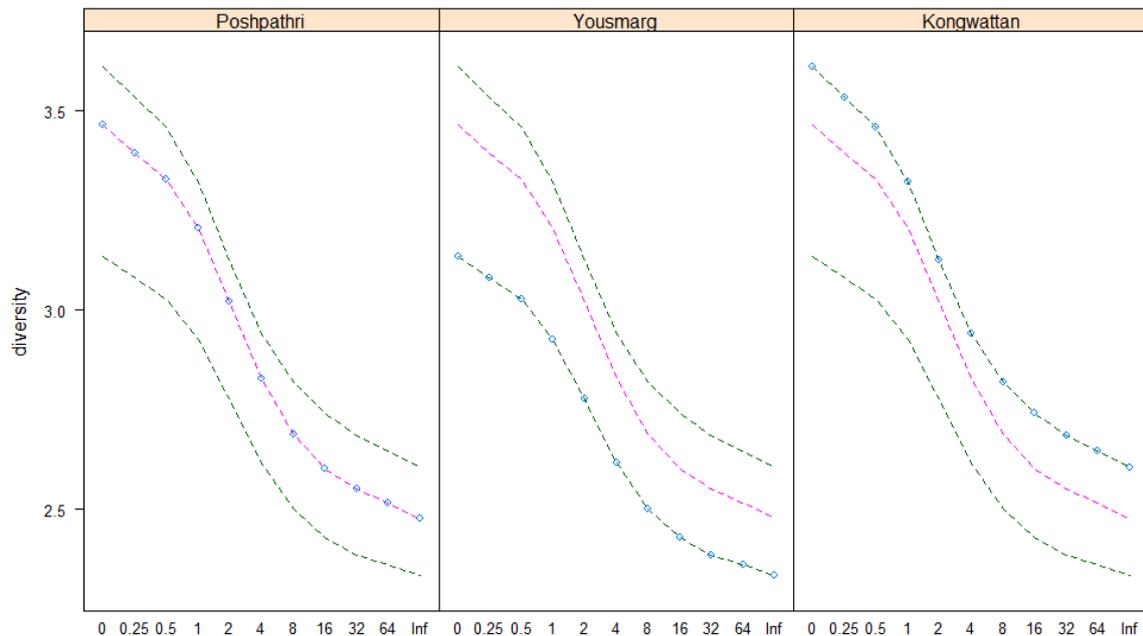


Figure 5. Renyi diversity profiles for the three studied alpine sites. The dots show the values for plots corresponding to given values of scaling parameter (α) and the lines represent the median and extremes in the data set.

3.8. β -Diversity

The results of the multiple-site dissimilarities are shown in Figure 6. The results indicate that the Sorensen dissimilarity among the three studied alpine sites was relatively low. Furthermore, the nestedness component (β_{sne}) was found to contribute largest to the overall dissimilarity among the studied sites, as shown by the higher peak of β_{sne} (nestedness-resultant dissimilarity) (Figure 6A). This in turn reflects that the observed dissimilarity among the sites is most likely a consequence of richness difference (i.e., nestedness component) and to a lesser extent because of species replacement (i.e., turnover component). Moreover, the clustering obtained from the dissimilarity matrices of the turnover component showed that Poshpathri was highly dissimilar to the other two sites (Yousmarg and Kongwattan), which were similar to a greater extent as they formed the same sub-cluster (Figure 6B). Contrary to this, the clustering obtained from the dissimilarity matrices of nestedness showed a quite different pattern in which the Yousmarg site was highly dissimilar to the other two sites (PP and KW), which were similar to a greater extent as they formed the same sub-cluster (Figure 6C).

3.9. Correlation

The results of the multiple correlation are presented in Figure 7. The results indicate that species richness is positively correlated with altitude, precipitation, Shannon diversity, and Simpson diversity ($r = 0.96, 0.72, 1, \text{ and } 1$, respectively), but the resultant correlation is significant only between species richness and Shannon diversity index ($p < 0.05$). Similarly, species richness is negatively correlated with temperature and Pielou's evenness ($r = -0.9$ and -1 , respectively), but the resultant correlation is significant between species richness and Pielou's evenness only ($p < 0.05$). In addition, there was a significant positive correlation

between Shannon diversity index and Simpson diversity index ($r = 1; p < 0.05$) and a significant negative correlation between Shannon diversity index and Pielou’s evenness ($r = -1; p < 0.05$) and between Simpson diversity index and Pielou’s evenness ($r = -1; p < 0.05$) (Figure 7).

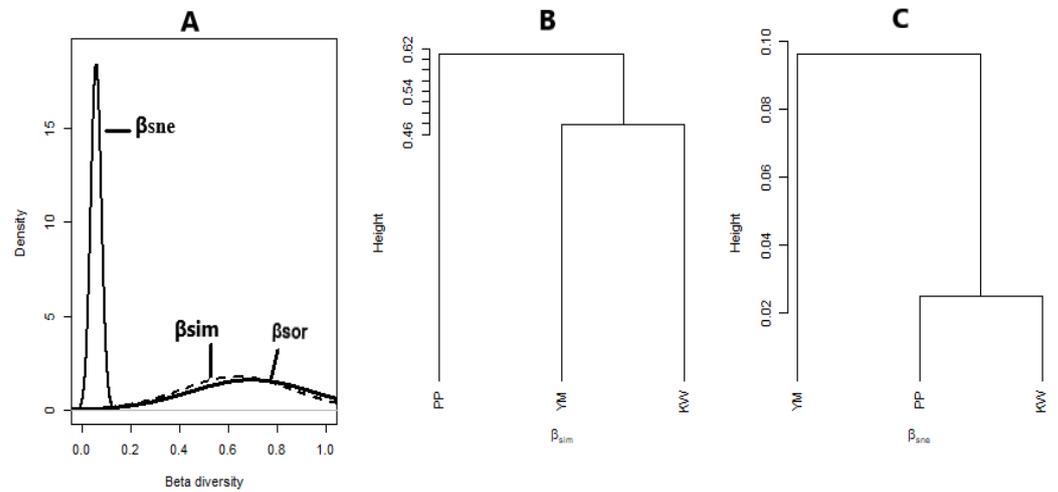


Figure 6. Dissimilarities across the three studied alpine sites. (A) Partitioning of β_{sor} (total dissimilarity—gray line) into β_{sim} (turnover or species replacement component of beta diversity—dashed line) and β_{sne} (nestedness or richness difference component of beta diversity—solid line) for the study sites and average clustering of (B) β_{sim} and (C) β_{sne} components of species dissimilarity among the studied sites.

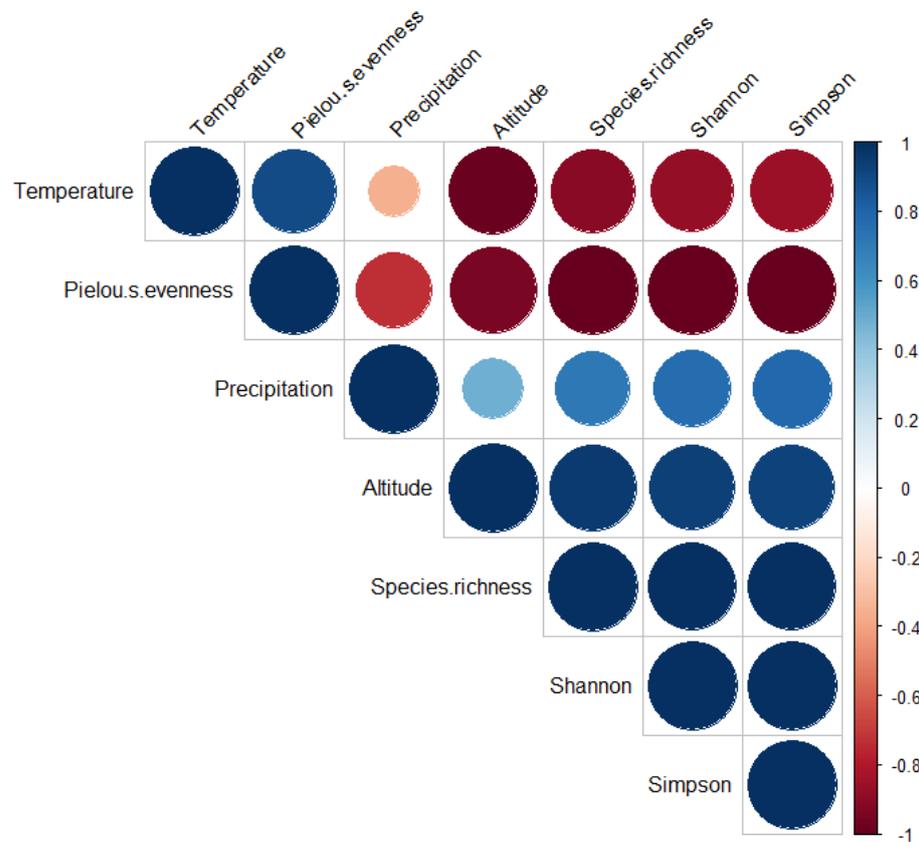


Figure 7. Correlation chart between community indices (i.e., plant species richness, diversity, and evenness measures) and environmental variables (temperature, precipitation, and altitude). Statistical significance $p < 0.05$.

4. Discussion

Grasslands account for about 40% of total land area and thus act as a significant ecosystem on a global scale [68]. Grasslands are a key component of Alpine and pre-Alpine landscapes, with a wide range of appearances ranging from heavily exploited grasslands in the lower areas to very diverse seasonal alpine pastures and specialized natural ecosystems at the higher elevations [69,70]. Besides providing significant economic value, alpine pastures deliver a wide range of ecosystem services, such as storage and cycling of different nutrients (prominently nitrogen and carbon) and water retention [71–73]. Previous studies have shown that species diversity decreases with increasing altitude [74]. In our study, species diversity increased with an increase in altitude. This is possibly due to the sedentary livestock grazing at the lower alpine sites, resulting in decreased species diversity as compared to higher diversity at upper alpine reaches where the pressure of grazing and trampling is less [75,76].

Soil pH values increased with the increase in altitude and the decrease in temperature at different grassland sites. These results are consistent with the findings of Yimer et al. [77], Oyonarte et al. [78], and Roukos et al. [79]. At warmer temperatures, decomposition results in greater soil organic carbon density [80] which tends to increase the concentration of H^+ ions [81], resulting in lower pH at lower altitudes with higher temperatures. Nitrogen, phosphorus, and potassium get significantly reduced with increased altitude. These findings are supported by the results of Bhandari and Zhang [82] who also reported similar findings on the grasslands of Tibet, China, and Manang District, Nepal.

The flora of alpine regions face demanding environmental conditions with cold soils having low moisture and low winter temperatures with extended permafrost and a brief growing season [83,84]. To cope with such adverse conditions, plants of alpine regions show various morphological and physiological adaptations and tend to complete their life cycle within a short duration during favorable climatic conditions [85–87]. The survival of most species in the grasslands can also be attributed to a modest degree of species competition during early regeneration, which has resulted in the dominance of only a few species [88]. In the present study, plant species from the Asteraceae, Fabaceae, Plantaginaceae, and Rosaceae families were dominant and this can be attributed to their adaptation to the alpine environment by producing large amount of seeds and showing diverse reproductive strategies.

Prolonged snow cover, reduced photoperiod, low temperature, and frost (hence, the lack of moisture) during the early months of the spring prevent the plant species from overcoming their dormancy [89–91]. The weather shift from late March results in a change in environmental conditions on the alpine grasslands. The increase in temperature not only results in the melting of snow, which makes optimum soil moisture available for the floral evocation of plant species, but also helps in breaking the dormancy of seeds and buds under the alpine conditions. During this study, species specific responses to environmental conditions were reported while analyzing the germination at these grasslands. About 76.26% of the plant species started germination in the month of April, while the remaining 23.74% of the plant species germinated in the month of May. While analyzing the effect of climate change on plant species at different alpine grasslands, various investigators reported that the change in the weather conditions (increase in temperature, melting of snow, change in photoperiod, melting of permafrost) during the early days of spring played a vital role in the germination of the plant species [92–94].

Observations made to assess the floral evocation of plants revealed that flowering attains its peak in July, exploiting the period of most favorable climatic conditions. Such observations are supported by the findings of different researchers who also reported direct proportionality of peak flowering with peak temperatures [89,95–98]. Plant species tend to complete their life cycle before they face a drastic decline in temperature and precipitation in the alpine regions. Completing their life cycle within the stipulated time of 7–8 months was observed to be an important adaptation by these alpine plants to survive the severe conditions of the alpine regions. Vashistha [98], while studying the phenology of plant

species at an alpine region of north-west Himalaya (India), also reported initiation of senescence from September onwards and reported that the plants of alpine regions exploit the favorable weather conditions in such a way that they complete their whole life cycle within this period (April–October).

IVI analysis gives information about a species' social interactions and may be identified as a pattern of dominating species in a population [99]. IVI analysis revealed distinct combinations of species with different dominants and co-dominants at different study sites. Based on the importance value index (IVI), dominance of the plant species reflects a considerable degree of variation. *Sibbaldia cuneata*, *Trifolium repens*, *Plantago major*, *Trifolium pratense*, *Poa compressa*, *Poa angustifolia*, and *Plantago lanceolata* depicted wide amplitude and showed greater IVI (more dominant) as compared to other plant species, reflecting their adaptation to the conditions of alpine regions. The species found to be dominant were rhizomatous and grow as tussocks. Such observations are supported by the results of different workers [100,101] who also reported these plant species to be dominant on other alpine grasslands of the western Himalayas. Changing environmental conditions and pressure caused by grazers and trampers exert an immense impact on the community characteristics of plants in alpine regions. A considerable increase in the values of density and frequency of plant species upto July was observed to be in accordance with the increase of favorable climatic conditions and minimal grazing pressure. A decline in such values from July onwards is attributed to the change in weather conditions (temperature, precipitation, and photoperiod) and the increase of anthropogenic pressure caused by the grazers and trampers. Such results are in accordance with the findings of other researchers [102,103] who also reported a decline in the density and frequency values due to climate change and an increase in grazing.

5. Conclusions

The present work on the nutrient analysis, species diversity, and ecological analysis allowed us to conclude that:

- Nutrient composition (N, P, K) of the alpine soil show negative correlation with altitude and precipitation and positive correlation with temperature.
- The altitudinal gradients and grazing intensity had a significant impact on the floristic diversity of alpine grasslands in Kashmir Himalaya.
- The phenology of the plant species is significantly affected by the altitude, temperature, and precipitation at different study areas.
- Observations related to the phenology have radically proved that certain factors such as low temperature, heavy snowfall, frost, and high altitude interfere with the vegetative growth of plant species, and are essential for the morphogenesis of flowering.
- Renyi diversity profiles show that the alpine grassland of Yousmarg has the least floristic diversity due to a sedentary system of livestock rearing.
- The Sorensen β diversity index reveals a relatively great similarity index among the studied sites which reflects the adaptability of the plant species in the higher altitudes of alpine regions.
- Stonliferous forbs and tussock forming graminoids such as *Sibbaldia cuneata*, *Trifolium repens*, *Plantago major*, *Trifolium pratense*, *Poa compressa*, *Poa angustifolia* and *Plantago lanceolata* showed greater importance value index (IVI) and dominated the floristic composition of these regions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su14020887/s1>, Table S1: Phenology of plant species at Yousmarg. Table S2: Phenology of plant species at Poshpathri. Table S3: Phenology of plant species at Kongwattan.

Author Contributions: Conceptualization, S.V., M.M.G. and G.N. methodology, I.A.W. and R.G.; software, I.A.W.; validation, S.V., I.A.W., M.M.G. and G.N.; formal analysis, M.M.G. and S.V.; investigation, I.A.W.; resources, M.M.G. and R.G.; data curation, S.V.; writing—original draft preparation, I.A.W. and S.V.; writing—review and editing, I.A.W. and R.G.; visualization, G.N. and M.M.G.; supervision, S.V. and G.N.; project administration, H.M.S. and F.A.A.-M.; funding acquisition, F.A.A.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP-2021/24), King Saud University, Riyadh, Saudi Arabia.

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

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