Impacts of slope aspects on altitudinal species richness and species composition of Narapani-Masina landscape, Arghakhanchi, West Nepal

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Abstract

This study aimed to find out the roles of altitude, slope aspect, and soil factors in species richness in the Narapani-Masina landscape, Arghakhanchi, Nepal. We surveyed forest plant communities by sampling 192 statistically representative 10 x 10 m sample plots from 64 sites representing all 100 elevation bands of 4 slope aspects (East, South, West and North) of the landscape. We considered the species present in all plots of each 100 m contour elevation of four aspects as species richness of that elevation band. We collected the soil sample from 10x10x10 cm plot of 10 cm below the ground level at four corners of plant sampled plots to estimate the soil nutrients in a laboratory. We estimated the correlation, regression, Tukey Post -Hoc test, PerMANOVA, and CCA to show the relationship between environment and response variables. We found a significant negative relationship between species richness and altitude and soil nitrogen. The elevation showed a unimodal structure with species richness. The slope aspect showed a significant effect on species composition, but not on species richness. This study concludes that the increasing trend of soil N, P, and K with altitude showed a negative relation with species richness.

Key Words: Elevational band, CCA, PerMANOVA, RRI, Soil nutrient

Introduction

Global patterns of species ranges and richness are the product of many interacting factors such as environmental conditions, competition, geographical area, and historical/evolutionary development (Criddle et al 2003). Elevation and latitude are well-known broad-scale factors affecting species richness (Hawkins et al 2003). The climatic factors (temperature, potential evapotranspiration, length of the growing season, humidity, air pressure, ultraviolet radiation, moisture index, and rainfall) vary with elevation (Funnell and Parish 2001; Chang-Ming et al 2005) and exert a strong controlling influence on the distribution in all biomes (Miao and Jianmeng 2015).

Rahbek (1995, 1997) showed the three types of response of species richness to altitude (monotonic decline, hump-shaped, and monotonic incline). Globally, more than half of the studies on elevational diversity pattern show unimodal responses (Hakwins et al 2003), which
may be explained by altitude (Zhou et al 2019), area (Lomolino 2000; Lee et al 2013), mid
domain effect (Sanders 2002; McCain 2004), mountain-mass effect (Flenly 1994), rainfall
(Rosenzweig 1992), resource diversity (Gentry 1988; Hrivnak et al 2014), productivity
(Sanders et al 2007), temperature (Pounds et al 2006; Vinka et al 2010), competition (Bryant
et al 2008) and environmental heterogeneity (Gerstner and Kreft 2014). In the Himalayas,
with high altitudinal gradients and extreme slopes, climatic zones may change rapidly and this
is reflected by noticeable changes in the community structures even at a small distance
(Chawla et al 2008; Sinha et al 2018). Patterns of altitudinal species richness are indicative of
broad-scale diversity mechanisms, which are affected by water and temperature (Chang-Ming
et al 2005; Grytnes and McCain; 2007). Altitude and aspect have effects on β-diversity
(Gallardo et al 2009) or may function as limiting factors on plant species or ecosystem
properties and processes in the mountains (Xiang et al 2017).

The observed elevational trends for species varies among groups of organisms and from
one area to another. Regional and local patterns in plant species richness differ concerning
resource availability (Cornwell and Grubb 2003). Nutrient availability plays a variable role in
germination or seedling establishment and species dominance according to species
composition along an altitudinal gradient (Wenk and Dawson 2007). High water availability
usually leads to higher species richness, but higher nutrient availability usually leads to lower
species richness (Palpurina et al 2016). There is an inverse correlation between nutrient
resorption efficiency of plants and soil nutrients content (Zhiqiang et al 2018). On the other
hand, forests are generally species-rich in high nutrient sites (Peet and Christensen 1988). All
plants and animals require nitrogen to make proteins in their body. Phosphorus is needed to
make phosphate compounds, and potassium plays an activation role in photosynthesis, CO2
uptake, and the opening and closing of stomata. Available soil nitrogen is the most limiting
factor for plant growth and plays a role in increasing the diversity of plants (Fisher et al 2013).
Soil pH shows influences on biogeochemical processes like trace element mobility,
nitrification and denitrification (Neina 2019) and it indicates soil condition and the expected
direction of many soil processes. Most of the plant nutrients are more available at slightly
acidic to slightly alkaline soil - pH 6.5 to 7.5 (Khadka et al 2016). Other environmental
factors: precipitation (Palpurina et al 2016), temperature, energy flow (Rosenzweig 1995),
latitude, altitude, and depth gradients (Rohde 1992) have a co-operating role with respect to
The effect of nutrients on species diversity. The interaction between water and energy provides a good explanation (over 60%) for globally extensive plant and animal diversity gradients (Hawkins et al 2003).

The presence of species in a small area depends mainly on a suitable local niche but the presence of species over larger geographical areas on depends on climatic conditions (Rahbek 2005). Local diversity bears a noticeable dependence upon regional diversity (Ricklefs 1987). Minor changes in microclimatic environment variables like slope, aspect and soil nutrients with altitude may create unusual modifications of the local diversity. The temperature shows negative correlation with species of large distribution range but positive relation with species of small distribution range (Pan et al., 2016). The contribution of many smaller landscapes or mountains to biodiversity conservation is not well known. The small areas also may help to add to global biodiversity conservation through matrix habitat improvement, connectivity, and preservation of localized ecosystems (Baldwin and Fouch 2018). The numerous studies based on altitudinal gradients have not focused on slope aspects. In the study of Maren et al (2015), the aspect (north and south) was found to be a main ecological driver in altitudinal species richness. There are less studies about species richness with relation to slope aspects in Nepalese Himalayas. This study is based on the hypothesis that the slope aspect brings significant differences in altitudinal species richness patterns.

The specific objectives of this study are: (1) quantify vascular plant species diversity (2) discuss the role of altitude, slope aspect, and soil factors in species richness and species composition.

**Material and methods**

**Study area**

The study site: Narapani-Masina landscape (approximately 27°0'45" - 27°0'57 N and 82°0'45" - 83°18'E), is centered in the south part in Arghakhanchi district, west Nepal and extending between about 210 to 2200 m asl (personnel field visit). This landscape extends from the east border (Palpa district) to Jhimruk and Rapti rivers (west border) an extends for approximately 62 km east-west and 39 km north-south. The north face of this hill (aerial distance 4.8 km) is
steeper than the south face (aerial distance about 17.5 km). This hill lies at Mahabharat range and occupies the total area of Sitganga municipality and Panena rural municipality and 2 wards of each Sandhikharka and Bhumikasthan municipalities of Arghakhanchi district.

According to the climatic records of station: Khanchikot, which is located on this area, average annual temperature and annual rainfall of the area are 14.9°C and 1627.7 mm (DHM, 2017) respectively. The south part of this hill is hot and warm and dominated with Shorea-Syzygium forest. The east and north faces are moist and covered by Shorea forest, Schima-Castonopsis forest, and Pinus forest, while the west face is covered by Shorea-Diploknema forest, Pinus forest, and Quercus-Xylosma forest (personnel observation). Most of the forest of Arghakhanchi district lies in the southern part of this hill. The region of the hill above than 1200 m is steeper and moister. Narapani (tourist area and former headquarter of Arghakhanchi district) and a famous Hindu temple - Supa Deuali are located at 1700 and 1380 m respectively of this landscape.

**Figure 1.** Map of Arghakhanchi district with sampling spots.
The overall altitudinal range of the Narapani-Masina landscape (200-2200) was divided into 20, 100 m wide elevation bands. The field was visited and sampled by a plot of 10 x 10 m at each 100 m elevation band in October-November 2018. Based on the availability of different forest types the number of sample plot laid varied from 2 to 3 in each elevation band. The distance between the two sample plots varied from 100 to 150 m.

**Figure 2.** Outline of sampling design for primary data collection

Voucher specimens were collected, properly dried and kept in herbarium sheets. In addition to GPS data, other micro-ecological characters were also recorded for each herbarium specimen. All herbarium specimens were identified with the help of relevant taxonomic literature (Polunin and Stainton 1984, DPR 2010, 2011, 2012, 2015; Fraser-Jenkins et al 2015; Rajbhandary et al 2017; Fraser-Jenkins and Kandel 2019). Some species were also identified with the help of consulting experts and compared to specimens deposited at KATH and TUCH. All these identified herbarium specimens were submitted in the TUCH. Each plot was divided into four subplots and the presence/absence of all rooted species of vascular plants (Pteridophytes, gymnosperms, and angiosperms) was enumerated. The presence of a species among four subplots within a plot was finally recorded as 1. All species that occurred inside the sampled plots were identified with the help of field guides such as
Mager and Burrow (2007). At least one sample of each plant specimen was collected as a voucher specimen in order to verify the identification.

**Environmental variables**

Environmental factors (Relative Radiation Index, altitude, aspect, soil pH, nitrogen, phosphorus, and potassium) are considered as explanatory variables in this study. The coordinates (longitude and latitude), aspect, and altitude of each plot location were also measured using GPS (eTrex). Similarly, the slope of the sampled plots was measured using a clinometer.

Soil composition effects on the distribution of plants because they are dependent on the soil to survive. About 1 kg soil sample was collected from 15 cm below the surface at 4 corners of each sampling plot.

The soil nutrients (pH, Nitrogen, Phosphorus, and Potassium) were estimated following the chemical analysis method by Jones (1991) in Soil, Water, and Air Testing Laboratories (SWAT), Kathmandu, Nepal.

**Statistical analysis**

The relative radiation index (RRI) can be used as a measure for comparison of the distribution of direct solar radiation throughout a specific studied area (Mammassis et al 2012).

The relative radiation index (RRI) was calculated by using the formula given by Óke (1987).

\[
\text{RRI} = \cos(180^\circ - \Omega) \cdot \sin\beta \cdot \sin\Phi + \cos\beta \cdot \cos\Phi.
\]

Where \(\Omega\) is an aspect, \(\beta\) is the slope, and \(\Phi\) is the latitude of each plot. It gives a relative value of how much solar radiation a particular spot receives at noon at the equinoxes. Its value ranges from +1 to –1.
The total species of all sampled plots of each 100 m elevational band was considered as species richness of that spot. The diversity indices (Shannon Wiener diversity and Simpson diversity) were estimated by using “diversity” function through the vegan package (Oksanen et al 2019).

Species diversity is the aggregate form of species richness and evenness. Hurlbert (1971) proposed the formula to calculate the evenness by using the Shannon–Weiner index:

\[ E = \frac{H}{\log(S)} \]

Where, \( E \) = Evenness of species, \( H \) = Shannon-Wiener Index and \( S \) = Species richness

The slope aspect is a qualitative environmental variable. So, dummy data of aspects (denoting by 1 for a particular aspect and 0 for others in the column of each aspect) was applied for correlation purposes. The correlation of species richness with environmental variables was determined through Pearson correlation (Kassambara 2018). We applied a generalized linear model (GLM) to express the relations of environmental variables with species richness of different aspects and in total (Hastie and Pregibon 1993). The quasi-poisson family of error distributions was applied to remove over dispersion.

Similarly, the effect of 4 aspects (east, west, north, and south) on species richness was tested by ANOVA using Tukey Post-Hoc test (Quick 2011). The effect of aspects on species richness was confirmed by the application of a generalized linear mixed model (GLMM) (Berridge and Crouchley 2011) in R version 4.0.2.

We tested relationships of altitude and aspects with vegetation community composition by Permutational multivariate analysis of variance (PerMANOVA) by function Adonis (Anders on 2001) on the Bray-Curtis distance matrix.

We started the ordination to show the environment species relation by applying DCA in R vegan. The length of the gradient of the DCA axis I for total data set was more than 2.5 standard deviation units, suggested that (according to Leps and Smilauer 2003) unimodal ordination methods (e.g. CCA) were preferable.

We tested the usefulness of CCA through the variance inflation factor (VIF) before CCA. Canonical Correspondence Analysis (CCA) is used to analyze species environmental
CCA is a direct gradient analysis that displays the variation of vegetation concerning the included environmental factors by using environmental data to order samples (Kent 2011). We included only the species of high species score to make the CCA plot. The species score (stand score) represent the centroid of the species or the mode of the unimodal species response curve. All these analyses were done in R (R Core Team 2020).

**Results**

*Vascular plant species diversity*

We found a total of 460 vascular plant species representing 112 families and 331 genera in the Narapani-Masina landscape. There was great variation in species richness as well as life form richness between the four aspects of the study area. South and north aspect had the highest (369) and the lowest (316) species richness, respectively (Table 1). As for diversity indices, the average Shannon-Winner index and Simpson index values ranged between 3.78-3.88 and 0.97-0.98 respectively. The value of both diversity indices is higher in east and west aspects.

**Table 1.** Species richness of different aspects of different plant life-forms

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Aspects</th>
<th>Species richness</th>
<th>Shannon Wiener index ($H^\prime$)</th>
<th>Evenness (E)</th>
<th>Simpson index (D)</th>
<th>Altitudinal range (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Herbs  Climbers Ferns Shrubs Trees Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>East</td>
<td>155  21  28  67  76  347</td>
<td>3.86+0.35</td>
<td>1.52</td>
<td>0.98+0.01</td>
<td>700-1850</td>
</tr>
<tr>
<td>2</td>
<td>South</td>
<td>168  20  31  71  79  369</td>
<td>3.78+0.46</td>
<td>1.47</td>
<td>0.97+0.02</td>
<td>200-2200</td>
</tr>
<tr>
<td>3</td>
<td>West</td>
<td>153  20  27  74  74  348</td>
<td>3.88+0.55</td>
<td>1.53</td>
<td>0.98+0.02</td>
<td>500-2000</td>
</tr>
<tr>
<td>4</td>
<td>North</td>
<td>152  15  23  65  61  316</td>
<td>3.78+0.42</td>
<td>1.50</td>
<td>0.97+0.01</td>
<td>800-2200</td>
</tr>
<tr>
<td></td>
<td>Grand total</td>
<td>207  26  40  93  94  460</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The correlation between explanatory and response variables

Pearson correlation analysis was used to show the correlation between environmental variables and species richness (Table 2). There were significant positive relations of altitude with soil nitrogen \((r = 0.61)\), phosphorus \((r = 0.26)\) and Potassium \((r = 0.4)\). On the other hand, species richness showed a significant negative correlation with soil nitrogen \((r = -0.29)\) and altitude \((r = -0.31)\).

In the case of aspects, soil pH showed significant negative relations with the east and west sides. RRI showed a statistically positive correlation in the west but negative in the south aspects. Similarly, nutrients N, P, K also showed positive in one aspect and negative in other aspects (Table 2).

**Table 2.** Correlations among Species richness and environmental variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Species richness</th>
<th>altitude</th>
<th>RRI</th>
<th>pH</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species richness</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>altitude</td>
<td>-0.31**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRI</td>
<td>-0.15</td>
<td>0.17</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.05</td>
<td>-0.21</td>
<td>-0.06</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>-0.29**</td>
<td>0.61***</td>
<td>-0.01</td>
<td>-0.05</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>-0.12</td>
<td>0.26*</td>
<td>-0.09</td>
<td>-0.19</td>
<td>0.22*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>-0.09</td>
<td>0.4***</td>
<td>0.19</td>
<td>-0.4</td>
<td>0.02</td>
<td>0.33**</td>
<td>1</td>
</tr>
<tr>
<td>South</td>
<td>-0.05</td>
<td>-0.13</td>
<td>-0.46***</td>
<td>0.34**</td>
<td>0.2</td>
<td>-0.13</td>
<td>-0.58***</td>
</tr>
<tr>
<td>East</td>
<td>0</td>
<td>-0.03</td>
<td>0.04</td>
<td>-0.32**</td>
<td>0.09</td>
<td>-0.23*</td>
<td>0.27**</td>
</tr>
<tr>
<td>North</td>
<td>-0.08</td>
<td>0.09</td>
<td>0.2</td>
<td>0.18</td>
<td>-0.1</td>
<td>0.16</td>
<td>0.28**</td>
</tr>
<tr>
<td>West</td>
<td>0.14</td>
<td>0.08</td>
<td>0.26*</td>
<td>-0.23*</td>
<td>-0.21*</td>
<td>0.21*</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Statistically significant (p value): ** ** < 0.001, ** < 0.01, * < 0.05

Relation between environmental variables and species richness

The relations of all environmental variables except aspect on species richness was regressed using GLM. Species richness increased with altitude at first and at more or less mid altitudinal range, the richness started to decrease with increasing altitude. This gives a
unimodal structure in total and all aspects even though the $R^2$ values vary (Figure 3 and Appendix 1). This model had best fit in the north aspect ($R^2=0.64$ & $p < 0.05$).

Other explanatory variables RRI, soil pH, nitrogen (N), Phosphorus (P), and Potassium (K) showed linear relationships with altitudinal species richness, except the unimodal structure of Phosphorus in the north aspect ($R^2=0.58$) and total species (Appendix 1). Soil pH, N and P showed stronger correlations with species richness in north than other aspects.

![Plant Species distribution against altitude in 4 aspects](image)

**Figure 3.** Plant Species distribution against altitude in 4 aspects (E-East, N-North, S- South and W-west)

The regression analysis of altitude on environmental factors (pH, N, P, and K) showed variable and insignificant effects.
The slope aspect is the qualitative variable. The Tukey Post-Hoc Test showed that the aspects showed no significant effect on species richness and altitudinal nitrogen content (Figure 4). But, the effect of aspects on RRI, pH, P and K distribution was statistically significant (p < 0.05).

The relation of species distance matrix with environmental variables

The environmental factors showed different effects on species richness and vegetation community composition. The distance-based tests of multivariate dispersions of species of the community showed that altitude ($R^2=0.073$ & $P = 0.001$) and aspects ($R^2=0.046$ & $p = 0.003$) had significant relations (Table 3) with species composition. Similarly, the soil P ($R^2=0.014$ & $p=0.046$) and K ($R^2=0.026$ & $p=0.001$) also showed significant relationships with species composition.
Table 3. Results of model test of PerMANOVA analysis between environmental variables and vegetation community composition in Narapani-Masina landscape, West Nepal. \((R^2\) shows each variable’s share of the total variation in the data set).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Df</th>
<th>F value</th>
<th>(R^2)</th>
<th>Pr ((&gt;F))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alt</td>
<td>1</td>
<td>7.12</td>
<td>0.07</td>
<td>0.001</td>
</tr>
<tr>
<td>Aspect</td>
<td>3</td>
<td>1.52</td>
<td>0.05</td>
<td>0.003</td>
</tr>
<tr>
<td>RRI</td>
<td>1</td>
<td>1.21</td>
<td>0.01</td>
<td>0.132</td>
</tr>
<tr>
<td>pH</td>
<td>1</td>
<td>1.02</td>
<td>0.01</td>
<td>0.439</td>
</tr>
<tr>
<td>N</td>
<td>1</td>
<td>1.01</td>
<td>0.01</td>
<td>0.441</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>1.37</td>
<td>0.02</td>
<td>0.046</td>
</tr>
<tr>
<td>K</td>
<td>1</td>
<td>2.52</td>
<td>0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>25</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Bold type face indicates statistically significant (p < 0.05)

Species environment composition

The relation between species composition and environmental variables analyzed by ordinate methods DCA and CCA showed that every species has different relationships with explanatory variables. The DCA result of species richness showed the axis length of DCA1 was 3.23 and a decreasing trend of axis length towards DCA2 and DCA3 (Table 4). It indicated that the CCA ordination method is suitable to show the relation of species-environment composition (Smilauer 2003).

Table 4. The table of DCA of species composition

<table>
<thead>
<tr>
<th></th>
<th>DCA1</th>
<th>DCA2</th>
<th>DCA3</th>
<th>DCA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigen value</td>
<td>0.35</td>
<td>0.18</td>
<td>0.18</td>
<td>0.16</td>
</tr>
<tr>
<td>Decorana value</td>
<td>0.36</td>
<td>0.2</td>
<td>0.17</td>
<td>0.16</td>
</tr>
<tr>
<td>Axis length</td>
<td>3.23</td>
<td>3.04</td>
<td>2.37</td>
<td>3.32</td>
</tr>
</tbody>
</table>

Table 5. Value of CCA1 and CCA2

<table>
<thead>
<tr>
<th></th>
<th>CCA1</th>
<th>CCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigen value</td>
<td>0.32</td>
<td>0.16</td>
</tr>
<tr>
<td>Proportion explained</td>
<td>0.27</td>
<td>0.14</td>
</tr>
</tbody>
</table>

The test of goodness of CCA through the variance inflation factor (VIF) showed that there was no multicollinearity among the environmental variables (Table 5).
The CCA analysis showed that the constrained variables consisted 18% (Inertia value 1.476) out of the total Inertia value (8.284). It showed that constraints (environmental variables) explain only 18% of the causes of the vegetation distribution. There was a significant relationship between species richness and environment variables in CCA (p < 0.05). The first two axes of CCA explained 27 and 14% (total 41%) variation in species richness. The eigenvalues of these axes 1 and 2 accounted for 0.32 and 0.16, respectively (Table 5).

The altitude (canonical correlation r = 0.99), nitrogen, (canonical r = 0.51), and phosphorus (canonical r = 0.24) variables were most correlated with CCA axis 1. Similarly, other variables: pH (canonical r = -0.30), potassium (canonical r = 0.56), RRI (canonical r = 0.39) and south, west and north aspects were most correlated with axis 2 of CCA (Table 6).

**Table 6.** CCA Biplot scores of species composition against environmental variables and variance inflation factor (Vif. CCA)

<table>
<thead>
<tr>
<th>Variables</th>
<th>CCA1</th>
<th>CCA2</th>
<th>Vif. CCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspect North</td>
<td>0.07</td>
<td><strong>0.35</strong></td>
<td>2.09</td>
</tr>
<tr>
<td>Aspect South</td>
<td>-0.24</td>
<td><strong>-0.96</strong></td>
<td>2.97</td>
</tr>
<tr>
<td>Aspect West</td>
<td>0.14</td>
<td><strong>0.28</strong></td>
<td>2.39</td>
</tr>
<tr>
<td>Alt</td>
<td><strong>0.99</strong></td>
<td>-0.05</td>
<td>2.09</td>
</tr>
<tr>
<td>RRI</td>
<td>0.15</td>
<td>0.39</td>
<td>1.45</td>
</tr>
<tr>
<td>pH</td>
<td>-0.19</td>
<td><strong>-0.30</strong></td>
<td>1.47</td>
</tr>
<tr>
<td>N</td>
<td><strong>0.51</strong></td>
<td>-0.27</td>
<td>2.01</td>
</tr>
<tr>
<td>P</td>
<td><strong>0.24</strong></td>
<td>-0.08</td>
<td>1.47</td>
</tr>
<tr>
<td>K</td>
<td>0.39</td>
<td><strong>0.56</strong></td>
<td>2.30</td>
</tr>
</tbody>
</table>

The CCA ordination shows the distribution of the species based on their weighted average of the present in 192 sampling sites. According to the weighted average of species present in each of the sites, their direct relation to environmental variables is determined for each site. The soil nitrogen and phosphorus showed a positive relation with altitude. Similarly, the soil pH showed a positive response mainly in the south aspect and negative with K and RRI as well as west and north aspects. South aspects showed a strong effect as altitude in species distribution.

The species score represents the centroid of the species, or the mode of the unimodal species response curve (ter Braak 1987). The species *Zanthoxylum armatum* (CCA species
score: 0.85), *Macaranga pastulosa* (0.90), *Melastoma malabathricum* (0.27), *Asperagus racemosus* (0.20), *Saccharum spontaneum* (0.14), *Pyrus pashia* (0.23) showed high frequency at high nitrogen-containing sites. Similarly, dominancy of the species *Fragaria nubicola* (0.51), *Centella asiatica* (0.18), *Reinwardtia indica*, (0.20), *Bauhinia purpurea* (0.02), *Semecarpus anacardium* (0.19) on sites of a high content of phosphorus indicated that they were phosphorus loving species. The species *Albizia lebbeck* (0.79), *Berberis aristata* (1.74), *Berberis asiatica* (0.98), *Dendrobium longicornu* (1.25), *Drynaria propinqua* (0.91), *Prunus ceraoides* (0.99), *Selinum wallichianum* (1.23), *Quercus semicarpifolia* (1.44), *Geranium nepalense* (1.08), *Oleandra wallichii* (0.81), *Myrica indica* (0.72) showed positive relation with altitude because they were present at high altitude (Figure 5 A and B).

The species *Berlaria cristata* (0.04), *Imperata cylindrica* (0.02), *Xylosma longifolium* (0.19), *Digitaria ciliaris* (-0.11) show the maximum abundance towards the direction of the highest content of potassium. The species scores indicated that these were potassium loving species. Similarly, *Innula cappa* (-0.07), *Euphorbia hirta* (-0.11), *Brachiaria villosa* (-0.10), *Gnapalium affine* (-0.16), etc. were dominantly present at sites of high RRI value. Some species showed strong negative relation with nitrogen, phosphorus, and potassium but exposed a positive relationship with pH. *Evolvulus numularia* (-0.08), *Setaria parvifilium* (-0.21), *Ageratum conyzoides* (-0.04), *Achyranthus aspera* (-0.20), *Cynoglossum zeylanicum* (-0.41), *Terminali tomentosa* (-0.43), *Desmodium elengens* (-0.24), *Indigofera atropurpurea* (-0.25) were more abundant in the high pH containing sites mainly in south aspect (Figure 5 A and B). This suggests that these are pH loving species.
Figure 5. Canonical correspondence analyses (CCA) plot showing the effect of significant environmental variables on the species richness of (A) Herbs & (B) combined form of Climber, Ferns, Shrubs & Trees.

Discussion

Species Richness and diversity indices

Biodiversity is a natural resource, which closely links to the economic well-being of any country or any region. Narapani-Masina landscape is small but rich in biodiversity (460 vascular species). The possible reasons may be large variation in topography, aspect and climate within the area. Panthi and Chaudhari (2002) listed 500 species of angiosperms from the Arghakhanchi district. The present results suggest that further detailed exploration of this biodiversity-rich area is necessary for documentation and conservation purposes.

We found the values of Shannon-Winner and Simpson indices higher in the forest of east and west aspects. Diversity is the aggregate form of species richness and evenness and high diversity indices indicate the characteristic of more diverse communities. If the species are uniformly distributed, then the diversity value would be high (Southwood and Lineacre 2015). The species richness and altitudinal range of the south face is more in comparison to other
aspects. Generally, the north slope aspect of hills in Nepal is moist, and the south is drier, which results in higher species richness in the north slope aspect than the south in Manang valley (Panthi et al. 2007). According to Aulander et al. (2003), south-facing slopes may receive six times the amount of solar radiation of north-facing slopes in the northern hemisphere. The growth rate of plants in the south-facing slope aspect may be less than the North aspect due to high solar radiation and less moisture. It is also supported by Maren et al. (2015) because water plays a deterministic role in the composition, structure, and density of plant communities (Kutiel and Lavee 1999). We found a high value of species richness and diversity indices at the east and west faces which may be due to the presence of sufficient both moisture and solar radiation. It confirms that water and solar radiation play a leading role in species richness and species distribution in different aspects of any landscape or all continents or countries. The species diversity is less in south aspect even there is high species richness, and the diversity is higher in east and west aspects due to their comparatively high evenness of species.

**Correlation among the variables**

According to the results of the correlation analyses, altitude shows a significant positive correlation with soil total nitrogen, phosphorus, and potassium. Qasba et al (2017) also reported that available N, P, and K showed a significantly positive relation with altitude. According to Anic et al (2010), the soil nutrients also showed a significant negative correlation with elevation in the Andes (1970 to 3330 m), Central Chile. Similarly, there is a negative correlation of pH with soil total nitrogen, available phosphorus (P$_2$O$_5$), and extractable potassium (K$_2$O) (Khadka et al 2016). The elevation and other climatic factors are responsible for controlling the carbon, nitrogen, and other minerals concentration (Shedayi et al 2016). The above- and belowground stocks of the total nitrogen increase significantly with elevation. The decrease in soil temperature (1°C) with the altitude showed a significant inverse relationship of nitrogen stocks (decrease rate of 1 Mg·ha$^{-1}$) in soil temperature (Vieira et al 2011). This increasing soil water content and lowering soil temperature help to decreases in soil N mineralization and nitrification rates which ultimately makes the high content of soil nitrogen due to the low rate of litter decomposition (Zhang et al 2012; Måren et
al 2015). The farmland was also found nearer to the forest in some parts of the study area where some portion of chemical fertilizer may reach the forest. These reasons support the positive relation of soil nitrogen, phosphorus, and potassium with the altitude of this research.

Species richness showed negative correlations with all environmental variables but significant with only altitude and nitrogen. Similarly, species richness showed a negative relationship with elevation (Bhandari and Zhang 2019) and Phosphorus (Riesch et al 2018). High availability of P favors a few competitive species that results in the exclusion of low productive species which lose out in the competition for light (Hautier et al 2009). The nutrient content of soil (C, N, P, K, etc.) varies with the topographic aspect and altitude with vegetation (Bangroo et al 2017).

All four aspects of the hill showed variable responses with respect to environmental factors. The south slope aspect has a significant negative, but the west slope aspect has a positive correlation with RRI. Similarly, the east and west aspects show statistically negative, but the south slope aspect shows a positive relationship with soil pH. Soil pH shows minor changes through the elevation gradient (Saeed et al 2014).

**Effect of environmental variables on species richness**

Altitude functions as the main decisive factor of ecosystem properties and processes in the mountains (He et al 2016) and altitude variations determines slope and aspects on land. The model result of regression showed that altitude had a significant effect, but the slope aspect has no significant influence on species richness. The studied altitudinal ranges varied between the four slope aspects. Each altitudinal range showed a significant unimodal richness pattern, but the peak of maximum richness differed between them. However, the unimodal nature of species against elevation was stronger in north and west than in other slope aspects. Many researchers (Rohde 1992; Bhattarai and Vetaas 2003; Bhattarai et al 2004; Grau et al 2007) reported that altitude showed a unimodal effect on species richness. The elevation having maximum species richness depended on altitudinal range, plant taxa etc. (Grau et al 2007). Generally, a right skewed altitudinal species richness pattern shows negative correlations and a left skewed pattern shows positive. When the gradient is shorter, a hump-
shaped pattern of longer elevational gradients would change to a monotonic decreasing trend (Nogue’s-Bravo et al 2008) or linearly increasing trend (Greatness and Vetaas 2002) or with increasing spatial scale of extent, the richness pattern changes from a monotonic to a hump-shaped pattern (Manish et al 2017). Most of the unimodal relationship between species richness and altitude are right skewed. The right skewed pattern in north and east aspect and left skewed pattern in west aspect suggest that no particular type of skewness present in the unimodal pattern in this study. There is evidence of a unimodal pattern in many regions at smaller spatial scales such as landscape and local gradients (Ooman and Shanker 2005). The hard-boundary effect (Colwell and Lees 2000), which results less area on top of the hills (Hua 2004) and overlapping of species of both lower and higher elevation range declares the unimodal nature is generally fit to describe the relationship between species richness and elevation well.

RRI score is an aggregate of latitude, slope, and aspect of any mountainous area. Spatial variation in slope and aspect function as determinants of vegetation pattern, species distribution, and ecosystem processes in many mountainous environments (Bennie et al 2008). RRI showed no linear relations with species in all aspects. The microclimatic conditions on the slope of an area vary dramatically, which may affect the biology of organisms at all levels. The distinct climate conditions and soil nutrients through the altitude gradient of slopes can influence the above-ground biomass and species richness (Bhandary and Zhang 2019).

The soil variables like pH, N, and K showed non-significant, linear relations with species richness in all aspects and total landscape. But, soil P showed a significant unimodal relationship with species richness in the north slope aspect but non-significant linear structures in other slope aspects. The soil nutrients are related to nutrient cycling through leaf litter fall and decomposition (Hicks and Frank 1984), and the litter decomposition has resulted from soil moisture, soil temperature, soil micro-organisms, and other factors. The nutrients reach the soil from the air through physical or biochemical processes or were present in the rocks. So, the slope aspect has no direct role in mineral distribution.

Litter fall regulates the accumulation of soil organic matter, the input of the nutrients, nutrient replacement, maintenance of biodiversity and other ecosystem functions in natural vegetation (Giewta 2020). Generally, dry litter decomposes more slowly in dry areas than in moist areas. At suitable moisture conditions, increasing temperature results in an exponential increase in decomposition rates (Salahab and Scholes 2010). The south aspect is drier than the
north slope aspect in Nepalese Himalaya. The moisture and suitable temperature of the northern slope aspect helps in continuous leaf litter decomposition, which results in high nutrients content on the soil. Plant available soil K, P and N are not limiting the productivity at south with respect to north-oriented slopes, because soil available water functions as the primarily limiting factor for plant productivity (Gong et al 2008). Generally, north-facing slopes are connected with higher biomass, coverage and height, and species diversity than south facing slopes (Yang et al 2020). We can say that altitude is the main factor affecting species richness. But, aspect indirectly affects species richness by creating a dry or moist environment and altering the rate of leaf litter decomposition.

**Environment Species Composition**

The result of Permutational multivariate analysis (PerMANOVA) suggested that altitude, aspect, phosphorus, and potassium showed a significant relationship with species composition. Anderson (2006) stated that distance-based tests are robust and useful for detecting real differences in the species spread.

The survival of plant species generally depends on nutrient requirements. Different plant groups may show different responses to nutrient availability (Ellenberg 1988; Johnson and Leopold 1994). Species scores are expressive forms of the united effect of all projecting variables represented by the controlled axes. The first eigenvalue of CCA is equal to the maximized dispersion of species scores along the first CCA axis (Ter Braak 1986). In the CCA diagram, altitude, soil N, and P are correlated with the first axis and show positive relation to each other. The canonical correlation r-value shows the effects of variables on species distribution in the following order: altitude > N > P. The high score bearing species: like: *Zanthoxylum armatum, Macaranga pastulosa*, etc. are present in sites higher in N and also show positive responses to altitude. Similarly, species like *Fragaria nubicola* and *Centella asiatica*, which have medium scores, are found at the sites having a high content of phosphorus. This result shows that soil nitrogen is more strongly correlated to altitude than phosphorus. Soil moisture is the main cause of the nitrogen effect in soil (Gornish and Miller 2015). The moisture generally increases with elevation in Nepal. The high nitrogen content makes the soil more acidic in combination with high moisture (Deutsch et al 2010; Ying et
This may be a result of the decreasing trend of species richness toward higher elevations. Vegetation composition is affected by nutrient limitation, which is governed by low soil temperatures and influenced by soil moisture conditions in the Himalayan region (Drollinger et al. 2017). The plants require (on a mass basis) about ten times more N than P to promote balanced plant growth (Aerts and Chapin 2013).

The canonical correlation value of potassium ($r = 0.56$) and pH ($r = -0.30$) shows that they have negative scores on the CCA 2 axis. The moderately positive scores of bearing species like; *Barleria cristata*, *Imperata cylindrica*, show high dominancy at high potassium-containing sites. The negative score bearing species on CCA2 are: *Evolvulus numularia*, *Setaria parviflora*, shows positive response with pH and are present at high pH sites. The linearly decreasing pattern of pH and the increasing patterns of nitrogen content against altitude shows that the high altitude sites have high N content and low pH value. Inouye et al (1987) also found a negative relationship between soil nitrogen and species richness. Crawley et al (2005) experimentally proved that species richness increases sharply with increasing pH (6-7) but decreases along with the addition of phosphorus and potassium. The presence of more nitrogen in soil may be the main cause of the loss of plant biodiversity in terrestrial ecosystems (Dise 2011). Similarly, the species *Inula cappa* and *Gnaphalium affine* have negative scores on CCA2 and are present at the high RRI score bearing area.

The comparative test of aspect on species richness by ANOVA and species composition by PerMANOVA shows that slope aspect has a significant effect on species distribution, not on species richness. The CCA also describes the relation of slope aspects with species and other environmental variables. The south slope aspect shows a strong role in species distribution than other slope aspects. The species: *Euphorbia hirta*, *Justicia adhatoda*, *Diospyrus spp*, *Cyanotis vag*, *Mallotus philippensis* have high dominancy in the south slope aspect. Other North and west aspects show a negative relationship with pH and a positive with RRI and potassium. The south slope aspect has a positive relation with only soil pH, which is also proved by the negative correlation of species richness with altitude and Nitrogen content. The topographical factors (elevation and aspect) affect mountain forests through their direct influence on radiation and moisture (Maren et al 2015).

The accurate estimation of soil nutrients helps to understand the interaction of biogeochemical cycles with the global climate (Shaw et al 2008). Changes in climatic
conditions (temperature and precipitation) along altitudinal gradients can influence nutrient content (Fisher et al 2013). The wild plants get nitrogen content from the soil nutrients via the nitrogen fixation and nitrification processes.

Some species are present at N, P, and K rich sites at high altitudes, and some are present at high pH value at low elevations. The nutrients (N & P) absorption efficiency of plants depends on latitude, mean annual temperature (MAT), and mean annual precipitation (MAP) (Yuan and Chen 2008). This study shows that the nutrient requirement of plants varies according to species, plant age, habitat, and soil type, but a few species have a positive response towards a high concentration of nutrients.

Conclusions

The Narapani-Masina landscape is inhabited by at least 460 vascular plant species, and there is a considerable variation in slope aspect wise species richness and nutrient contents. The species richness showed a unimodal pattern against altitude, but the slope aspect did not have a significant impact on species richness and diversity indices. The increasing trend of soil N, P, and K with altitude showed negative relations with species richness. CCA analysis gave ordination axes that were strongly correlated to environmental variables: elevation, soil N, K, and south aspect and related to the pattern of species distribution. The plant species that are present in the high concentrations of nitrogen, phosphorus, and potassium in the soil also show positive responses to altitude. On the other hand, the species which are present in soil with high pH (especially at the south aspect) showed a negative relation with elevation. In mountainous regions, both altitude and slope aspects show effects on species composition rather than species richness through the changes in edaphic and climatic factors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Acknowledgment

Our sincere thank goes to Prof. Dr. Ram Kailash Prasad Yadav, Chairman of Central Department of Botany, Tribhuvan University, Nepal, who helped us with official and technical work. We acknowledge Mr. Subhas Khatri and Mr. Dhanaraj Kandel Senior officer and Scientist, National Herbarium, Godawari, who provided laboratory facilities for plant identification.

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Appendix 1. The impact of aspect wise environmental parameters on species richness obtained by generalized linear model (GLM) (p < 0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>South Model R²</th>
<th>East Model R²</th>
<th>North Model R²</th>
<th>West Model R²</th>
<th>Aggregate Model R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude</td>
<td>0.3 2</td>
<td>0.46 2</td>
<td>0.64 2</td>
<td>0.59 2</td>
<td>0.67</td>
</tr>
<tr>
<td>RRI</td>
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<td>0.36 1</td>
<td>0.1 1</td>
<td>0.02 1</td>
<td>0.02</td>
</tr>
<tr>
<td>pH</td>
<td>0.0 4</td>
<td>&lt; 0.001</td>
<td>0.08 1</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Nitrogen</td>
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<td>0.11 1</td>
<td>0.26 1</td>
<td>0.02 1</td>
<td>0.09</td>
</tr>
<tr>
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<td>0.58 1</td>
<td>0.00 5</td>
<td>0.1</td>
</tr>
<tr>
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<td>0.003 1</td>
<td>0.03 1</td>
<td>0.02 1</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Declaration of interests

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