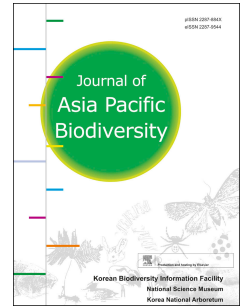


# Journal Pre-proof

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

Babu Ram Nepali, John Skartveit, Chitra Bahadur Baniya



PII: S2287-884X(21)00048-0

DOI: <https://doi.org/10.1016/j.japb.2021.04.005>

Reference: JAPB 616

To appear in: *Journal of Asia-Pacific Biodiversity*

Received Date: 11 December 2020

Revised Date: 17 April 2021

Accepted Date: 28 April 2021

Please cite this article as: Nepali BR, Skartveit J, Baniya CB, Impacts of slope aspects on altitudinal species richness and species composition of Narapani-Masina landscape, Arghakhanchi, West Nepal, *Journal of Asia-Pacific Biodiversity*, <https://doi.org/10.1016/j.japb.2021.04.005>.

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1 Original Article

2

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

5

6 **Babu Ram Nepali<sup>a, b, \*</sup>, John Skartveit<sup>c</sup>, Chitra Bahadur Baniya<sup>a</sup>**

7

8 <sup>a</sup>*Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal*

9 <sup>b</sup>*Butwal Multiple Campus, Tribhuvan University, Butwal, Nepal*

10 <sup>c</sup>*University College, NLA, Bergen, Norway*

11

12 \*Corresponding author.

13 *E-mail address: nepalibaburam7@gmail.com*

14

15

## 16 **Abstract**

17

18 This study aimed to find out the roles of altitude, slope aspect, and soil factors in species  
19 richness in the Narapani-Masina landscape, Arghakhanchi, Nepal. We surveyed forest plant  
20 communities by sampling 192 statistically representative 10 x 10 m sample plots from 64 sites  
21 representing all 100 elevation bands of 4 slope aspects (East, South, West and North) of the  
22 landscape. We considered the species present in all plots of each 100 m contour elevation of  
23 four aspects as species richness of that elevation band. We collected the soil sample from  
24 10x10x10 cm plot of 10 cm below the ground level at four corners of plant sampled plots to  
25 estimate the soil nutrients in a laboratory. We estimated the correlation, regression, Tukey  
26 Post -Hoc test, PerMANOVA, and CCA to show the relationship between environment and  
27 response variables. We found a significant negative relationship between species richness and  
28 altitude and soil nitrogen. The elevation showed a unimodal structure with species richness.  
29 The slope aspect showed a significant effect on species composition, but not on species  
30 richness. This study concludes that the increasing trend of soil N, P, and K with altitude  
31 showed a negative relation with species richness.

32

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

34

35

## 36 **Introduction**

37

38 Global patterns of species ranges and richness are the product of many interacting factors  
39 such as environmental conditions, competition, geographical area, and historical/evolutionary  
40 development (Criddle et al 2003). Elevation and latitude are well-known broad-scale factors  
41 affecting species richness (Hawkins et al 2003). The climatic factors (temperature, potential  
42 evapotranspiration, length of the growing season, humidity, air pressure, ultraviolet radiation,  
43 moisture index, and rainfall) vary with elevation (Funnell and Parish 2001; Chang-Ming et al  
44 2005) and exert a strong controlling influence on the distribution in all biomes (Miao and  
45 Jianmeng 2015).

46 Rahbek (1995, 1997) showed the three types of response of species richness to altitude  
47 (monotonic decline, hump-shaped, and monotonic incline). Globally, more than half of the  
48 studies on elevational diversity pattern show unimodal responses (Hakwins et al 2003), which

49 may be explained by altitude (Zhou et al 2019), area (Lomolino 2000; Lee et al 2013), mid  
50 domain effect (Sanders 2002; McCain 2004), mountain-mass effect (Flenly 1994), rainfall  
51 (Rosenzweig 1992), resource diversity (Gentry 1988; Hrivnak et al 2014), productivity  
52 (Sanders et al 2007), temperature (Pounds et al 2006; Vinka et al 2010), competition (Bryant  
53 et al 2008) and environmental heterogeneity (Gerstner and Kreft 2014). In the Himalayas,  
54 with high altitudinal gradients and extreme slopes, climatic zones may change rapidly and this  
55 is reflected by noticeable changes in the community structures even at a small distance  
56 (Chawla et al 2008; Sinha et al 2018). Patterns of altitudinal species richness are indicative of  
57 broad-scale diversity mechanisms, which are affected by water and temperature (Chang-Ming  
58 et al 2005; Grytnes and McCain; 2007). Altitude and aspect have effects on  $\beta$ -diversity  
59 (Gallardo et al 2009) or may function as limiting factors on plant species or ecosystem  
60 properties and processes in the mountains (Xiang et al 2017).

61 The observed elevational trends for species varies among groups of organisms and from  
62 one area to another. Regional and local patterns in plant species richness differ concerning  
63 resource availability (Cornwell and Grubb 2003). Nutrient availability plays a variable role in  
64 germination or seedling establishment and species dominance according to species  
65 composition along an altitudinal gradient (Wenk and Dawson 2007). High water availability  
66 usually leads to higher species richness, but higher nutrient availability usually leads to lower  
67 species richness (Palpurina et al 2016). There is an inverse correlation between nutrient  
68 resorption efficiency of plants and soil nutrients content (Zhiqiang et al 2018). On the other  
69 hand, forests are generally species-rich in high nutrient sites (Peet and Christensen 1988). All  
70 plants and animals require nitrogen to make proteins in their body. Phosphorus is needed to  
71 make phosphate compounds, and potassium plays an activation role in photosynthesis, CO<sub>2</sub>  
72 uptake, and the opening and closing of stomata. Available soil nitrogen is the most limiting  
73 factor for plant growth and plays a role in increasing the diversity of plants (Fisher et al 2013).  
74 Soil pH shows influences on biogeochemical processes like trace element mobility,  
75 nitrification and denitrification (Neina 2019) and it indicates soil condition and the expected  
76 direction of many soil processes. Most of the plant nutrients are more available at slightly  
77 acidic to slightly alkaline soil - pH 6.5 to 7.5 (Khadka et al 2016). Other environmental  
78 factors: precipitation (Palpurina et al 2016), temperature, energy flow (Rosenzweig 1995),  
79 latitude, altitude, and depth gradients (Rohde 1992) have a co-operating role with respect to

80 the effect of nutrients on species diversity. The interaction between water and energy provides  
81 a good explanation (over 60%) for globally extensive plant and animal diversity gradients  
82 (Hawkins et al 2003).

83 The presence of species in a small area depends mainly on a suitable local niche but the  
84 distribution of species over larger geographical areas on depends on climatic conditions  
85 (Rahbek 2005). Local diversity bears a noticeable dependence upon regional diversity  
86 (Ricklefs 1987). Minor changes in microclimatic environment variables like slope, aspect and  
87 soil nutrients with altitude may create unusual modifications of the local diversity. The  
88 temperature shows negative correlation with species of large distribution range but positive  
89 relation with species of small distribution range (Pan et al., 2016). The contribution of many  
90 smaller landscapes or mountains to biodiversity conservation is not well known. The small  
91 areas also may help to add to global biodiversity conservation through matrix habitat  
92 improvement, connectivity, and preservation of localized ecosystems (Baldwin and Fouch  
93 2018). The numerous studies based on altitudinal gradients have not focused on slope aspects.  
94 In the study of Maren et al (2015), the aspect (north and south) was found to be a main  
95 ecological driver in altitudinal species richness. There are less studies about species richness  
96 with relation to slope aspects in Nepalese Himalayas. This study is based on the hypothesis  
97 that the slope aspect brings significant differences in altitudinal species richness patterns.

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

101

## 102 **Material and methods**

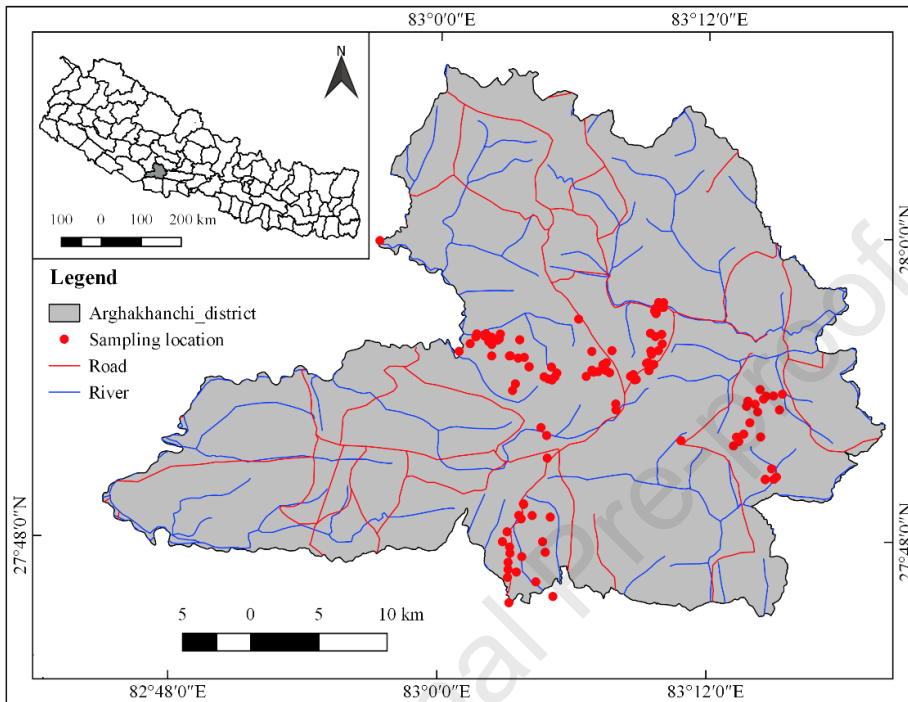
103

### 104 *Study area*

105

106 The study site: Narapani-Masina landscape (approximately  $27^{\circ}45'$ -  $27^{\circ}57'$  N and  $82^{\circ}45'$ -  
107  $83^{\circ}18'E$ ), is centered in the south part in Arghakhanchi district, west Nepal and extending  
108 between about 210 to 2200 m asl (personnel field visit). This landscape extends from the east  
109 border (Palpa district) to Jhimruk and Rapti rivers (west border) an extends for approximately  
110 62 km east-west and 39 km north-south. The north face of this hill (aerial distance 4.8 km) is

111 steeper than the south face (aerial distance about 17.5 km). This hill lies at Mahabharat range  
 112 and occupies the total area of Sitganga municipality and Panena rural municipality and 2  
 113 wards of each Sandhikharka and Bhumikasthan municipalities of Arghakhanchi district.  
 114



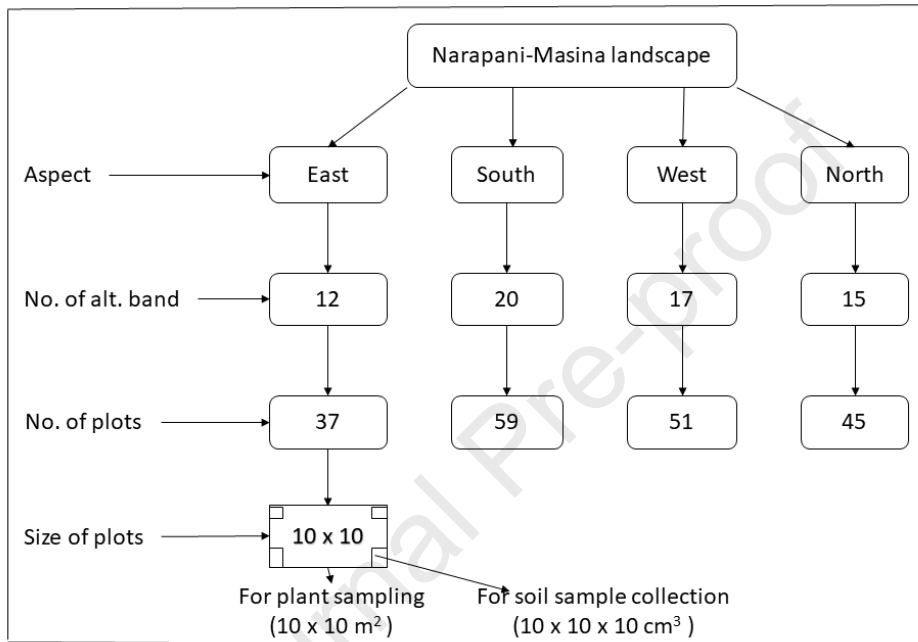
115  
 116 **Figure 1.** Map of Arghakhanchi district with sampling spots.

117  
 118 According to the climatic records of station: Khanchikot, which is located on this area,  
 119 average annual temperature and annual rainfall of the area are 14.9<sup>0</sup>C and 1627.7 mm (DHM,  
 120 2017) respectively. The south part of this hill is hot and warm and dominated with *Shorea-*  
 121 *Syzygium* forest. The east and north faces are moist and covered by *Shorea* forest, *Schima-*  
 122 *Castonopsis* forest, and *Pinus* forest, while the west face is covered by *Shorea-Diploknema*  
 123 forest, *Pinus* forest, and *Quercus-Xylosma* forest (personnel observation). Most of the forest  
 124 of Arghakhanchi district lies in the southern part of this hill. The region of the hill above than  
 125 1200 m is steeper and moister. Narapani (tourist area and former headquarter of Arghakhanchi  
 126 district) and a famous Hindu temple - Supa Deuali are located at 1700 and 1380 m  
 127 respectively of this landscape.

128  
 129 *Sampling design and plant collection*

130

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



136

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

138

139 Voucher specimens were collected, properly dried and kept in herbarium sheets. In  
 140 addition to GPS data, other micro-ecological characters were also recorded for each herbarium  
 141 specimen. All herbarium specimens were identified with the help of relevant taxonomic  
 142 literature (Polunin and Stainton 1984, DPR 2010, 2011, 2012, 2015; Fraser-Jenkins et al 2015;  
 143 Rajbhandary et al 2017; Fraser-Jenkins and Kandel 2019). Some species were also identified  
 144 with the help of consulting experts and compared to specimens deposited at KATH and  
 145 TUCH. All these identified herbarium specimens were submitted in the TUCH.

146

147 Each plot was divided into four subplots and the presence/absence of all rooted species of  
 148 vascular plants (Pteridophytes, gymnosperms, and angiosperms) was enumerated. The  
 149 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

150 Mager and Burrow (2007). At least one sample of each plant specimen was collected as a  
 151 voucher specimen in order to verify the identification

152

153 *Environmental variables*

154

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

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

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

166

167 *Statistical analysis*

168

169 The relative radiation index (RRI) can be used as a measure for comparison of the  
 170 distribution of direct solar radiation throughout a specific studied area (Mammassis et al  
 171 2012).

172 The relative radiation index (RRI) was calculated by using the formula given by Ôke  
 173 (1987).

174

$$175 \quad \text{RRI} = \text{Cos} (180^{\circ} - \Omega) \cdot \text{Sin}\beta \cdot \text{Sin}\Phi + \text{Cos}\beta \cdot \text{Cos}\Phi.$$

176

177 Where  $\Omega$  is an aspect,  $\beta$  is the slope, and  $\Phi$  is the latitude of each plot. It gives a relative  
 178 value of how much solar radiation a particular spot receives at noon at the equinoxes. Its value  
 179 ranges from +1 to -1.



180 The total species of all sampled plots of each 100 m elevational band was considered as  
 181 species richness of that spot. The diversity indices (Shannon Wiener diversity and Simpson  
 182 diversity) were estimated by using “diversity” function through the *vegan* package (Oksanen  
 183 et al 2019).

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

186

$$E = \frac{H}{\log(S)}$$

187

188 Where,  $E$  = Evenness of species,  $H$  = Shannon-Wiener Index and  $S$  = Species richness

189

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

197 Similarly, the effect of 4 aspects (east, west, north, and south) on species richness was test  
 198 ed by ANOVA using Tukey Post-Hoc test (Quick 2011). The effect of aspects on species rich  
 199 ness was confirmed by the application of a generalized linear mixed model (*GLMM*) (Berridg  
 200 e and Crouchley 2011) in R version 4.0.2.

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

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

208 We tested the usefulness of CCA through the variance inflation factor (VIF) before CCA.  
 209 Canonical Correspondence Analysis (CCA) is used to analyze species environmental

210 composition. CCA is a direct gradient analysis that displays the variation of vegetation  
 211 concerning the included environmental factors by using environmental data to order samples  
 212 (Kent 2011). We included only the species of high species score to make the CCA plot. The  
 213 species score (stand score) represent the centroid of the species or the mode of the unimodal  
 214 species response curve. All these analyses were done in R (R Core Team 2020).

215

## 216 **Results**

217

### 218 *Vascular plant species diversity*

219

220 We found a total of 460 vascular plant species representing 112 families and 331 genera  
 221 in the Narapani-Masina landscape. There was great variation in species richness as well as life  
 222 form richness between the four aspects of the study area. South and north aspect had the  
 223 highest (369) and the lowest (316) species richness, respectively (Table 1). As for diversity  
 224 indices, the average Shannon-Wiener index and Simpson index values ranged between 3.78-  
 225 3.88 and 0.97- 0.98 respectively. The value of both diversity indices is higher in east and west  
 226 aspects.

227

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

S. N.	Aspects	Species richness						Shannon Wiener index (H')	Evenness (E)	Simpson index (D)	Altitudina range (m)
		Herbs	Climbers	Ferns	Shrubs	Trees	Total				
1	East	155	21	28	67	76	347	3.86±0.35	1.52	0.98±0.01	700-1850
2	South	168	20	31	71	79	369	3.78±0.46	1.47	0.97±0.02	200-2200
3	West	153	20	27	74	74	348	3.88±0.55	1.53	0.98±0.02	500-2000
4	North	152	15	23	65	61	316	3.78±0.42	1.50	0.97±0.01	800-2200
Grand total		207	26	40	93	94	460				

229

230 *The correlation between explanatory and response variables*

231

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

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

241

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

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

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

244

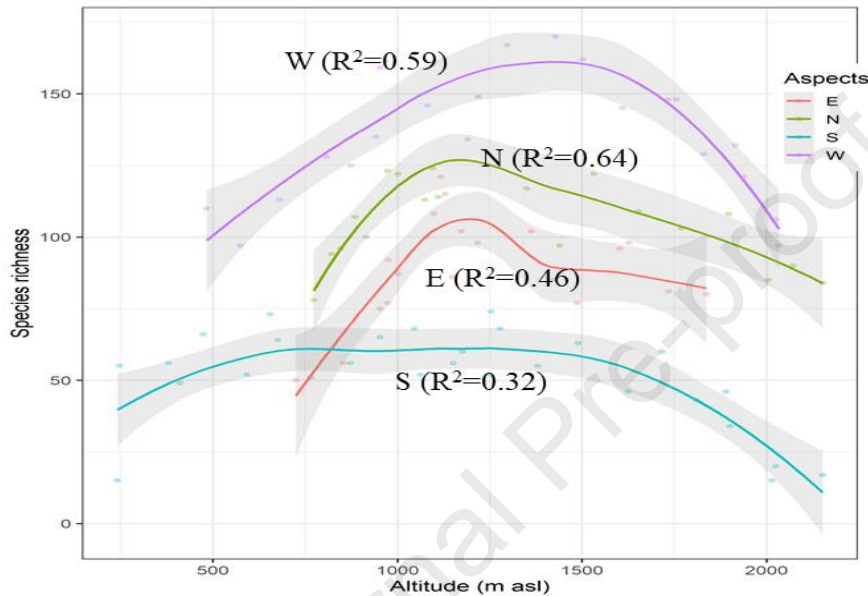
245 *Relation between environmental variables and species richness*

246

247 The relations of all environmental variables except aspect on species richness was  
 248 regressed using GLM. Species richness increased with altitude at first and at more or less mid  
 249 altitudinal range, the richness started to decrease with increasing altitude. This gives a

250 unimodal structure in total and all aspects even though the  $R^2$  values vary (Figure 3 and  
 251 Appendix 1). This model had best fit in the north aspect ( $R^2=0.64$  &  $p < 0.05$ ).

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

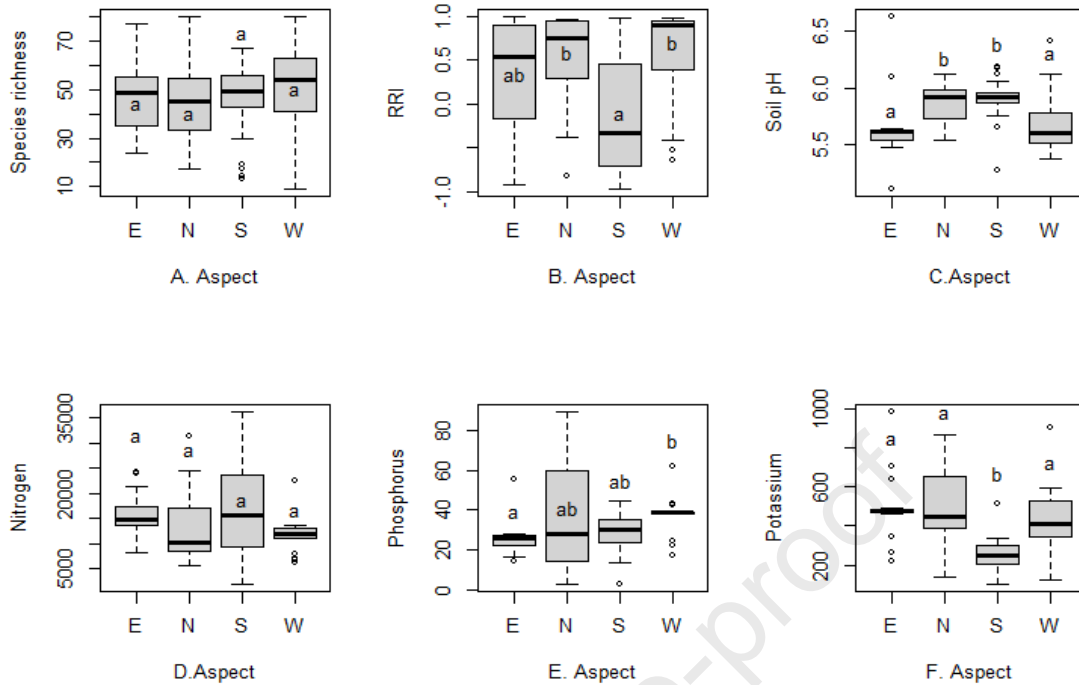


256

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

259

260 The regression analysis of altitude on environmental factors (pH, N, P, and K) showed  
 261 variable and insignificant effects.



262

263 **Figure 4.** The Post-Hoc Analysis with Tukey's Test

264

265 The slope aspect is the qualitative variable. The Tukey Post-Hoc Test showed that the  
 266 aspects showed no significant effect on species richness and altitudinal nitrogen content  
 267 (Figure 4). But, the effect of aspects on RRI, pH, P and K distribution was statistically  
 268 significant ( $p < 0.05$ ).

269

270 *The relation of species distance matrix with environmental variables*

271

272 The environmental factors showed different effects on species richness and vegetation  
 273 community composition. The distance-based tests of multivariate dispersions of species of the  
 274 community showed that altitude ( $R^2=0.073$  &  $P = 0.001$ ) and aspects ( $R^2=0.046$  &  $p = 0.003$ )  
 275 had significant relations (Table 3) with species composition. Similarly, the soil P ( $R^2=0.014$  &  
 276  $p=0.046$ ) and K ( $R^2=0.026$  &  $p=0.001$ ) also showed significant relationships with species  
 277 composition.

278

279 **Table 3.** Results of model test of PerMANOVA analysis between environmental variables  
 280 and vegetation community composition in Narapani-Masina landscape, West Nepal. ( $R^2$   
 281 shows each variable's share of the total variation in the data set).

282

Parameter	Df	F value	$R^2$	Pr (>F)
Alt	1	<b>7.12</b>	<b>0.07</b>	<b>0.001</b>
Aspect	3	<b>1.52</b>	<b>0.05</b>	<b>0.003</b>
RRI	1	1.21	0.01	0.132
pH	1	1.02	0.01	0.439
N	1	1.01	0.01	0.441
P	1	<b>1.37</b>	<b>0.02</b>	<b>0.046</b>
K	1	<b>2.52</b>	<b>0.03</b>	<b>0.001</b>
Residuals	25		0.26	

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

284

285 *Species environment composition*

286

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

293

294 **Table 4.** The table of DCA of species composition

**Table 5.** Value of CCA1 and

295 CCA2

	DCA1	DCA2	DCA3	DCA4		CCA1	CCA2
Eigen value	0.35	0.18	0.18	0.16	Eigen value	0.32	0.16
Decorana value	0.36	0.2	0.17	0.16	Proportion explained	0.27	0.14
Axis length	3.23	3.04	2.37	3.32			

296

297 The test of goodness of CCA through the *variance inflation factor* (VIF) showed that  
 298 there was no multicollinearity among the environmental variables (Table 5).

299 The CCA analysis showed that the constrained variables consisted 18 % (Inertia value  
 300 1.476) out of the total Inertia value (8.284). It showed that constraints (environmental  
 301 variables) explain only 18 % of the causes of the vegetation distribution. There was a  
 302 significant relationship between species richness and environment variables in CCA ( $p <$   
 303 0.05). The first two axes of CCA explained 27 and 14 % (total 41 %) variation in species  
 304 richness. The eigenvalues of these axes 1 and 2 accounted for 0.32 and 0.16, respectively  
 305 (Table 5).

306 The altitude (canonical correlation  $r = 0.99$ ), nitrogen, (canonical  $r = 0.51$ ), and  
 307 phosphorus (canonical  $r = 0.24$ ) variables were most correlated with CCA axis 1. Similarly,  
 308 other variables: pH (canonical  $r = -0.30$ ), potassium (canonical  $r = 0.56$ ), RRI (canonical  $r =$   
 309 0.39) and south, west and north aspects were most correlated with axis 2 of CCA (Table 6).

310

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

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

313

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

321 The species score represents the centroid of the species, or the mode of the unimodal  
 322 species response curve (ter Braak 1987). The species *Zanthoxylum armatum* (CCA species

323 score: 0.85), *Macaranga pastulosa* (0.90), *Melastoma malabathricum* (0.27), *Asperagus*  
324 *racemosus* (0.20), *Saccharum spontaneus* (0.14), *Pyrus pashia* (0.23) showed high frequency  
325 at high nitrogen-containing sites. Similarly, dominance of the species *Fragaria nubicola*  
326 (0.51), *Centella asiatica* (0.18), *Reinwardtia indica*, (0.20), *Bauhinia purpurea* (0.02),  
327 *Semecarpus anacardium* (0.19) on sites of a high content of phosphorus indicated that they  
328 were phosphorus loving species. The species *Albizia lebbeck* (0.79), *Berberis aristata*  
329 (1.74), *Berberis asiatica* (0.98), *Dendrobium longicornu* (1.25), *Drynaria propinqua*  
330 (0.91), *Prunus ceraoides* (0.99), *Selinum wallichianum* (1.23), *Quercus semicarpifolia*  
331 (1.44), *Geranium nepalense* (1.08), *Oleandra wallichii* (0.81), *Myrica indica* (0.72) showed  
332 positive relation with altitude because they were present at high altitude (Figure 5 A and B).

333 The species *Berlaria cristata* (0.04), *Imperata cylindrica* (0.02), *Xylosma longifolium*  
334 (0.19), *Digitaria ciliaris* (-0.11) show the maximum abundance towards the direction of the  
335 highest content of potassium. The species scores indicated that these were potassium loving  
336 species. Similarly, *Innula cappa* (-0.07), *Euphorbia hirta* (-0.11), *Brachiaria villosa* (-  
337 0.10), *Gnapalium affine* (-0.16), etc. were dominantly present at sites of high RRI value.  
338 Some species showed strong negative relation with nitrogen, phosphorus, and potassium but  
339 exposed a positive relationship with pH. *Evolvulus numularia* (-0.08), *Setaria parvifilium* (-  
340 0.21), *Ageratum conyzoides* (-0.04), *Achyranthus aspera* (-0.20), *Cynoglossum zeylanicum* (-  
341 0.41), *Terminali tomentosa* (-0.43), *Desmodium elengens* (-0.24), *Indigofera atropurpurea* (-  
342 0.25) were more abundant in the high pH containing sites mainly in south aspect (Figure 5 A  
343 and B). This suggests that these are pH loving species.

344



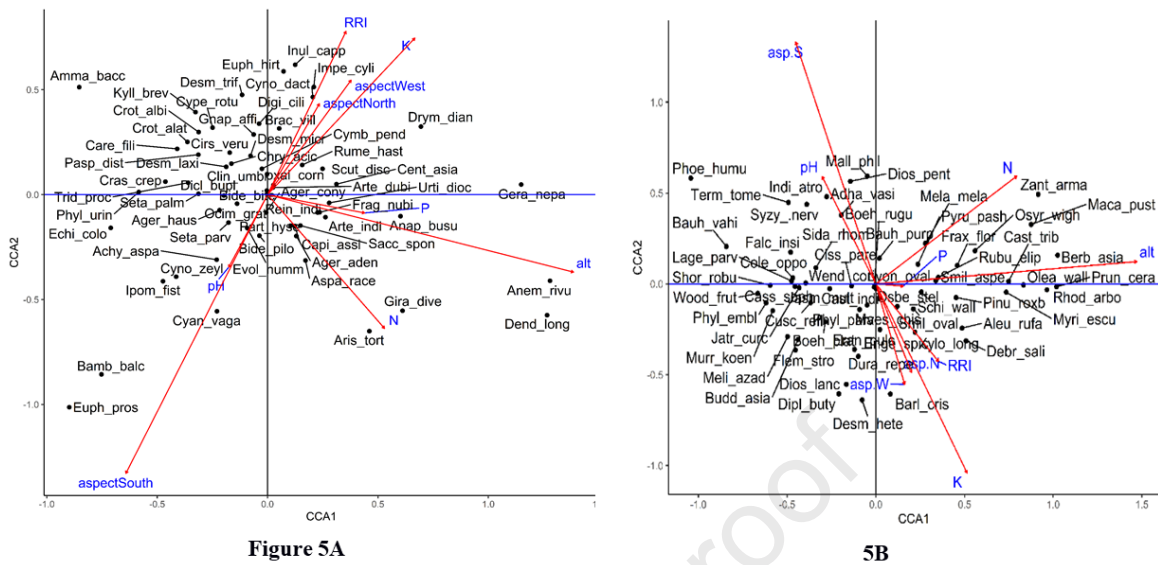


Figure 5A

5B

345

346

347 **Figure 5.** Canonical correspondence analyses (CCA) plot showing the effect of significant  
 348 environmental variables on the species richness of (A) Herbs & (B) combined form of  
 349 Climber, Ferns, Shrubs & Trees.

350

## 351 Discussion

352

### 353 *Species Richness and diversity indices*

354

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

361

362 We found the values of Shannon-Winner and Simpson indices higher in the forest of east  
 363 and west aspects. Diversity is the aggregate form of species richness and evenness and high  
 364 diversity indices indicate the characteristic of more diverse communities. If the species are  
 365 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

366 aspects. Generally, the north slope aspect of hills in Nepal is moist, and the south is drier,  
367 which results in higher species richness in the north slope aspect than the south in Manang  
368 valley (Panthi et al 2007). According to Aulander et al. (2003), south-facing slopes may  
369 receive six times the amount of solar radiation of north-facing slopes in the northern  
370 hemisphere. The growth rate of plants in the south-facing slope aspect may be less than the  
371 North aspect due to high solar radiation and less moisture. It is also supported by Maren et al.  
372 (2015) because water plays a deterministic role in the composition, structure, and density of  
373 plant communities (Kutiel and Lavee 1999). We found a high value of species richness and  
374 diversity indices at the east and west faces which may be due to the presence of sufficient both  
375 moisture and solar radiation. It confirms that water and solar radiation play a leading role in  
376 species richness and species distribution in different aspects of any landscape or all continents  
377 or countries. The species diversity is less in south aspect even there is high species richness,  
378 and the diversity is higher in east and west aspects due to their comparatively high evenness  
379 of species.

380

### 381 *Correlation among the variables*

382

383 According to the results of the correlation analyses, altitude shows a significant positive  
384 correlation with soil total nitrogen, phosphorus, and potassium. Qasba et al (2017) also  
385 reported that available N, P, and K showed a significantly positive relation with altitude.  
386 According to Anic et al (2010), the soil nutrients also showed a significant negative  
387 correlation with elevation in the Andes (1970 to 3330 m), Central Chile. Similarly, there is a  
388 negative correlation of pH with soil total nitrogen, available phosphorus ( $P_2O_5$ ), and  
389 extractable potassium ( $K_2O$ ) (Khadka et al 2016). The elevation and other climatic factors are  
390 responsible for controlling the carbon, nitrogen, and other minerals concentration (Shedayi et  
391 al 2016). The above- and belowground stocks of the total nitrogen increase significantly with  
392 elevation. The decrease in soil temperature ( $1^\circ C$ ) with the altitude showed a significant  
393 inverse relationship of nitrogen stocks (decrease rate of  $1 \text{ Mg}\cdot\text{ha}^{-1}$ ) in soil temperature  
394 (Vieira et al 2011). This increasing soil water content and lowering soil temperature help to  
395 decrease in soil N mineralization and nitrification rates which ultimately makes the high  
396 content of soil nitrogen due to the low rate of litter decomposition (Zhang et al 2012; Måren et

397 al 2015). The farmland was also found nearer to the forest in some parts of the study area  
398 where some portion of chemical fertilizer may reach the forest. These reasons support the  
399 positive relation of soil nitrogen, phosphorus, and potassium with the altitude of this research.

400

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

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

413

414 *Effect of environmental variables on species richness*

415

416 Altitude functions as the main decisive factor of ecosystem properties and processes in  
417 the mountains (He et al 2016) and altitude variations determines slope and aspects on land.  
418 The model result of regression showed that altitude had a significant effect, but the slope  
419 aspect has no significant influence on species richness. The studied altitudinal ranges varied  
420 between the four slope aspects. Each altitudinal range showed a significant unimodal richness  
421 pattern, but the peak of maximum richness differed between them. However, the unimodal  
422 nature of species against elevation was stronger in north and west than in other slope aspects.  
423 Many researchers (Rohde 1992; Bhattarai and Vetaas 2003; Bhattarai et al 2004; Grau et al  
424 2007) reported that altitude showed a unimodal effect on species richness. The elevation  
425 having maximum species richness depended on altitudinal range, plant taxa etc. (Grau et al  
426 2007). Generally, a right skewed altitudinal species richness pattern shows negative  
427 correlations and a left skewed pattern shows positive. When the gradient is shorter, a hump-

428 shaped pattern of longer elevational gradients would change to a monotonic decreasing  
429 (Nogue´s-Bravo et al 2008) or linearly increasing trend (Greatness and Vetaas 2002) or with  
430 increasing spatial scale of extent, the richness pattern changes from a monotonic to a hump-  
431 shaped pattern (Manish et al 2017). Most of the unimodal relationship between species richness  
432 and altitude are right skewed. The right skewed pattern in north and east aspect and left skewed  
433 pattern in west aspect suggest that no particular type of skewness present in the unimodal pattern in  
434 this study. There is evidence of a unimodal pattern in many regions at smaller spatial scales  
435 such as landscape and local gradients (Ooman and Shanker 2005). The hard- boundary effect  
436 (Colwell and Lees 2000), which results less area on top of the hills (Hua 2004) and  
437 overlapping of species of both lower and higher elevation range declares the unimodal nature  
438 is generally fit to describe the relationship between species richness and elevation well.

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

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

454 Litter fall regulates the accumulation of soil organic matter, the input of the nutrients,  
455 nutrient replacement, maintenance of biodiversity and other ecosystem functions in natural  
456 vegetation (Giewta 2020). Generally, dry litter decomposes more slowly in dry areas than in  
457 moist areas. At suitable moisture conditions, increasing temperature results in an exponential  
458 increase in decomposition rates (Salahab and Scholes 2010). The south aspect is drier than the

459 north slope aspect in Nepalese Himalaya. The moisture and suitable temperature of the  
460 northern slope aspect helps in continuous leaf litter decomposition, which results in high  
461 nutrients content on the soil. Plant available soil K, P and N are not limiting the productivity  
462 at south with respect to north-oriented slopes, because soil available water functions as the  
463 primarily limiting factor for plant productivity (Gong et al 2008). Generally, north-facing  
464 slopes are connected with higher biomass, coverage and height, and species diversity than  
465 south facing slopes (Yang et al 2020). We can say that altitude is the main factor affecting  
466 species richness. But, aspect indirectly affects species richness by creating a dry or moist  
467 environment and altering the rate of leaf litter decomposition.

468

469 *Environment Species Composition*

470

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

475 The survival of plant species generally depends on nutrient requirements. Different plant  
476 groups may show different responses to nutrient availability (Ellenberg 1988; Johnson and  
477 Leopold 1994). Species scores are expressive forms of the united effect of all projecting  
478 variables represented by the controlled axes. The first eigenvalue of CCA is equal to the  
479 maximized dispersion of species scores along the first CCA axis (Ter Braak 1986). In the  
480 CCA diagram, altitude, soil N, and P are correlated with the first axis and show positive  
481 relation to each other. The canonical correlation  $r$ - value shows the effects of variables on  
482 species distribution in the following order: altitude > N >P. The high score bearing  
483 species: like: *Zanthoxylum armatum*, *Macaranga pastulosa*, etc. are present in sites higher in  
484 N and also show positive responses to altitude. Similarly, species like *Fragaria nubicola* and  
485 *Centella asiatica*, which have medium scores, are found at the sites having a high content of  
486 phosphorus. This result shows that soil nitrogen is more strongly correlated to altitude than  
487 phosphorus. Soil moisture is the main cause of the nitrogen effect in soil (Gornish and Miller  
488 2015). The moisture generally increases with elevation in Nepal. The high nitrogen content  
489 makes the soil more acidic in combination with high moisture (Deutsch et al 2010; Ying et

490 al 2012). This may a cause of the decreasing trend of species richness toward higher  
491 elevations. Vegetation composition are affected by nutrient limitation, which is governed by  
492 low soil temperatures and influenced by soil moisture conditions in Himalayan region  
493 (Drollinger et al 2017). The plants require (on a mass basis) about ten times more N than P to  
494 promote balanced plant growth (Aerts and Chapin 2013).

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

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

519 The accurate estimation of soil nutrients helps to understand the interaction of  
520 biogeochemical cycles with the global climate (Shaw et al 2008). Changes in climatic

521 conditions (temperature and precipitation) along altitudinal gradients can influence nutrient  
522 content (Fisher et al 2013). The wild plants get nitrogen content from the soil nutrients via the  
523 nitrogen fixation and nitrification processes.

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

530

531

## 532 **Conclusions**

533

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

546

## 547 **Declaration of Competing Interest**

548

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



551

552 **Acknowledgment**

553

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

559

560

561

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

812

	South		East		North		West		Aggregate	
	Mode 1	R <sup>2</sup>	Mode 1	R <sup>2</sup>	Mode 1	R <sup>2</sup>	Model	R <sup>2</sup>	Mode 1	R <sup>2</sup>
Altitude	2	0.32	2	0.46	2	<b>0.64</b>	2	<b>0.59</b>	2	<b>0.67</b>
RRI	1	0.09	1	0.36	1	0.1	1	0.02	1	0.02
pH	1	0.04	1	< 0.001	1	0.08	1	< 0.001	1	< 0.001
Nitrogen	1	0.12	1	0.11	1	0.26	1	0.02	1	0.09
Phosphorus	1	0.05	1	0.14	2	<b>0.58</b>	1	0.005	2	0.1
Potassium	1	0.04	1	0.003	1	0.03	1	0.02	1	0.005

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**Declaration of interests**

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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