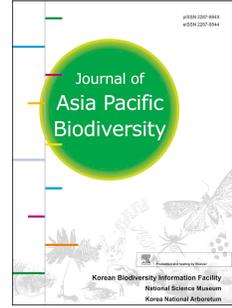


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Impacts of slope aspects on altitudinal species richness and species composition of Narapani-Masina landscape, Arghakhanchi, West Nepal

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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

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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 β -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, CO₂
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**

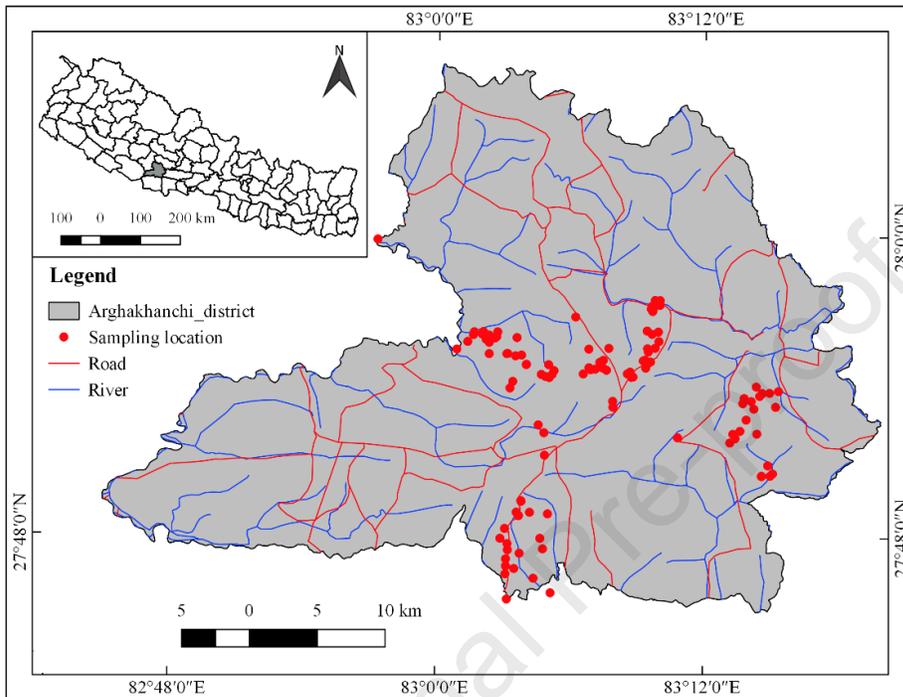
103

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



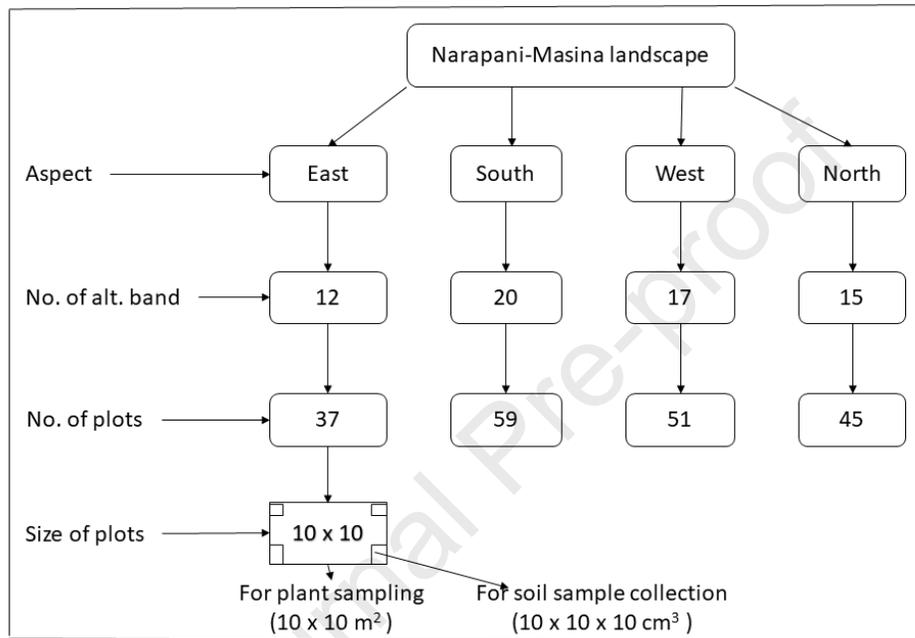
115
 116 **Figure 1.** Map of Arghakhanchi district with sampling spots.

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 14.9⁰C 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

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

176

177 Where Ω is an aspect, β is the slope, and Φ 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)	Altitudinal 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

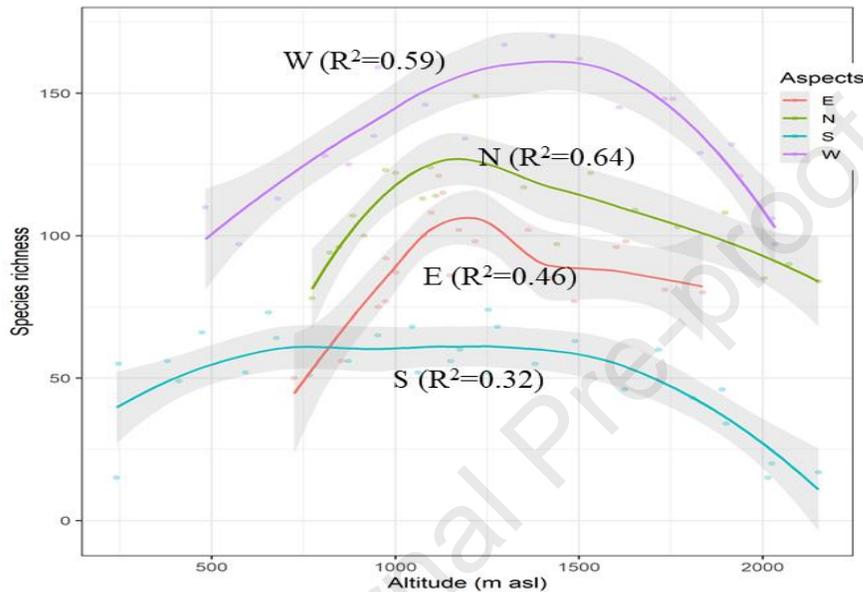
245 *Relation between environmental variables and species richness*

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.

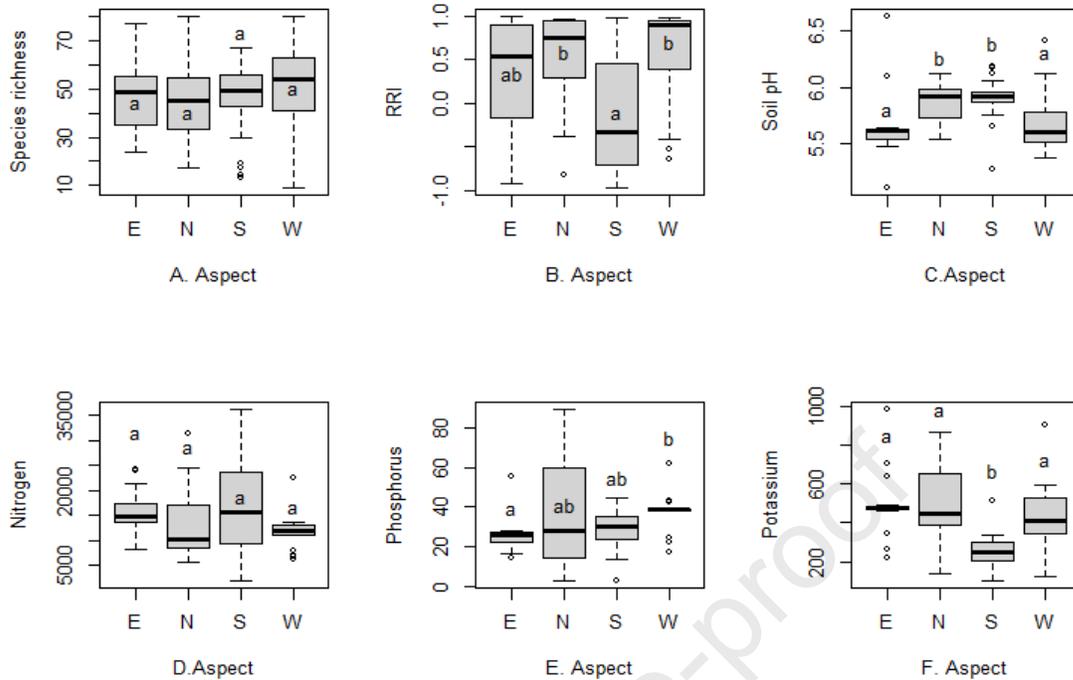


256

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	7.12	0.07	0.001
Aspect	3	1.52	0.05	0.003
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	1.37	0.02	0.046
K	1	2.52	0.03	0.001
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	0.35	2.09
Aspect South	-0.24	-0.96	2.97
Aspect West	0.14	0.28	2.39
Alt	0.99	-0.05	2.09
RRI	0.15	0.39	1.45
pH	-0.19	-0.30	1.47
N	0.51	-0.27	2.01
P	0.24	-0.08	1.47
K	0.39	0.56	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 lebbbeck* (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

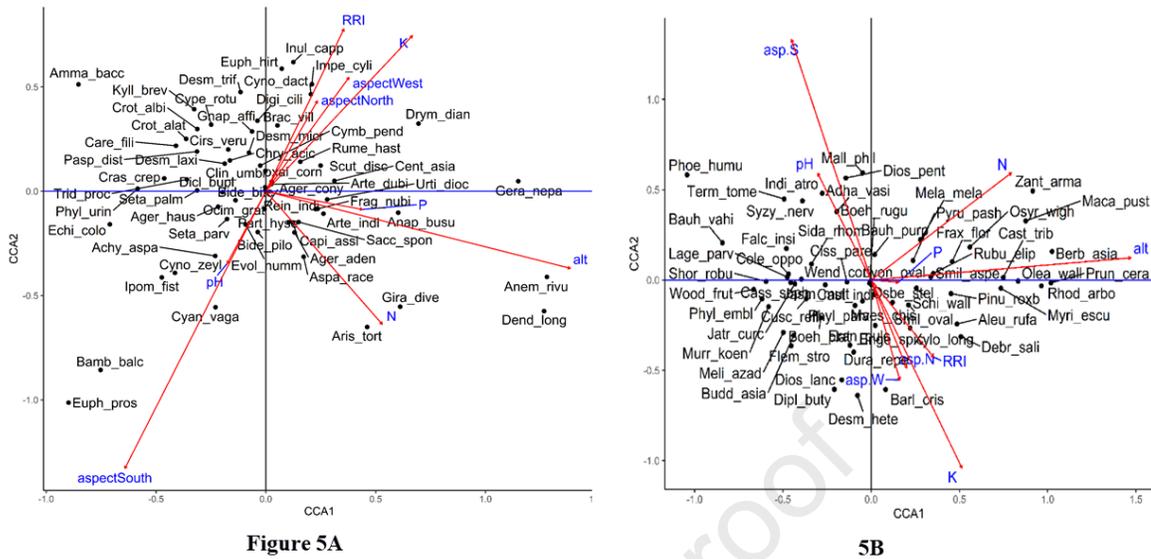


Figure 5A

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

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559

560

561

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809

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 ²	Mode 1	R ²	Mode 1	R ²	Model	R ²	Mode 1	R ²
Altitude	2	0.32	2	0.46	2	0.64	2	0.59	2	0.67
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	0.58	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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