Journal of Institute of Science and Technology, 27(2), 13-21 (2022)
ISSN: 2467-9062 (print), e-ISSN: 2467-9240
https://doi.org/10.3126/jist.v27i2.51214

Research Article

CHIR PINE (*Pinus roxburghii*) AND BLUE PINE (*P. wallichiana*) FORESTS IN WEST NEPAL: COMPARING ASSOCIATED PLANT SPECIES

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(***Received:*** *August 05, 2021;* ***Final Revision:*** *March 29, 2022;* ***Accepted:*** *October 15, 2022)*

ABSTRACT

Chir pine (*Pinus roxburghii*) and blue pine (*P. wallichiana*) are two dominant tree species in the mid-hill forests of Nepal that also contribute significantly to the country's total forest area. The associated vegetation pattern in pine forests is influenced by environmental and edaphic factors. The objective of this study was to determine the correlation between soil chemical properties and the composition of associated plant species in two pine forests in the Kailash Sacred Landscape, Nepal. We used the quadrat sampling method to collect vegetation and soil data. Pearson's correlation was used to determine the relationship between soil chemical properties, Generalized Linear Model (GLM) to assess the effect of soil properties on species richness, and Canonical Correlation Analysis (CCA) for species composition. Our analysis showed that soil pH was negatively correlated with soil chemical properties and species richness of associated species. There was a higher number of associated plant species in the blue pine forest than in chir pine forest. Associated plant species generally prefer high nitrogen and phosphorus content. Soil nutrients play an important role in determining the species richness and composition but may not be only factors affecting these patterns. Pine forests play an important role in the socio-economy from the national to community levels while the associated plant species have more significant functions in the livelihoods of local communities.

Keywords: Associated species, Himalaya, pine forests, species composition, soil chemical properties

INTRODUCTION

Understanding plant diversity patterns and the relationship between environmental factors is important in the field of plant ecology. Knowledge on plant diversity patterns serves as baseline evidence for management and conservation of biodiversity (Gutiérrez & Huth, 2012; Sutherland *et al.*, 2013). Important factors that determine the specific patterns of diversity and composition of plant species include temperature, precipitation, altitude, slope and site orientation (Gracia *et al.*, 2007), light, water, soil nutrients, microclimatic condition and nutrient cycling (Hart & Chen, 2006), seedling germination, recruitment and survival of plant communities (Parker & Muller, 1979; Barbier *et al.*, 2008). Generally, dominant plant species play an important role in shaping plant species association (Iason *et al.*, 2005), but exceptions have been reported where dominant tree species play a secondary role after environmental variables (Hu *et al.*, 2012). The understory vegetation in the forest is directly influenced by the upper layer of vegetation as they regulate light (Halpern *et al.*, 1995; Palik & Engstrom, 1999; Barbier *et al.*, 2008).

Forest types are categorized on the basis of their dominant species, but associated plant species are very important as they help in the functioning of forests as a whole ecosystem (Lamsal *et al.*, 2018). Many of these species are important as they provide timber, firewood, fodder, medicinal plants, and raw materials that provide livelihoods for millions of people worldwide (Oldekop *et al.*, 2020). In Nepal, the forestry sector contributes to the national economy through the productivity of both its dominant and associated species in the form of timber and non-timber forest products (Storrs & Storrs, 1998; Manandhar, 2002; Baral & Kurmi, 2006). Therefore, a comprehensive understanding of forest associated species is important to better determine the socioeconomic potential of existing forests (Pandey *et al.*, 2016).

Pine forests are important forest types in Nepal (Stainton, 1972; Miehe et al., 2015). Pine forests are generally dominated by two species, i.e., Chir pine (*Pinus roxburghii*) and blue pine (*P. wallichiana*) which collectively contribute to almost 12% of the total forest area in Nepal (DFRS, 2015). Chir pine is distributed from 500 to 2,700 m asl (meters above sea level) and blue pine from 1,400-4,000 m asl (Press *et al.*, 2000; Shrestha *et al.*, 2018; Rajbhandari *et al.*, 2020). Pine forests provide provisioning services that are timber, fuel wood, medicinal and aromatic plants, and livestock bedding. In addition, they provide regulatory services for maintaining water quality, carbon sequestration and soil erosion control (Price *et al.*, 2011). Pine forests are vulnerable to human disturbances and are at high risk of growth decline and die-back in the Himalayan region (Sigdel *et al.*, 2018). In order to increase our knowledge base on highly exploited chir and blue pine forests of western Nepal, we observed how environmental factors affect the diversity and composition of associated plant species in these forests. Specifically, we asked: How do the species richness and composition pattern of associated plant species differ, and are they affected by different soil nutrients in two types of pine forests?

MATERIALS AND METHODS

Study area

The study area (Fig. 1) of the Chir pine forest was in Kirmade Sinnedi Community Forest in Malikaarjun Rural Municipality, Darchula District, Nepal. This community forest covers an area of 50.76 hectares with elevation ranging from 1,808 m asl. to 1,958 m asl. The associated tree species were *Lyonia ovalifolia*, *Rhododendron arboreum*, *Symplocos paniculata*, and *Viburnum erubescens*. The blue pine forest was in Kailash Kachaharikot Women's Community Forest in Jayaprithvi Municipality of Bajhang District. This forest is 20 hectares in area with an elevation ranging from 1,800 m asl to 2,000 m asl. The major associated tree species were *Quercus lanata*, *Rhododendron arboreum* and *Myrica esculenta*.

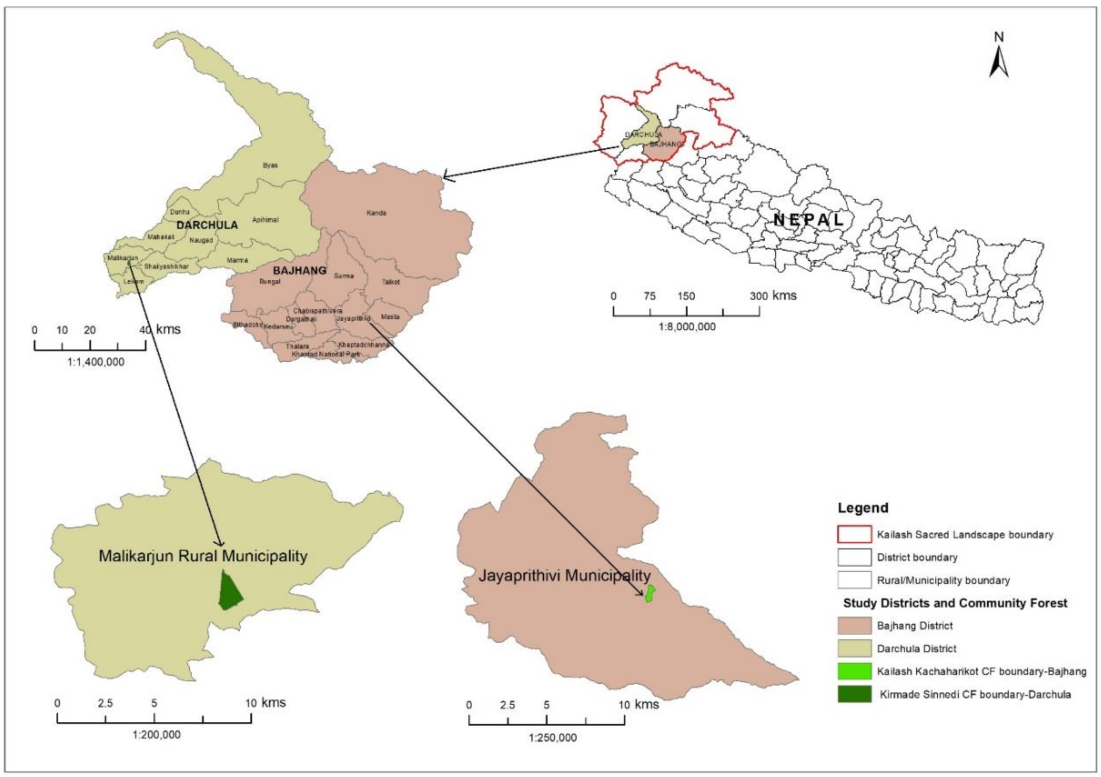

Figure 1. Study area in western Nepal

Both the forests were located within the Kailash Sacred Landscape in Nepal (KSL-Nepal). The KSL-Nepal covers an area of 13,289 square kilometers (42% of the total area spread across Nepal, China, and India) and includes four mountain districts *viz.* Baitadi, Bajhang, Darchula, and Humla. In the KSL-Nepal, only 8.6% of the area is available for cultivation (CDB, 2010). Hence, people in the KSL-Nepal are highly dependent on natural resources, particularly forest resources that are often under high pressure of exploitation (Kunwar *et al.*, 2013; Kunwar *et al.*, 2019). Annual temperature in the study area averages a high of 18.6°C and low of 7.7°C, while the annual rainfall averages 2129 mm. Vegetation is characterized by subtropical broadleaved, montane broadleaved and subalpine broadleaved forest types (CDB, 2010).

Data collection

Field work was conducted from May to June 2016. We used 20 m × 25 m quadrat to include maximum number of species (DFRS, 2000). We collected data in four quadrats in blue pine and 10 quadrats in chir pine forests. In each quadrat, we collected necessary physiographic information (altitude, latitude, longitude, slope and aspect). Altitude was recorded by using altimeter (Sunto), longitude and latitude by a portable GPS receiver (eTrex Vista, Garmin), and slope and aspect by compass (Sunto). The presence of shrubs and herbs was recorded. All recorded plant species were collected, placed between newspaper sheets, pressed by a herbarium press and dried in the sunlight (Forman & Bridson, 1989). These specimens were later identified and verified with the help of available literature (Sharma & Kachroo, 1983; Polunin & Stainton, 2000) and by comparing with herbarium specimens deposited at the Tribhuvan University Central Herbarium (TUCH) and the National Herbarium and Plant Laboratory (KATH). We followed Press *et al*. (2000) for species nomenclature.

Soil sampling and analysis

Soil samples were collected from the top 20 cm of surface soil from a 20 cm×20 cm grid that was set up at the center of each quadrat. The collected soil samples were stored in zip-lock plastic bags (Carter & Gregorich, 2007) and transported to Kathmandu where they were air dried and sieved through a 2 mm mesh. Soil chemical analysis was done in Kathmandu University's Soil and Water Analysis Laboratory. Soil pH was measured by the Probe method, soil organic carbon (SOC) by dry combustion method (Walkley & Black, 1934), total nitrogen (N) by Kjeldhal method (Jacobs, 1951), available phosphorus (P) by modified Olsen's bicarbonate method (Olsens & Somers, 1982), and available potassium (K) by Ammonium Acetate followed by Atomic Absorption spectrophotometer (AAS) method (Walkley & Black, 1934).

Statistical analysis

We used Pearson's correlation test to determine relationships between different types of soil nutrients as the data was uniformly distributed. The correlation tests were performed using STATISTICA (StaSoft Inc, 2015).

To determine the effect of environmental factors (pH, SOC, N, P, K) and forest types on species richness of associated understorey plant species in the two forest types, we used the generalized linear model (GLM) in R 4.1.2 (R Development Core Team, 2021). In the model, species richness was used as response variable and environmental factors as predictors. Species richness was square-root transformed to normalize the data. In GLM, the R-squared (R^2) values for each significant variable was obtained by dividing the sum of squares of each environmental factor by total sum of squares of all environmental factors.

As plant species were collected in present absent categories (Šmilauer & Lepš, 2014) multivariate tests of species composition of different plant species were conducted using canonical correspondence analysis (CCA) with Canoco 5.04 (ter Barrak & Smilauer, 2012). Significance of the effect of environmental factors (pH, SOC, N, P, K) and forest types on associated species composition was tested by performing Monte Carlo permutation test (n=4999). Rarely occurring species were down-weighted in all the multivariate analyses to reduce their effect on the results.

RESULTS

Soil pH was negatively correlated with soil organic carbon, total nitrogen, available phosphorus, and available potassium. On the other hand, soil organic carbon was positively correlated with total nitrogen, available phosphorus, and available potassium. Both total nitrogen and available phosphorus were positively correlated with available potassium (Table 1). Species richness of the associated plants decreased significantly with increasing soil pH, while species richness increased with increasing soil potassium (Table 2).

The composition of associated plant species was variable in the two pine forests as illustrated in Figure 2. Furthermore, species composition was also significantly influenced by soil pH, soil organic carbon, nitrogen, phosphorus and potassium (Table 3). Most of the species preferred sites with high soil organic carbon, nitrogen, potassium, and phosphorus contents. These species included *Myrsine africans*, *Anaphalis triplinervis*, *Viola serpens*, *Rubus ellipticus*, *Daphne papyracea*, *Oplismenu scompositus*, *Spiraea bella*, *Potentilla sundaica*, *Erigeron karvinskianus*, *Berberis asiatica*, *Taraxacum parvulumrum* and *Gaultheria nummularioides*. Only a few species preferred sites with high pH and included *Flemingia strobilifera*, *Ageratina adenophora* and *Gonostegia hirta* (Fig. 2).

Table 1. Correlation between different soil chemical properties (N=280). Bold figures are significant at p < 0.050

	pH	SOC	Total N	P
pH	1.000000			
SOC	**-0.483456**	1.000000		
Total N	**-0.312008**	**0.736686**	1.000000	
P	**-0.162220**	**0.415354**	-0.016934	1.000000
K	**-0.305529**	**0.638400**	**0.344865**	**0.640736**

Table 2. Effects of environmental factors on species richness of associated plant species

	df	Deviance	Resid. Df	Resid. Dev	p-value	R^2
pH	1	31.21	66	89.99	<0.001	0.258
Nitrogen	1	2.49	65	87.49	0.114	-

Carbon	1	1.51	64	85.98	0.219	-
Phosphorus	1	1.98	63	84.00	0.160	-
Potassium	1	10.70	62	73.30	0.001	0.088
Forest types	1	19.22	61	54.08	<0.001	0.159

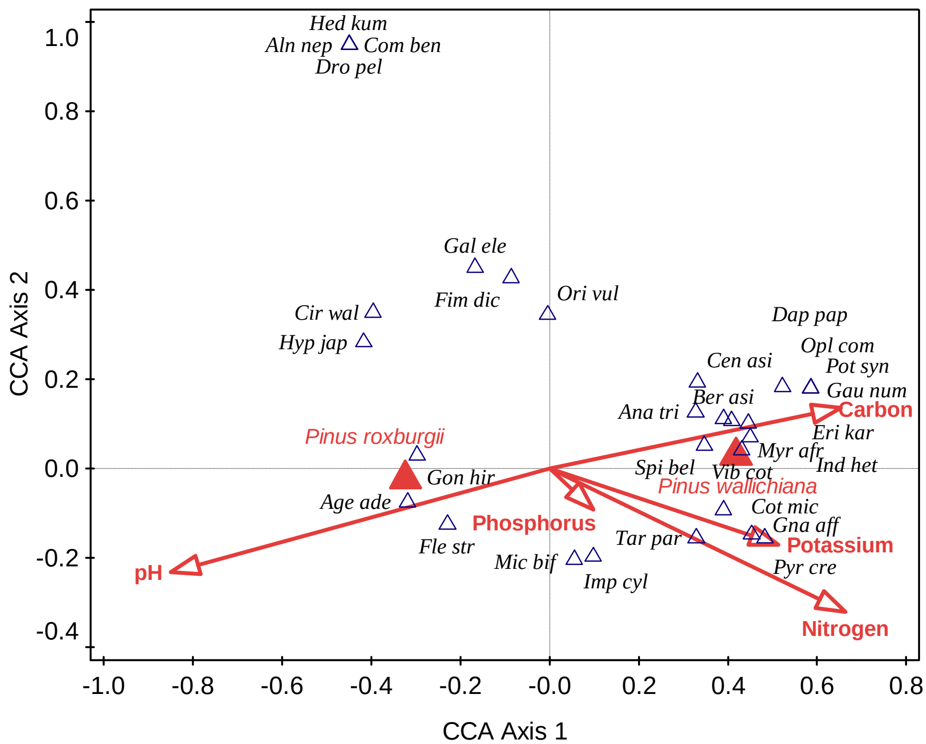

Figure 2. Association between species composition of associated plants and soil chemical properties of two pine forests

Table 3. Relationship between environmental factors and species composition of associated plants of two pine forests

	Df	p-value	R^2
pH	1	0.0002	0.0010
Nitrogen	1	0.0004	0.0004
Carbon	1	0.0002	0.0008
Phosphorus	1	0.0004	0.0004
Potassium	1	0.0002	0.0012
Forest types	1	0.0002	0.0013

DISCUSSION

Similar to our findings, negative correlation between soil pH and soil chemical properties was observed in other forest in southern China (Wang *et al.*, 2010). Soil pH affects the decomposition process, which in turn affects the formation of soil nutrients. The activities of microbial communities (decomposers) are lower in soils with high pH value (Alexander, 1991; Wang *et al.*, 2010). On the other hand, since carbon, nitrogen, phosphorus and potassium are attributes of the humus content of soil, there is a positive correlation between these variables. Similar results have been resulted from other forests in the Himalaya (Aber & Melilo, 2001; Gupta & Shrama, 2008; Wang *et al.*, 2010; Gairola *et al.*, 2012). A strong positive correlation between these nutrients was also reported by Pausas (1994) in the *P. sylvestris* forest in Pyrenes.

Vascular plant species richness is generally positively correlated with pH in a monotonic curve pattern, (Dupré *et al.*, 2002; Pärtel, 2002; Schuster & Diekmann, 2005; Zinko *et al.*, 2006; Chytrý *et al.*, 2007), but our finding shows a negative correlation between species richness and pH. Such results have also been reported by other studies (Palmer *et al*., 2003; Van Couwenberghe *et al*., 2011). Since both forests in the present study are monodominant of pine, the soil condition is acidic. Negative correlation between pH and associated species in this study is most probably due to deficiency of soil nutrients for the growth of ground vegetation as the study sites are relatively dry and are in slopes. Potassium is an important nutrient that enhances growth of plants (Fenn *et al*., 1998; Venterink, 2011; Wright *et al*., 2011). Potassium abundance helps the growth of plant species through the number of forms of these nutrients in the soil (Venterink, 2011). In addition, different soil nutrients provide suitable environment for the growth of associated plant species resulting in increase in richness of associated plant species in studied pine forests. While comparing between two pine forests, the species richness of associated plant species is higher in blue pine forest than in chir pine forest. It is due to a lower pH value in blue pine forest than in chir pine forest. Chir pine is invasive in nature, encroaches broadleaved plant species to form monoculture or pure stand (Bhandari, 2003; Siddiqui *et al*., 2009). On the other hand, blue pine has tendency to share common habitat with other broadleaved plant species (Bhandari, 2003).

Since the nature and soil properties of blue pine and chir pine forests are different, variation is found in species composition of both forests. Species composition is regulated by different environmental factors found by different studies in the world (Parker & Muller, 1979; Halpern *et al*., 1995; Gracia *et al*., 2007; Barbier *et al*., 2008). The understorey plant composition is mainly regulated by multiple factors rather than one single factor. These factors are overstorey canopy, light availability, substrates, soil nutrients and pH (Légaré *et al*., 2002; Hart & Chen, 2006).

CONCLUSIONS

Soil chemical properties play an important role in affecting the species richness of associated plants in pine forests. However, other factors, especially human-induced disturbance factors, play a significant role in their composition and structure. Both chir and blue pine forests have socio-economic importance in western Nepal by directly providing services as socio-economically valuable tree species, but their associated plant species also provide valuable services, especially to the local communities. The present study can act as baseline information for formulation of management plan intended for pine and associated plant species conservation**.**

ACKNOWLEDGEMENTS

This work was supported by Kailash Sacred Landscape Conservation and Development Initiative (KSLCDI) Nepal. MBR was supported by the Czech Science Foundation (project no. 17-10280S) and partly by institutional support RVO 67985939. BT was supported by National Sustainability Program I (NPU I) (grant number LO1415) of MSMT. We are thankful to Binaya Pasakhala, Santosh Thapa, Mohan Pandey, Neha Bist, and community forest user group for their help during data collection and Sunil Thapa for preparing study area map. We also acknowledge District forest office Bajhang and Darchula for their support.

AUTHOR CONTRIBUTIONS

CKS, RPC, and JG conceived and designed the study. CKS, JG, and PB collected the field data. MBR and BT carried out statistical analysis. CKS, MRB, BT, and PB wrote the manuscript. All authors approve the manuscript for publication.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Supplementary Table 1. List of different plant species recorded in two Pine forests in west Nepal

Latin name	Abbreviations	Family
Ageratina adenophora (Sprengel) R. M. King & H. Robinson	Age ade	Asteraceae
Alnus nepalensis D. Don	Aln nep	Betulaceae
Anaphalis busua (Buch.-Ham. ex D. Don) DC.	Ana bus	Asteraceae
Anaphalis triplinervis (Sims) C. B. Clarke	Ana tri	Asteraceae
Aster Sp.	Ast sp	Asteraceae
Berberis asiatica Roxb. ex DC.	Ber asi	Berberidaceae
Carex filicina Nees	Car fil	Cyperaceae
Centella asiatica (L.) Urb.	Cen asi	Apiaceae
Cirsium wallichii DC.	Cir wal	Asteraceae
Clematis Sp.	Cle sp	Ranunculaceae
Commelina benghalensis L.	Com ben	Commmelinaceae
Cotoneaster microphyllus Wall. ex Lindl.	Cot mic	Rosaceae
Daphne papyracea Wall. ex Steud.	Dap pap	Thymelaeaceae
Drosera peltata Sm.	Dropel	Droseraceae
Eragrostis Sp.	Era sp	Poaceae
Erigeron karvinskianus DC.	Eri kar	Asteraceae
Chelanthus Sp.	Che sp	Pteridaceae
*Fimbristylis dichotom*a (L.) Vahl	Fim dic	Cyperaceae
Flemingia strobilifera (L.) W. T. Aiton	Fle str	Fabaceae
Fragaria indica Andrews	Fra ind	Rosaceae
Galium elegans Wall. ex Roxb.	Gal ele	Rubiaceae
Gaultheria nummularioides D. Don	Gau num	Ericaceae
Gnaphalium affine D. Don	Gna aff	Asteraceae
Gonostegia hirta (Blume) Miq.	Gon hir	Urticaceae
Hedysarum kumaonense Benth. ex Baker	Hed kum	Fabaceae
Hypericum japonicum Thunb. ex Murray	Hyp jap	Clusiaceae
Hypoxis aurea Lour.	Hyp aur	Hypoxidaceae
Imperata cylindrica (L.) P. Beauv.	Imp cyl	Poaceae
Indigofera heterantha Wall. ex Brandis	Ind het	Fabaceae
Inula cappa (Buch.-Ham. ex D. Don) DC.	Inu cap	Asteraceae
Lyonia ovalifolia (Wall.) Drude	Lyo ova	Ericaceae
Micromeria biflora (Buch.-Ham. ex D. Don) Benth.	Mic bif	Lamiaceae
Myrsine africana L.	Myr afr	Myrsinaceae
Ophioglossum Sp.	Oph sp	Ophioglossaceae
Oplismenus compositus (L.) P. Beauv.	Opl com	Poaceae

Origanum vulgare L.	Ori vul	Lamiaceae
Oxalis corniculata L.	Oxacor	Oxalidaceae
Poa Sp.	Poasp	Poaceae
Potentilla sundaica (Blume) Kuntze	Pot syn	Rosaceae
Prinsepia utilis Royle	Priuti	Rosaceae
Pyracantha crenulata (D. Don) M. Roem.	Pyrcre	Rosaceae
Pyrus pashia Buch.-Ham. ex D. Don	Pyr pas	Rosaceae
Reinwardtia indica Dumort.	Rei ind	Linaceae
Rhododendron arboreum Sm.	Rho arb	Ericaceae
Rosa Sp.	Ros sp	Rosaceae
Rubus ellipticus Sm.	Rub ell	Rosaceae
Rubus paniculatus Sm.	Rub pan	Rosaceae
Smilax aspera L.	Smi asp	Smilacaceae
Spiraea bella Sims	Spi bel	Rosaceae
Symplocos pyrifolia Wall. ex G. Don	Sym pie	Symplocaceae
Taraxacum parvulum DC.	Tar par	Asteraceae
Viburnum cotinifolium D. Don	Vib cot	Sambucaceae
Viola serpens Wall. ex Ging.	Vio ser	Violaceae