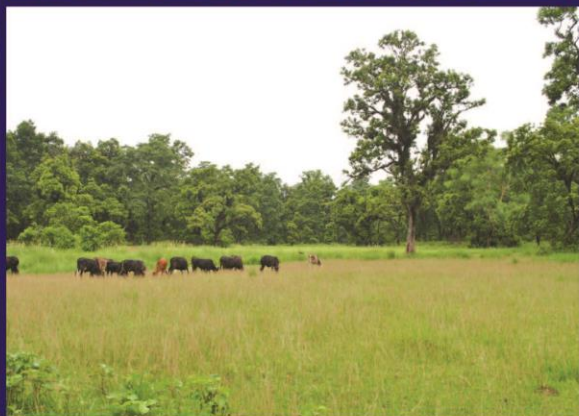


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Research

Fuelwood resources and their use pattern by Bhotia community in Niti valley, Western Himalaya

Monideepa Mitra, Amit Kumar, B.S. Adhikari* and G.S. Rawat

Wildlife Institute of India, P.O. Box # 18, Dehradun 248001, Uttarakhand, India

Abstract

The local communities of the Himalaya have been using fuelwood as one of the major sources of energy since millennia. Their dependency on these resources as primary source of fuelwood has resulted in unsustainable pressures on the forests. The present communication aims to access the fuelwood resource and consumption pattern of Bhotia, an ethnic community in a cold arid and buffer zone of Nanda Devi Biosphere Reserve, i.e. Niti valley in the Western Himalaya. Within the Niti valley, 87 households in six villages were surveyed using open and close-ended structured questionnaire. Use Index (I%) of each fuelwood species was calculated to evaluate the key species used by the inhabitants and their preferences. The study revealed use of 10 species for fuelwood (five species each of tree and shrub) by the Bhotias. *Pinus wallichiana* (I = 96.6%) followed by *Cedrus deodara* (93.1%) were the preferred tree species for fuelwood, while among shrubs, *Juniperus indica* and *J. communis* were extensively used. Fuelwood consumption in the Niti valley was much lower (1.6 ± 0.2 kg household⁻¹ day⁻¹) than other villages in the Greater Himalaya (4.9 ± 0.4 kg household⁻¹ day⁻¹). The seasonally employed which formed 23% of the surveyed households, collected almost twice as much fuelwood than the employed households (9.2 ± 0.4 and 5.2 ± 0.4 quintals season⁻¹, respectively). As the area is characterized by sparse vegetation cover, low primary productivity and short growing season, and is thus highly susceptible to irreversible changes of natural habitats. The study suggests that providing alternate and non-conventional energy sources such as solar cookers and fuel efficient portable ovens to the inhabitants at subsidized rates could reduce the pressure on nearby forests.

Key-words: Bhotia community, Nanda Devi National Park, Trans-Himalaya, Use Index.

Introduction

Leach (1992) explained that household fuel choice is mostly determined by income and passes through a linear 3-stage switching process that initially involves solid biomass fuels, but with increased economic prosperity finally leads to liquefied petroleum gas (LPG) and electricity, usually via a transition phase involving kerosene, coal and charcoal. Household behaviour can be explained in terms of wealth and substitution effects of increases in household income (Bardhan *et al.* 2001). Current empirical evidence suggests a more complicated process at work than the simple linearized version of the energy ladder model. First, the phenomenon of ‘fuel stacking’ suggests that richer rural households opt for a mix of modern and traditional fuel types to meet larger energy requirements (UNDP/ESMAP 2003). Similar evidences are recorded from all across the globe (Heltberg 2005; Masera *et al.* 2000).

According to the 55th Round National Sample Survey (NSSO 2001), from a country wide sample of 71,385 rural households, 75% of households used firewood and wood chips as a primary energy source for cooking and lighting, and close to 90% of households used one or the other form of solid biomass-based fuels for meeting their domestic energy

needs. Degradation of forests in the Indian mid-Himalaya is reported to be primarily due to collection of fuelwood and fodder by residents of neighboring villages (Baland *et al.* 2006-07). Several parameters, *viz.* socio-economic, demographic, geographic and dwelling characteristics of the inhabitants determine villagers’ energy requirements (Pachauri 2004; Rao and Reddy 2007). According to Rai and Chakrabarty (1996), the per capita fuelwood consumption in India ranged between 423–1320 kg. In various parts of the Himalaya, the fuelwood consumption is reported to be between 4.55–23.88 kg person⁻¹ day⁻¹ (Khuman *et al.* 2011; Dhanai *et al.* 2014; Dhanai *et al.* 2015).

In the Indian Himalayan region, a major part of the total household energy demand is met through non-commercial energy (98.6%), while commercial components such as domestic LPG and kerosene form only 1.4% of the total energy demand (Kumar 2005). By all accounts, the energy transition from biomass to clean fuel types (kerosene, electricity and LPG) has been extremely slow in rural India (Pachauri and Jiang 2008; TERI 2008). The village population in these areas are totally dependent on wood resources as a non-commercial energy source (Bhatt and Sachan 2004).

*Corresponding author. e-mail - adhikaribs@wii.gov.in

Forest resources form an integral part of the social and cultural lives of the Bhotia community inhabiting Niti valley in the Indian Trans-Himalaya. They are highly dependent on the surrounding forests for their fuelwood and fodder requirements (Kumar *et al.* 2015a). In the Trans-Himalayan regions, anthropogenic pressure along with climatic constraints like low precipitation, low temperature and short growing season adversely affects the growth and regeneration of plants (Murti 2001). The present study was undertaken to understand the fuelwood diversity and consumption pattern of the Bhotia community in the Trans-Himalayan region of Nanda Devi Biosphere Reserve to provide a basis for developing conservation strategies in the region where there is scarcity of forest resources.

Materials and Methods

STUDY AREA

Nanda Devi Biosphere Reserve (hereafter referred as NDBR), located in the northern part of the Western Himalaya, has two core zones *viz.*, Nanda Devi National Park (NDNP, 630 km²) and Valley of Flowers National Park (VoFNP, 87.5 km²), which were inscribed a 'World Heritage Site' in the year 1988 by UNESCO (<http://whc.unesco.org/en/list/335/>). The study was conducted in Niti valley, a buffer zone of NDBR (Figure 1). The valley, with elevations ranging from 3500 m amsl to 5000

m amsl is spread over an area of *ca.* 727.7 km². The picturesque valley falls under the Trans-Himalayan region in Uttarakhand state of India. The valley is also known as Upper Dhauri valley and is named after the river Dhauri Ganga that forms one of the major catchments of Alaknanda river (a sub-catchment of the river Ganga). It has three sub-watersheds namely, Amrit Ganga, Satyagad and Ganesh Ganga, which are used for transhumant pastoralism (Rawat 2005; Mitra *et al.* 2013).

The valley has seven migratory villages namely, Kailashpur, Mahergaon, Gurgutti, Farkia, Bampa, Gamshali and Niti, which are mainly inhabited seasonally by the 'Bhotia', an ethnic community of Indo-Mongoloid origin. These migratory villagers have two settlements - winter settlement in the lower elevation of Chamoli district, and summer settlement in the higher elevation in Niti valley. The total number of households in the valley was 292 with a population of 864 individuals (47.5% males and 52.5% females) and family size ranges between 4-5 persons (Census India 2011).

This area is situated in the rain-shadow zone of NDBR and dryness increases towards the upper reaches of the valley, which remain snow bound for more than six months in a year. Growing season is very short and generally lasts from June to August. The region receives low amount of precipitation and remains dry and dusty above 3200 mamsl. The vegetation of

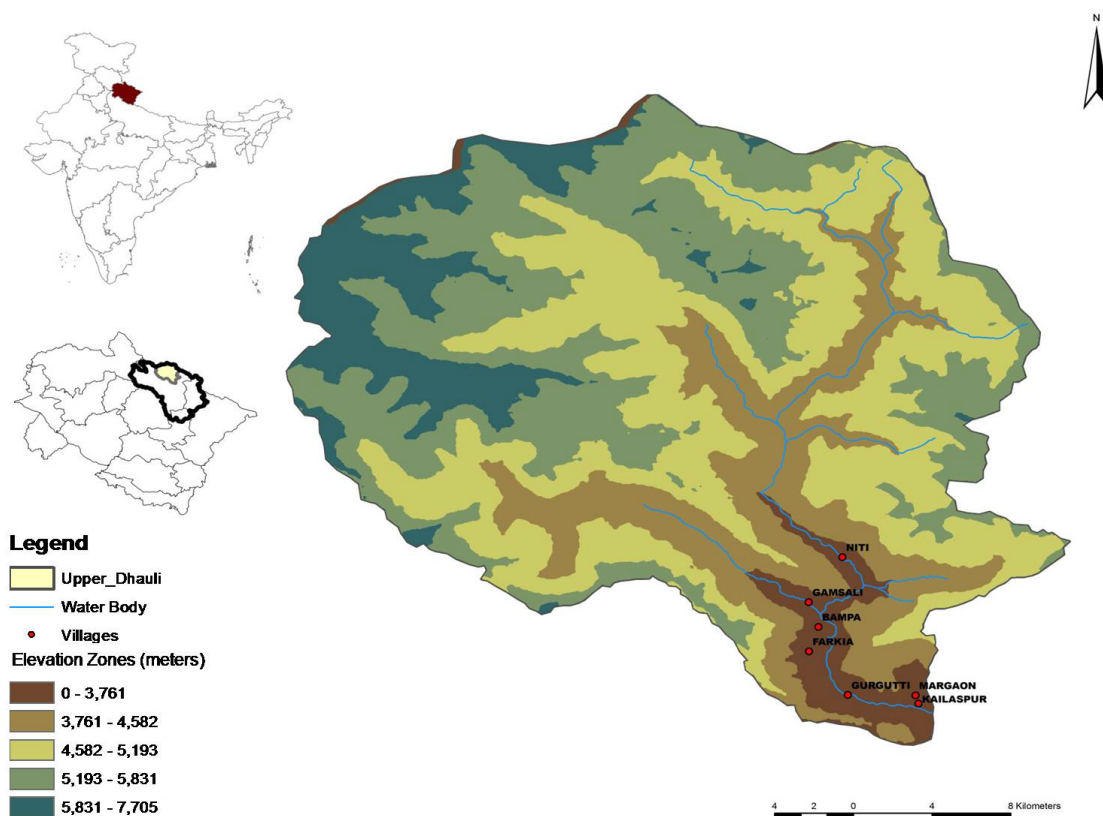


Figure 1. Map showing location of villages in Niti valley, Western Himalaya.

the study area is broadly divisible into the following classes: (i) dry temperate forests, (ii) sub-alpine forests, (iii) riverine scrub, (iv) alpine dry scrub, and (v) alpine mixed herbaceous formations. The alpine dry scrub and alpine mixed herbaceous formations cover >70% of the study area (Kumar et al. 2015b, 2016).

DATA COLLECTION AND ANALYSIS

In this study, 87 households representing 30% of total household were surveyed from six villages in Niti valley- Niti, Gamshali, Bampa, Farkia, Mahergaon and Kailashpur, which were located between 3200 to 3600m amsl (Figure 2). Information was gathered through semi-structured questionnaire surveys with open and close-ended questions following Bernard (2006) during June to October 2012. Use of major fuelwood species and their preference by the local inhabitants along with their relationship between employment and fuelwood consumption was studied. To evaluate the major fuelwood species used by the villagers and their preference, Use Index (I%) of each species was calculated following Lance et al. (1994):

$$I\% = n/N \times 100$$

Where, I% represents percentage index of use, n represents the number of people citing the use of the tree species, and N represents the total number of people surveyed. If the value of I% is between 60 and 100%, the species is widely used; if I% is between 30 and 60%, the species is moderately used; if I% is less than 30%, the species is not used or minimally used.

Results

SPECIES USED AND THEIR PREFERENCE

A detailed phytosociological investigation was conducted in the study area for the assessment and availability of fuelwood species. A total of 495 species of vascular plants (angiosperms, gymnosperms and pteridophytes) belonging to 267 genera and

73 families have been recorded from the study area (Kumar et al. 2016). *Pinus wallichiana* (806 trees ha⁻¹) followed by *Cedrus deodara* (389 trees ha⁻¹) were dominant tree species in the valley and forms the forest.

Among the surveyed villages, a total of 10 species (five species each of tree and shrub) belonging to eight families were primarily used as fuelwood by the Bhotia community. The use of fuelwood species depended on their quality, availability and accessibility in the area. Inhabitants, mostly women, collected fuelwood between 3000-4000 m, which is mainly used for cooking, boiling water and lighting, as well as space heating. The use value ranged from 2.3 to 96.6%, which was highest for *Pinus wallichiana* (96.6%) followed by *Cedrus deodara* (93.10%) indicates their great acceptability as fuelwood and high anthropogenic pressure on the species. The remaining species such as *Viburnum cotinifolium*, *Rosa sericea*, *Fraxinus xanthoxyloides*, *Prunus cornuta* and *Picea smithiana* showed <20% use value, reflected their low availability or low preference. Use of species such as, *Fraxinus xanthoxyloides* and *Picea smithiana* were reported from Kailashpur and Mahargaon villages. *Fraxinus* was distributed along steep riverside slopes of Dhauli Ganga while *Picea smithiana* was the co-dominant species in *Pinus-Cedrus* forest. Species of *Rosa* and *Viburnum* were also collected along roadside and nearby forested areas, while *Betula* was collected in three villages namely, Farkia, Bampa and Niti by the locals. Among the shrub species, *Rhododendron campanulatum*, *Juniperus indica* and *J. communis* were used extensively by the villagers of Gamshali and Niti. The major fuelwood species used and their preference by Bhotias in the valley is shown in Table 1.

EMPLOYMENT, LPG AND FUELWOOD EXTRACTION

The employment status of villagers and the availability of domestic LPG (each cylinder of 14.8 kg) in the study area play important roles in determining their dependency on forests for fuelwood. The employed strata have an option of buying domestic LPG, thus decreasing their requirement for fuelwood.

Table 1. Major fuelwood species and their use in Niti valley, NDBR.

Species	Niti	Gamshali	Bampa	Farkia	Mahergaon	Kailashpur
<i>Pinus wallichiana</i> ^a	√	√	√	√	√	√
<i>Cedrus deodara</i> ^a	√	√	√	√	√	√
<i>Picea smithiana</i> ^a	×	×	×	×	√	√
<i>Betula utilis</i> ^a	√	×	√	√	×	×
<i>Fraxinus xanthoxyloides</i> ^a	×	×	×	×	√	√
<i>Prunus cornuta</i> ^a	×	×	×	×	√	√
<i>Rhododendron campanulatum</i> ^b	√	√	×	×	×	×
<i>Viburnum cotinifolium</i> ^b	×	×	×	×	√	√
<i>Juniperus</i> spp. ^{b*}	√	√	√	√	√	√
<i>Rosa sericea</i> ^b	√	√	√	×	×	√

Symbols used: a = tree, b = shrub; √ = species is commonly used; × = species not used or not found in the area; * villagers refer both species of *Juniperus* by the same name, *Bitaru*.

Table 2. Fuelwood species with their Use Value (UV) based on quality, characteristics and availability in Niti valley, NDBR.

Species	Family	Density (individuals ha ⁻¹)	Availability*	Use Value (%)
<i>Pinus wallichiana</i> ^a	Pinaceae	805.7	Very high	96.6
<i>Cedrus deodara</i> ^a	Pinaceae	388.6	High	93.1
<i>Picea smithiana</i> ^a	Pinaceae	108.57	Low	2.30
<i>Betula utilis</i> ^a	Betulaceae	95.7	Very low	26.4
<i>Fraxinus xanthoxyloides</i> ^a	Oleaceae	Sparse distribution	Very low	5.8
<i>Prunus cornuta</i> ^a	Rosaceae	Sparse distribution	-	3.45
<i>Rosa sericea</i> ^b	Rosaceae	191.4	Low	13.8
<i>Juniperus</i> spp. ^b	Cupressaceae	281.2	Medium	47.1
<i>Rhododendron campanulatum</i> ^b	Ericaceae	134.2	Low	20.7
<i>Viburnum cotinifolium</i> ^b	Caprifoliaceae	Sparse distribution	Very low	14.9

*Availability (individuals ha⁻¹): Very low = <100, Low = 100-200, Medium = 200-300, High = 300-400, and Very high = >400. Species: a = tree, b = shrub.

The category of seasonally employed people consists of daily labourers, porters and people rearing domestic livestock for commercial purposes. 77% of households, which has at least one member employed is considered as employed, of which 44% use domestic LPG as well as fuelwood, while 56% are totally dependent on fuelwood for energy. The maximum fuelwood was extracted by the seasonally employed category i.e. 23% of the total sampled population. The fuelwood collected by the employed and unemployed category was 5.2±0.4 and 9.2±0.44 quintals season⁻¹, respectively. Likewise, in villages of Kailashpur and Mahergaon, where the percentage of employed people exceeds the percentage of unemployed, the fuelwood extracted was reported less (1.8±0.2 kg household⁻¹ day⁻¹ and 1.6±0.2 kg household⁻¹ day⁻¹, respectively) as compared to Farkia and Bampa villages (4.5±0.3 kg household⁻¹ day⁻¹ and 4.9± 0.4 household⁻¹ day⁻¹, respectively). The fuelwood consumption pattern with respect to employment status across different villages is shown in Figure 2.

Discussion

The villagers used blue pine (*Pinus wallichiana*) and deodar (*Cedrus deodara*) more (use value 96.6 and 93.1, respectively) than *Betula* (use value 26.4) because the lower reaches of the valley and the area adjoining villages are dominated by dry temperate forest of blue pine (*Pinus wallichiana*) and deodar (*Cedrus deodara*). The local inhabitants prefer to use these species compared to dry sub-alpine forests predominately of Birch (*Betula utilis*, remnant patches), because the former species are easily available. Although, when asked which species they considered as better fuelwood, *Betula* was considered a better fuelwood species by the villagers. The low use value of a potentially good fuelwood species is chiefly attributed due to its inaccessibility, as *Betula* grows alongside steep ridges protruding towards hill tops. The villages such as Niti, Bampa and Farkia which have *Betula* forests close by reported extensive use of it along with *Pinus* and *Cedrus*.

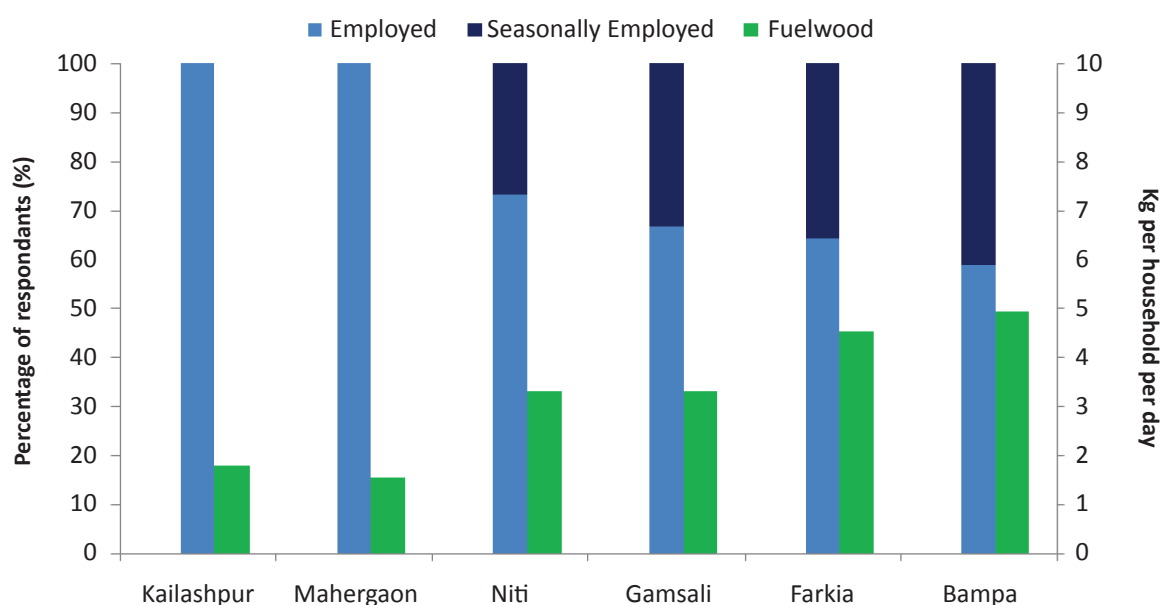


Figure 2. Fuelwood consumption and employment status across different villages in Niti valley, Nanda Devi Biosphere Reserve.

The fuelwood consumption in cold arid region of Niti valley was recorded to be much lower (1.6 ± 0.2 kg household⁻¹ day⁻¹) compared to villages of the Greater Himalaya (4.9 ± 0.4 kg household⁻¹ day⁻¹) (Samant et al. 2000; Awasthi et al. 2003; Bhatt and Sachan 2004; Singh et al. 2010; Dhanai et al. 2015). This is mainly attributed to sparse vegetation cover, fewer species and migratory settlements of the villagers in the Trans-Himalayan regions such as Niti valley. These regions are floristically impoverished as compared to adjacent high altitude areas of Greater Himalaya (Mani 1978; Schweinfurth 1984).

Fuelwood consumption is affected by the employment status and availability of alternative fuel to the villagers. Employment provides people with financial means to purchase alternative energy such as LPG and kerosene. This not only relieves them of the labour required in obtaining wood from forests, but also provides health benefits through the absence of smoke and ease of their use. Employed households in most cases also have better access to education and towns/cities, and they tend to minimize their stay in the remote valleys. They are likely to spend more time in towns where they are employed, hence, decreasing the demand for wood for cooking and heating. Likewise, if alternative energy is available for cooking, the amount of wood consumed will decrease. Therefore, providing alternative fuel options such as, LPG, solar cookers and fuel efficient portable ovens to the local and pastoral communities in such remote regions of the Himalaya would not only reduce the dependency on the forests for fuelwood, but also in turn save the forests from degradation.

Acknowledgements

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Research

Herbivory damage in native *Alnus nepalensis* and invasive *Ageratina adenophora*

Sujan Balami¹ and Lal B Thapa^{2*}

¹Department of Botany, Amrit Science Campus, Tribhuvan University, Kathmandu, Nepal

²Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Abstract

There are numbers of invasive and naturalized alien species in Nepal but studies related to herbivory effects on such species are scarce. An issue of debate is whether invasive alien species get benefited by less herbivory damage in their introduced range. In this study, we investigated the level of herbivory damage in *Alnus nepalensis* in an area invaded by *Ageratina adenophora* in Nepal. The damage was compared between invasive *A. adenophora* and native *Alnus nepalensis*. Results showed that *A. adenophora* experienced lower level of leaf damage by herbivores than that of *A. nepalensis*. This indicated that the invasive *A. adenophora* might have benefited from reduced herbivory damage behind its successful invasion in Nepalese forest. Further studies are needed to confirm whether controlling of herbivores to lessen the damage in native *Alnus nepalensis* could enhance its competitive ability against *Ageratina adenophora* in native vegetation of Nepal.

Key-words: alien invasion, enemy release hypothesis, herbivory damage, native species.

Introduction

Enemy release hypothesis (ERH) is commonly accepted mechanism for invasion success of alien species, which is corroborated by different experimental evidences (Agrawal *et al.* 2005; Vila *et al.* 2005). ERH predicts that invasive alien species (IAS) are capable of attaining vigorous growth at their introduced range and exhibit an increase in the distribution and abundance due to a decrease in regulation by natural enemies (Keane and Crawley 2002; Liu and Stiling 2006; Roy *et al.* 2011).

Enemy, in general, represents herbivores of different guilds (insects, nematodes, and microbes). In general, if native and invasive species are distant phylogenetically, the native herbivores (e.g., insects) can hardly switch to the invading species (Bertheau *et al.* 2010). Consequently, IAS show competitive advancement over native species by release out from natural enemies; i.e. they are benefited, such as in allocation of resources for growth and reproduction than in herbivore defense activities (Blossey and Notzold 1995; Dietz *et al.* 2004; Lake and Leishman 2004). This strategic trait of IAS affects growth and development of native species (Gorchov and Trisel 2003; Stinson *et al.* 2006; Hejda *et al.* 2009).

In the context of Nepal, ecological impacts caused by aggressively invading and naturalized alien species have been documented (e.g. Tiwari *et al.* 2005; Thapa *et al.* 2015; Shrestha 2016; Thapa *et al.* 2016a), but the studies on

specific mechanism of alien invasiveness including ERH are untouched. In this regard, it would be interesting to study the damage caused by natural enemies to native and invasive species and explore whether IAS are taking the benefit of less herbivory damage for their rapid growth. Studies on ERH in invasion ecology generally involve comparing IAS with its native congeners regarding their growth and herbivory damage (Agrawal and Kotanen 2003). However, for the purpose to generate a simple idea on ERH, taxonomically different, but co-occurring, native and invasive pairs can also be selected for comparing enemy damage level if there is lack of congeneric member.

In this study we compare the level of herbivory damage between invasive *Ageratina adenophora* (Spreng.) King & H. Rob. and native *Alnus nepalensis* D. Don. *Ageratina adenophora* (hereafter referred to as *Ageratina*) of family Asteraceae was introduced in Nepal around 1950s and now it has been naturalized in most parts of the country between 850 and 2200 m asl (Press *et al.* 2000; Tiwari *et al.* 2005). *Alnus nepalensis* (hereafter referred to as *Alnus*) of the family Betulaceae was selected as a native species to compare herbivory effect along with *Ageratina* because both grow on degraded forest patches with varying climatic and soil conditions (Orwa *et al.* 2009; Tripathi *et al.* 2011; Thapa *et al.* 2016b). A hypothesis set in the study was that the native *Alnus* suffer from high level of herbivory damage than the invasive *Ageratina*.

*Corresponding author. e-mail - lal_thapa25@yahoo.com

Materials and Methods

STUDY SITE

The study was conducted in Champadevi Community Forest (elevation range: 1400-2300 m asl; location: 27°42'N and 85°19' E) at southwest of Kathmandu valley. The community forest is invaded by *Ageratina*. The forest was highly degraded in the past, but its natural recovery has been achieved after conservation efforts of local people for two decades (Thapa *et al.* 2016b). A competition between growing *Alnus* saplings and *Ageratina* can be observed in field condition.

DAMAGE ASSESSMENT

The assessment was conducted in August 2016. Leaf damage in both native and invasive species was measured in the form of damage percentage. The damage was categorized into herbivory bites and necrotic spots. A total of 20 individuals of each *Ageratina* and *Alnus* were selected from *Ageratina*-invaded forest patches along a line transect. Distance between the patches was approximately 10 m. *Alnus* saplings having similar height as *Ageratina* (height ranges from 0.5 to 1.5 m) were selected for the study. Four different branches (one branch from base, two from middle and one from top of each plant) were selected from each individual of *Alnus*. Total number of leaves and number of damaged leaves were counted. In case of *Ageratina*, four different ramets were selected randomly from the same patches where *Alnus* saplings were associated. Afterward, percentage of damaged leaves was calculated.

$$\text{Percentage leaf damage} = \frac{\text{Total no. of damaged leaves in all branches}}{\text{Total number of leaves in all branches}} \times 100$$

The damage was also calculated in terms of percentage leaf area. A total of four damaged leaves from each selected branch were collected (20 individuals \times 4 branches \times 4 leaves = 320 leaves) for each native and invasive species. Out of 320 leaves, 100 were selected randomly. The leaves were photographed and percentages of leaf area damaged were assessed by using following formula.

$$\text{Leaf area damage} = \frac{\text{Area of damaged portion of leaf}}{\text{Total area of leaf}} \times 100\%$$

The calculation of leaf area was accompanied with image analyzing software ImageJ (version 1.49t).

STATISTICAL ANALYSIS

As the data were not normal, Mann-Whitney-Wilcoxon test was used as a test statistic for analyzing significant difference in damage severity and extent of damage in leaf area. Statistical tests were made by using software R (R Core Team 2015).

Results

PERCENTAGE OF DAMAGED LEAVES

Alnus exhibited high percentage of leaf damage than that of *Ageratina* ($p < 0.001$). In *Alnus*, leaf damage was $91.00 \pm 2.62\%$. Mostly, the damage was by herbivores with characteristics biting signs. In the case of *Ageratina*, $25.00 \pm 2.18\%$ of leaves showed herbivore damage (Figure 1). The results revealed that there was about 66% less leaf damage in *Ageratina* than in *Alnus*.

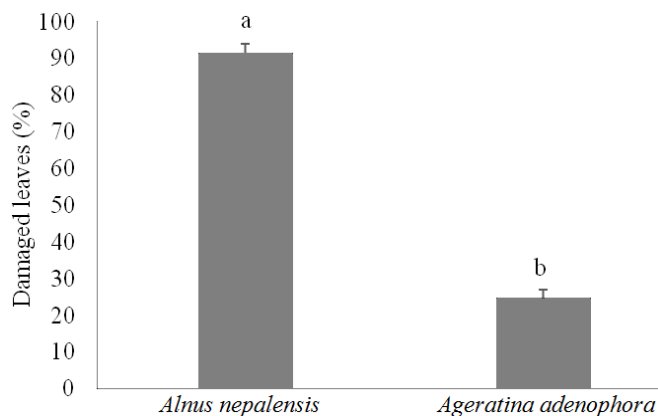


Figure 1. Percentage of damaged leaves in *Alnus* and *Ageratina* (the letters above error bar shows significant differences).

PERCENTAGE OF LEAF AREA DAMAGE

The herbivory damaged leaf area in *Alnus* was $16.00 \pm 1.18\%$ of total leaf area. In contrast, the damaged leaf area in *Ageratina* was negligible (i.e., only $0.65 \pm 0.26\%$; $p < 0.001$; Figure 2). On comparing leaf area damage type in *Ageratina*, the area of necrotic spots was significantly greater ($9.00 \pm 0.88\%$) than the herbivory bites ($p < 0.001$; Figure 3).

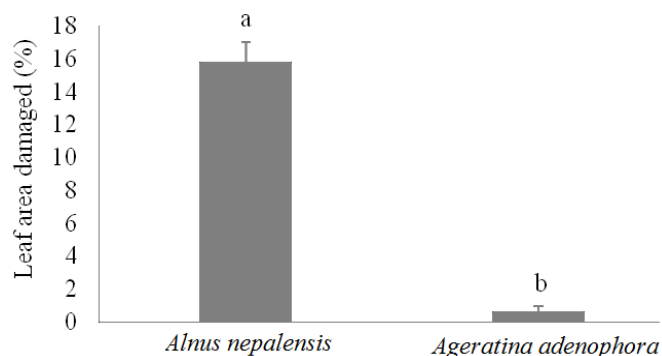


Figure 2. Percentage of leaf area damage in *Alnus* and *Ageratina* (the letters above error bar shows significant differences).

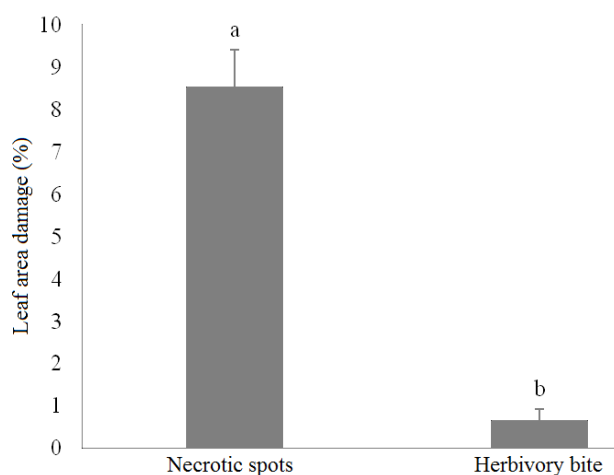


Figure 3. Leaf area damage type in *Ageratina* (the letters above error bar shows significant differences).

CORRELATION BETWEEN LEAF DAMAGE AND LEAF SIZE

Correlation analysis showed significant relationship between leaf size and degree of damage in both the native and invasive species. However, the correlation coefficient was much higher in native than in invasive species (Table 1).

Table 1. Spearman rank correlation between leaf size and damaged leaf area.

Species	Correlation coefficient	P value
<i>Alnus nepalensis</i>	0.64	<0.001
<i>Ageratina adenophora</i>	0.37	<0.001

Discussion

In accordance with the enemy release hypothesis, *Ageratina* in this study shows minimal herbivory damage. Contrasting to this, the damage in co-occurring *Alnus* was the worst. Percentage of damaged leaves was significantly high in *Alnus* in comparison to *Ageratina* in terms of number of damaged leaves (Figure 1). Similarly, comparing leaf area damage the difference was also significantly higher in *Alnus* than in *Ageratina* (Figure 2).

There are natural enemies of *Ageratina* in its native range, such as tephritid gall fly, lepidopteran stem borer and curculionid feeding on its shoot tips (Osborne 1924). As a biological control of *Ageratina*, the gall fly (*Procecidochares utilis*) has been introduced to other countries but its effect was insignificant, such as in South Africa (Kluge 1991) indicating that the introduced enemies may not effectively damage their host out of their native range. The gall fly was also observed during our field observation but only few numbers of galls were seen developed on *Ageratina* stem. Although we did not measure the galls developed in the plants, the condition was not likely to cause significant damage in *Ageratina*. Bites were also observed on leaves of *Ageratina* which might belong to

the fly but they were not severe as compared to the bites of herbivores on *Alnus* leaves (Figure 1 and 2).

Our results support previous findings; for example, a study conducted by Carpenter and Cappuccino (2005) on herbivory damage between exotic and native plant species in Ottawa, Canada found that the exotics suffered by less herbivores than the native ones. MacKay and Kotanen (2008) observed release of enemy of ragweed (*Ambrosia artemisiifolia*), where ragweed populations experienced significantly less damage relative to within-population plots. Similarly, test of ERH on *Hypericum perforatum* showed 58% of insect damage in native range with only 28% damage in introduced range (Vila *et al.* 2005).

There are several other explanations for prolific growth and successful invasion of alien species. *Ageratina* in their exotic range exhibits vegetative means of reproduction and allelopathy (Wan *et al.* 2010; Del Fabbro *et al.* 2014; Thapa *et al.* 2017). Our study indicates that one of the reasons behind a prolific growth and invasion of *Ageratina* in Nepal is reduced herbivory also.

Invasion of *Ageratina* in Nepal dates back to 1950s (Tiwari *et al.* 2005). It would be interesting to hypothesize that some pests or parasites might have adapted on feeding to *Ageratina* during this course of long time introduction and establishment in Nepal (about 70 years). We cannot assure that the bites belong to only its natural enemy (*Procecidochares utilis*), they might also belong to other insects that are co-evolved pests of native plants. We recommend further studies for its confirmation.

In addition, the stronger correlation of leaf size and damage in *Alnus* and weaker in *Ageratina* suggest that apart from leaf volatiles and other factors, leaf trait (relatively smaller size) of *Ageratina* might have aided them to lowered surface area for insects' ovulation and larval attachment. This result creates another opportunity to explore leaf trait and its relation with leaf damage in *Ageratina*.

Alnus could be a better candidate to compete *Ageratina* prolific growth regarding habitat preference as both of these species prefer disturbed and varied soil type. Our explanation is that phenological coincidences of native and invasive species are also related with herbivory damage. Usually the months of August-September represent time of active plant growth but at the meantime there is a high herbivore activity during this period. With end of these months, winter starts and all the plants lower their metabolic machinery, consequently there is less chance to recover/compensate the leaf damage/loss during whole winter. Thereby growth and development would be sufficiently low in herbivory-fed *Alnus*. As the growing season starts *Ageratina* already makes its way to the soil for their germination (March is the flowering month of *Ageratina*) where *Alnus* may still remain defoliated. After germination,

Ageratina gets well adapted even under *Alnus* canopy and may develop shade tolerant ability.

In conclusion, all these results imply that native *Alnus* which co-occur with invasive *Ageratina* is affected by severe herbivore damage. Native herbivores rarely switch to the invasive *Ageratina* even for these numbers of years since invasion. All the strategic development for escape, defense tolerance from herbivore is well developed in *Ageratina* that makes them successful invader in Nepalese forests. Therefore, constrain on *Alnus* due to herbivory might have hindered its competitive ability against *Ageratina* prolific growth.

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Research

Floodplain succession pattern along Budhi-Rapti River bank, Chitwan, Nepal

Dhiraj Chhetri Kunwar and Chitra Bahadur Baniya*

Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Abstract

Riverine floodplain is one of the most productive lowland ecosystems in Nepal. However, floodplain ecology is less understood due to its fluctuation. Budhi-Rapti River in Chitwan, central Nepal formed a floodplain along the Khorsor zone of Barandabhar corridor. This study was carried out to understand the floodplain ecosystem development after plant succession. The space for time substitution method of vegetation sampling was adopted in order to sample the floodplain created at different chronosequence. The floodplain that lies perpendicular to and 200 m away from the Budhi-Rapti river was sampled. Systematic sampling was done along two parallel transects, almost 200 m apart from each other. Sampling along the transect started right after 200 m away from the Budhi-River bank. Initial position of these transects towards Budhi-Rapti river was believed to be the youngest floodplain, which slowly getting older after passing away from the river. Abundance of vascular plant species was recorded in sample plots of 20 × 20 m each subdivided equally into 4 subplots (each of 100 m²). Along each transect, vegetation data was recorded from a series of 20 plots, placed 50 m apart from each other. Successional scores were calculated and utilized as environmental variables after applying non-metric multi-dimensional scaling (NMDS) through metaMDS. Total and life form (herbs, shrubs and trees) richness patterns were calculated. Altogether, 158 species of vascular plants under 60 families and 136 genera were recorded. Gramineae was the richest family followed by Leguminosae, Asteraceae and Cyperaceae. Total species richness showed significant negative correlation with the NMDS1 and NMDS2, which justified a convergent pattern of succession. Herb, orchid and shrub species richness also showed significant declining pattern with NMDS1. *Persicaria barbata*, *Parthenium hysterophorus*, *Ageratum conyzoides* and *Typha angustifolia* were early succession indicator species; whereas *Albizia lucidior*, *Miliusa velutina*, *Ficus hispida*, *Bauhinia purpurea* and *Brassaiopsis glomerulata* were the late succession tree species. This study agreed with the convergent model of succession.

Key-words: chronosequence, convergence, NMDS, primary succession, RDA, spatio-temporal, species richness.

Introduction

Ecological succession – the science of ecosystem development after colonization by different plant species, their life forms and their gradual changes in degrees of dominance, diversity and abundance over time – remains paradox in the biological science (Pielou 1966; Sousa 1979). Ecological succession has its root in the early 19th century. Myriads of scientific researches have been conducted to answer the question related to successional changes (Pidgeon 1940; Connell and Slatyer 1977; Chapin *et al.* 1994; Caccianiga *et al.* 2006). Barren land succession pattern after volcano (Vitousek *et al.* 1993; Nara *et al.* 2003; Walker and del Moral 2003), glacier moraine succession pattern after deglaciation (Chapin *et al.* 1994; Fastie 1995; Dolezal *et al.* 2008), river basin succession pattern after floods (Bryant 1987; Salo *et al.* 1986; Johansson *et al.* 1996; Schimel *et al.* 1996) are some of the important studies to understand science of primary succession.

Various biological colonization models have been proposed to unravel the mechanism of succession by plants and their life forms (Kitayama *et al.* 1995; Wardle *et al.*

2004). Facilitation, inhibition and tolerance are three common mechanisms to explain biological colonization patterns in succession (Connell and Slatyer 1977; Sigler *et al.* 2002; Bruno *et al.* 2003). Earlier studies unveiled the importance of colonizing species' life-form composition (Dolezal *et al.* 2008), habitat conditions (Tilman 1985), soil chemistry and soil nutrients (Carson and Barrett 1988), and below ground microbial activities (Ohtonen *et al.* 1999) in the succession pattern. Likewise, properties of succession such as species richness patterns along temporal gradient was studied by Álvarez-Molina *et al.* (2012).

Development of complex communities through simple plant life forms such as lichen crusts to stands of trees are the characteristic features of the primary succession (Grime 1977) where herbs, sub-shrubs and shrubs are their intermediate stages (Wiegleb and Felinks 2001; Zhang 2005). It is obvious that there is a variation in the species composition (Dzwonko and Loster 1992; Dolezal *et al.* 2008) and richness (Grubb 1977; Álvarez-Molina *et al.* 2012) of each plant life form through temporal gradient during succession. Particularly,

*Corresponding author. e-mail - cbbaniya@gmail.com.

the number of species are changing through time. Succession pattern can either be converging when number of species decreases with time (Rydin and Borgegård 1988; Lichter 1998; Fukami *et al.* 2005) or diverging when number of species increases with time (Wood and del Moral 1987; Sarmiento *et al.* 2003; Nicol *et al.* 2005; Baniya *et al.* 2009). Convergence pattern exemplifies the condition where there is decrease in the total species richness through time (Tilman 1987; Martínez *et al.* 2001). In contrast, the divergence pattern represents the increase in total species richness through time (Glenn-Lewin and van der Maarel 1992).

During succession, patterns of species richness might also be different according to life forms (Wardle *et al.* 1995). For instance, herbaceous species showed decreasing richness pattern with temporal gradient (Walker and del Moral 2003), but tree species showed both increasing (Nemergut *et al.* 2007) as well as unimodal (Guo 2003) richness patterns with temporal gradient. According to the life-history strategies during colonization, species are ruderals (*r*) and competitors (*k*) during succession. All early colonizing species are called ruderals or '*r*' selected species whereas the late colonizing species are competitors or '*k*' selected species.

Above and below ground habitat conditions undergo a series of modifications during successional events (McLendon and Redente 1992; Olf *et al.* 1993; Lichter 1998). Canopy cover fluctuates with time (Pena Claros 2003; Pugnaire *et al.* 1996) that varies amount of light fallen on the ground. These properties ultimately bring changes primarily on the higher life forms, like trees and climbers, but limits abundance and richness of understory herbs and shrubs (Grubb 1977). Meanwhile, there are changes in below ground properties, such as physiochemical properties of soil and its fertility (Crews *et al.* 1995) as well as changes in soil microbial activities (Ohtonen *et al.* 1999) during succession.

River basin is an ecosystem where colonization pattern can be clearly visualized from early to late stages of succession. Lowland in Nepal is almost flat, formed after deposition of sediments carried by upstream rivers. River basin succession study is one of the unique sectors where soil texture changes from sandy (nutrient poor, high moisture, high minerals, and low humus content) to alluvial soil (nutrient rich, low moisture, low minerals and high humus content) (McLendon and Redente 1992; Vetaas 1994; Lichter 1998; Ohtonen *et al.* 1999; Jones and Henry 2003). These respective changes facilitate the plants of different strategies, i.e. *r*- and *k*-selected species (Grime 1977). At the same time, disturbance (such as fire and grazing) may play a pivotal role in seed dispersal in the river basin succession (Salo *et al.* 1986; Ward and Stanford 1995; Turner *et al.* 1998).

Chitwan National Park and its Buffer Zone in south-central Nepal encompasses a unique ecosystem for distinct fauna such as Bengal Tiger and One horned Rhino, and flora

such as *Shorea robusta*. Rapti and Budhi-Rapti rivers passing through this national park and buffer zone shifted frequently in different directions at different periods that fragmented the landscape each year. Habitats thus created after shifting of river is a good site to study plant colonization.

Succession study is always constrained by measurement of temporal gradient since it was built. Shorter temporal gradient can be measured after direct measurement. However, direct measurement of longer temporal gradient is not feasible. Thus, indirect measurement of succession by means spatio-temporal ordination method is a good choice (Matthews 1978; Vetaas, 1994; Aikio *et al.* 2000; Mesquita *et al.* 2001; Caccianiga *et al.* 2006). In this method, space is substituted by time which is a highly adopted method of measuring succession. This study of primary succession along the Budhi-Rapti floodplain has been initiated with the general objective of deciphering the succession pattern of colonizing plant species. The specific objectives were to find the life form species richness and species composition pattern along Budhi-Rapti river bank, Chitwan National Park, Central Nepal.

Materials and Methods

STUDY AREA

Budhi-Rapti river bank lies at the Khorsor zone of Barandabhar corridor in Chitwan National Park (CNP), south-central Nepal (Figure 1). CNP is the first national park in Nepal, established in 1973, covering an area of 932 km² (DNPWC 2010). CNP comprises diverse ecosystems from lowland tropical forests to floodplains and Churia hills draining by Rapti, Reu and Narayani rivers. Altitude of CNP ranges from 100 to 815 m above sea level (asl). Almost 70% of the park area is dominated by Tarai hardwood sal (*Shorea robusta*) forest, followed by deciduous riverine forest (7%), and *Pinus roxburghii* forest (3%) (DNPWC 2010). Budhi-Rapti floodplain acts as a wildlife corridor between CNP and Mahabharat foothills (Panwar 1986; Litvaitis *et al.* 1996; Aryal *et al.* 2012). The east-west national highway passes through the Barandabhar forest. This is a highly disturbed forest under severe human pressure.

The study area lies in the tropical monsoon climatic zone with three seasons: summer, winter and monsoon. During winter (October to February) northerly dry winds enter here from the Himalayas and the Tibetan plateau so that the temperature is reduced up to 8°C. Rainfall is scanty during winter. Temperature reaches up to 37°C (DNPWC 2015) during summer (February to mid-June). The hottest months are May to early June. Monsoon starts from mid-June and lasts in late September during which 80% of the annual rainfall occurs. July and August receive maximum rains. Mean daily temperature ranges from 25°C to 34°C (DNPWC 2015) during this season. Monsoon rain causes dramatic floods and changes in the character and courses of rivers.

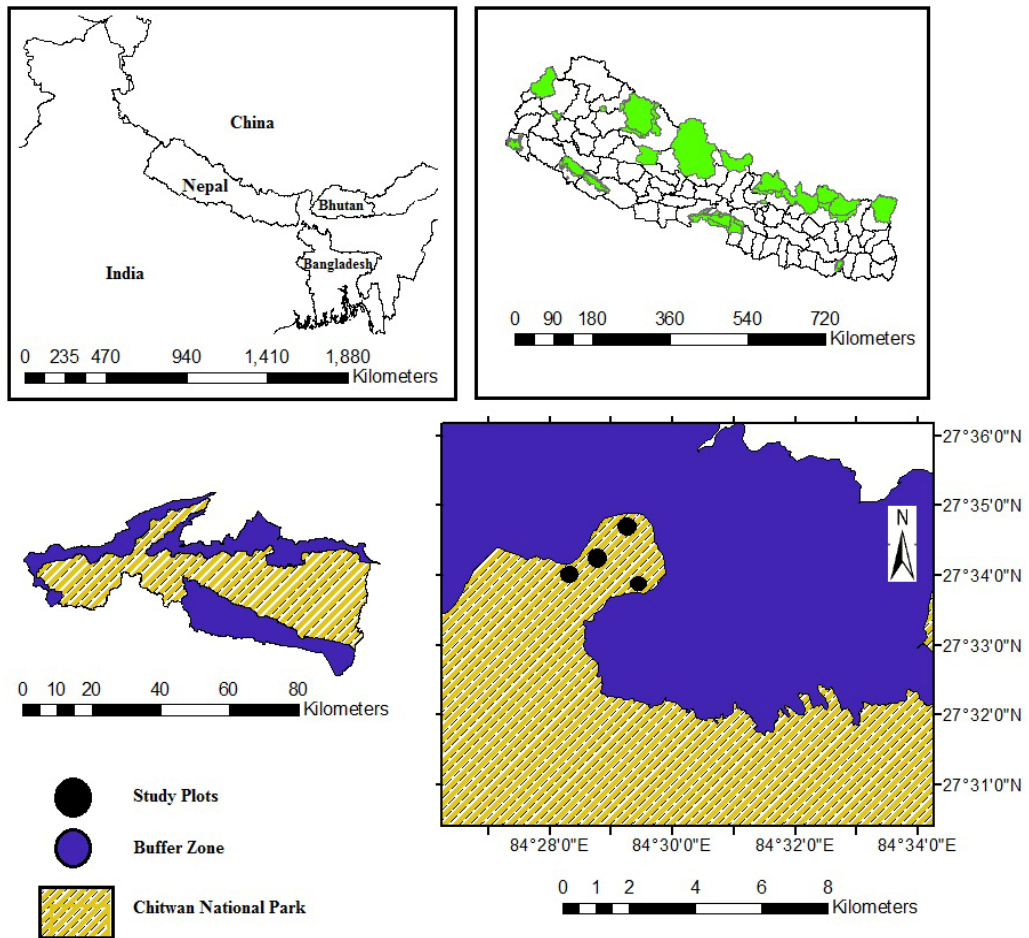


Figure 1. Map of the study area

LOCATION OF THE SAMPLING PLOT AND MEASUREMENT

The field work for this study was conducted during June and October of 2014. First, a reconnaissance survey was made for the preliminary idea about the floodplain formation, orientation and location. In addition, information about the formation of the Budhi-Rapti floodplain was also obtained through review of relevant literature and interview with CNP authorities and local elderly people. The floodplain was believed to be the youngest and slowly getting matured with increasing distance from the river. Thus, sampling was done on the floodplain after relying on the principle of space-with-time substitution method as developed by Matthews (1978).

Data on the plant species colonization pattern was obtained after sampling the vegetated floodplain that lied perpendicular to the Budhi-Rapti river but almost 200 m away from the river bank. Systematic sampling was applied along two parallel transects (T1 and T2) separated almost 200 m apart from each other (Figure 2). Sampling along the transect started right after 200 m away from the Budhi-Rapti river bank. Initial position of these transects towards Budhi-Rapti river was believed to be the youngest floodplain, which slowly getting

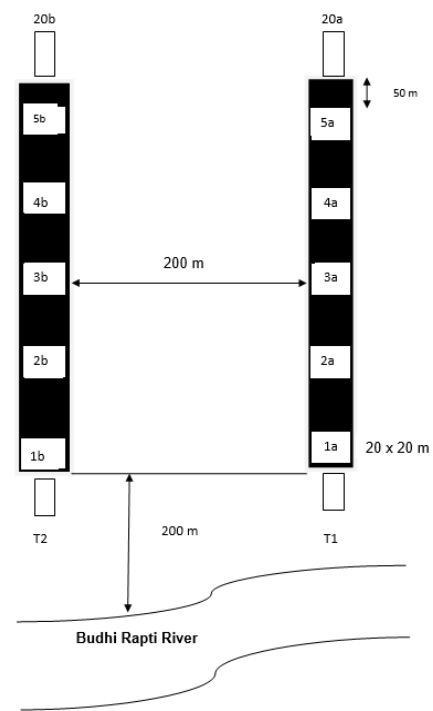


Figure 2. Sampling design.

older after passing away from the river. Abundance of vascular plant species was recorded in sample plots of 20×20 m each subdivided equally into 4 subplots (each of 100 m^2). In each transect, 20 such plots were placed consecutively and 50 m apart from each other (Figure 2). Consecutive plots, along the parallel transects (T1 and T2), were believed to have similar temporal scale, thus assigned same number but different alphabets i.e., 1a and 1b; 2a and 2b, and so on. Subplots were marked as 1, 2, 3 and 4 in the clockwise direction. Presence and absence of all vascular plant species within each subplot was recorded in the scale of 1 or 0 respectively. Thus, presence of a species in a plot means an abundance score of 1 to 4. In total, 40 plots were sampled in two transects with a total sampling area of 1.6 ha.

PLANT COLLECTION, HERBARIUM PREPARATION AND IDENTIFICATION

Voucher specimens of all species encountered in sample plots were collected and dried properly. Preliminary identification of plant species was done with the help of field guides (e.g., Polunin and Stainton 1984; Stainton 1988) and through expert consultation in Biodiversity Conservation Center (BCC), Chitwan, Nepal. Species identity was later confirmed by comparing with the specimens deposited at the national herbaria (TUCH and KATH). However, some species, mainly belonging to the genera *Vanda*, *Oberonia*, *Bulbophyllum* and *Carex* remained unidentified. They were definitely separate species but were ranked here by generic name only. Plant species were categorized into six life-forms: herb, shrub, climber, tree, orchid and fern (Baniya *et al.* 2009). Press *et al.* (2000) was followed for the nomenclature of flowering plants and Gurung (1991) was followed for the nomenclature of pteridophytes.

MEASUREMENT OF TEMPORAL GRADIENT

Spatio-temporal gradient was the main predictor variable in this study, which was indirectly obtained as the first axis sample score value after non-metric multidimensional scaling (NMDS) analysis of samples by species dataset (Matthews 1978). We believed NMDS orders samples based upon species abundance as sample scores value after simple indirect ordination method. Position of each sample plot indicated temporal score. No priori or transformation was considered during NMDS. Previous studies (e.g., Whittaker 1987; Zhang *et al.* 2016) applied NMDS sample scores to measure succession in their studies. Lower NMDS first axis sample score represents early succession and higher NMDS first axis sample score represents late succession. Likewise, zero NMDS first axis score represents the mid succession. We acknowledge the value of detrended correspondence analysis (DCA) for the analysis of similar dataset. However, we understood DCA

applies transformation by segments and thus we did not use it in our case.

NUMERICAL ANALYSIS

Sample by species data matrix was prepared prior to the quantitative analysis. Sample plots 1a and 1b entered first were believed to be the youngest gradient representing plots, likewise plots 20a and 20b represented the oldest gradient in this study. Since two-two plots represented the same temporal gradient, thus these were made one-one after total averaging. This averaging was done with abundance score from 0 to 8. Thus, total plots became 20 with area of 800 m^2 ($2 \times 20 \times 20$ m) after averaging. Reason for this total averaging was just to avoid spatial autocorrelation. All analyses were based on 800 m^2 ($2 \times 20 \times 20$ m) plots.

Pearson's correlation coefficients were calculated between response variables [total species richness and its derivatives (climber richness, fern richness, herb richness, orchid richness, shrub richness and tree richness)] and predictors variables, such as NMDS1, NMDS2 and elevation. Correlation coefficient matrix was prepared with probability value (p) (Oksanen *et al.* 2015).

Normality among the response variables were checked by using *Shapiro Wilcox* test. The generalized linear model (GLM; MacCullagh and Nelder 1989) was used for the simple linear regression analysis between response and predictor variables. The GLM up to the second order models were tested. The F -statistics was used to select the statistically best significant model ($p \leq 0.05$). The graphics was prepared from the best selected model.

Non-metric multidimensional scaling (NMDS) is an indirect ordination method in which samples and species are ordered in an ordination space based on various types of distances (Euclidean distance in this case). Its axes are representative of underlying gradients. This gradient has been used to map samples in simplified, two-dimensional ordination space (Schmidtlein *et al.* 2007). Thus, sample score values presented by NMDS1 and NMDS2 were utilized as environmental variables as suggested by previous researchers (Matthews 1978; Aikio *et al.* 2000; Mesquita *et al.* 2001; Caccianiga *et al.* 2006; Sahu *et al.* 2008).

In this study NMDS1 sample score was found highly correlated with the total species richness than was with NMDS2 (see Table 1). Thus, the total species richness change with NMDS1 must likely represents a temporal gradient.

Zero inflation in the data set was detected after the changing trend in the axis length value during the detrended correspondence analysis (DCA). The axis length value found higher in some of the higher axis. This error in the dataset caused multi-collinearity. Thus, this error was corrected after removing single or double times occurring species throughout

the whole dataset prior to the multivariate analysis. Thus, performed DCA resulted the first axis length value less than 2.5 SD units. This allowed us to apply redundancy analysis (RDA) for the direct gradient analysis to see the pattern of species composition along the floodplain. During RDA, the best fitted statistically significant environment variables were chosen after internal regression, forward selection and 999 times permutations (Oksanen *et al.* 2015). Statistically significant results obtained after RDA were shown through graphics.

All operating systems were performed through Ubuntu and libreoffice as office package. All statistical analyses were done in R program (R Core Team 2016). Ordination was done by using *vegan* package (Oksanen *et al.* 2015).

Results

PLANT SPECIES DIVERSITY

A total of 158 plant species under 60 families and 136 genera were recorded. Gramineae was the richest family followed by Fabaceae, Asteraceae, Cyperaceae, Lamiaceae, Euphorbiaceae and Verbenaceae (Appendix 1). Herb was the most dominant life form with 66 species, followed by tree (48 species), shrub (23), climber (12), fern (6) and orchid (3) (Figure 3).

PEARSON CORRELATION COEFFICIENTS AMONG VARIABLES

The total species richness showed statistically significant ($p \leq 0.05$) positive correlation with herb richness ($r = 0.94$, Table 1). Likewise, total species richness showed significant positive correlation with orchid richness ($r = 0.62$) and shrub richness ($r = 0.56$). Negative correlations were found between NMDS1 and total species richness ($r = -0.69$), herb richness ($r = -0.58$), orchid richness ($r = -0.78$) and shrub richness ($r = -0.72$) (Table 1). Similarly, orchid richness showed statistically

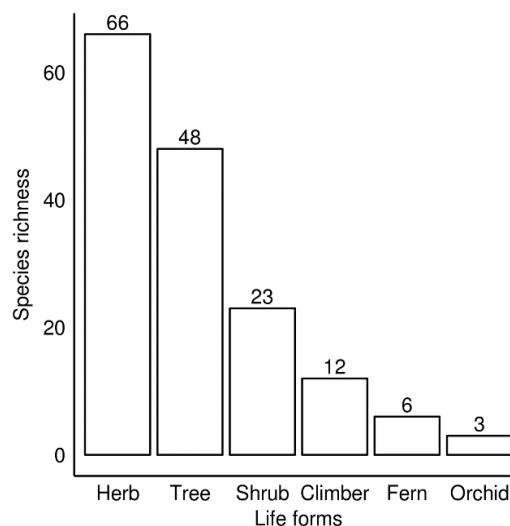


Figure 3. Species richness pattern with different life forms.

significant positive relationships with herb richness ($r = 0.50$) and shrub richness ($r = 0.58$).

REGRESSION ANALYSIS

Total species richness showed significant negative linear relationships ($R^2 = 1.0$) with NMDS1 and NMDS2 (Figures 4a, 4b; Appendix 2). No significant relationship obtained between climber species richness and NMDS1 and NMDS2 (Appendix 2). Fern species richness also showed insignificant relationship with both NMDS1 and NMDS2 (Appendix 2). Herb species richness showed significant negative linear relationship ($R^2 = 0.3$ and 0.4 respectively) with NMDS1 and NMDS2 (Figure 5a, 5b; Appendix 2). Orchid species richness also showed significant negative linear relationship ($R^2 = 0.5$) with NMDS1 (Figure 6), but the relationship with NMDS2 was insignificant (Appendix 2). Similarly, shrub species richness

Table 1. Pearson correlation coefficient matrix showing relationships among variables. The significant correlation coefficients ($p \leq 0.05$) are marked in bold.

Variables*	NMDS1	NMDS2	Alt	Sppn	Clim_rich	Fern_rich	Herb_rich	Orchid_rich	Shrub_rich	Tree_rich
NMDS1	1	0	-0.03	-0.69	0.06	0.38	-0.58	-0.78	-0.72	0.08
NMDS2		1	0.17	-0.47	0.02	-0.27	-0.59	0.03	0.28	-0.19
Alt			1	-0.13	0.03	0.3	-0.22	0.13	-0.13	0.22
Sppn				1	-0.12	0.03	0.94	0.62	0.56	0.21
Clim_rich					1	-0.11	-0.24	0	-0.04	-0.22
Fern_rich						1	0	-0.35	-0.32	0.38
Herb_rich							1	0.5	0.37	0.08
Orchid_rich								1	0.58	-0.04
Shrub_rich									1	-0.16
Tree_rich										1

* Alt – altitude; Clim-rich – climber species richness; Fern-rich – fern species richness; Herb_rich – herbaceous species richness; NMDS1 - non-metric multidimensional scaling axis 1; NMDS2 - non-metric multidimensional scaling axis 2; Orchid_rich – orchid species richness; Shrub_rich – shrub species richness; Sppn – total species richness; Tree_rich – tree species richness.

showed significant negative linear relationship ($R^2 = 0.5$) with NMDS1 (Figure 7) and insignificant relationship with NMDS2 (Appendix 2). Relationships of tree species richness with NMDS1 and NMDS2 were also insignificant (Appendix 2). There was no significant pattern between altitude and both axis values of NMDS (Appendix 2).

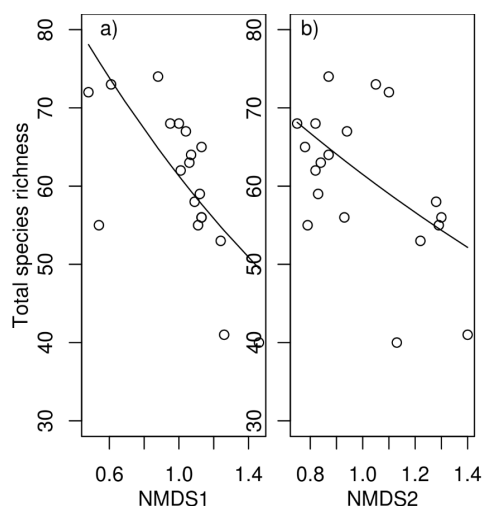


Figure 4. Relationship between total species richness and NMDS1 (a) and NMDS2 (b) based on simple linear regression analysis using generalized linear model.

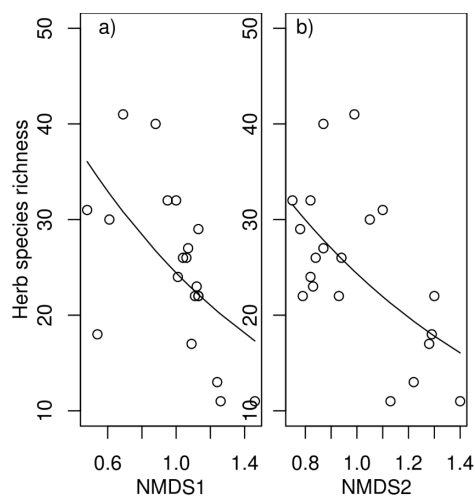


Figure 5. Relationship between herb species richness and NMDS1 (a) and NMDS2 (b) based on simple linear regression analysis using generalized linear model.

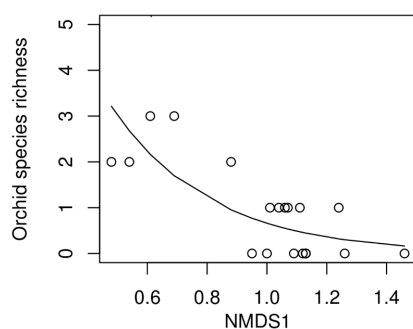


Figure 6. Relationship between orchid species richness and NMDS1 based on simple linear regression analysis using generalized linear model.

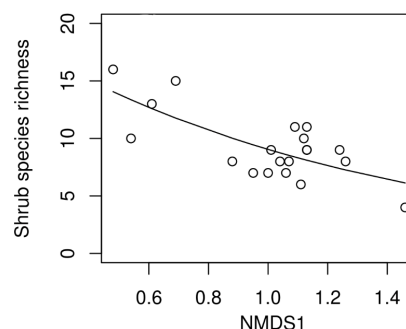


Figure 7. Relationship between shrub species richness and NMDS1 based on simple linear regression analysis using generalized linear model.

ORDINATION

DCA summary – The sample by species data matrix showed shorter lengths of gradient, 1.7 standard deviation unit (SD unit) by the first axis of DCA (Table 2). The length of gradient was found decreasing gradually with increasing axis. The variance explained by other axes was also found gradually decreasing with increasing axis. These results confirmed the linear pattern among species along the main succession gradient studied and allowed to choose linear direct ordination method, which is redundancy analysis (RDA).

Table 2. DCA summary.

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.2	0.14	0.07	0.06
Decorana values	0.23	0.12	0.07	0.04
Axis lengths	1.65	1.41	1.33	1.26

RDA analysis – Two statistically significant environmental axes, viz. NMDS1 and NMDS2 were obtained after 999 times permutations with species score. These number of permutations normalized the correlation value. The NMDS1 represented significantly the first axis of RDA that corresponded to the main gradient of this study which was the temporal gradient. The negative end of NMDS1 represented indicators to younger succession whereas the positive end represented indicators to the oldest gradient. All species as well as samples nearer around these respective ends of the gradient were indicators of that particular type of temporal gradient.

Abundance of *Albizia lucidior* (abbreviated as Albi. Luci in the ordination diagram), *Lygodium japonicum* (Lygo. japo), and *Fimbristylis dichotoma* (Fimb.dich) were highly significant towards plots with the highest value of NMDS1 (Figure 8; Appendix 1). The highest abundance of *Parthenium hysterophorus* (Part.hyst), *Persicaria barbata* (Pers.barb) and *Ficus religiosa* (Ficu.reli) towards the negative end of RDA first axis supported the plots with the least value of NMDS1, which were early colonizers. The abundance of *Albizia lucidior* and *Lygodium japonicum* towards the positive end of RDA first axis may indicate that these were the late succession indicator species. NMDS2 represented by the second axis

of RDA (Table 1, Figure 8). The abundance of *Commelina benghalensis* (Comm.beng), *Hypoxis aurea* (Hypo.aure), *Cyperus niveus* (Cype.nive) and *Alternanthera philoxeroides* (Alte.phil) supported by the least value of NMDS2 indicated high disturbance and less moisture towards negative end of RDA second axis (Figure 8; Appendix 1). The highest abundance of *Persicaria pubescens* (Pers.pune), *Flemingia macrophylla* (Flem.macr) and *Ageratum conyzoides* (Ager.cony) towards positive end of RDA second axis may support more moisture loving and less disturb indicators.

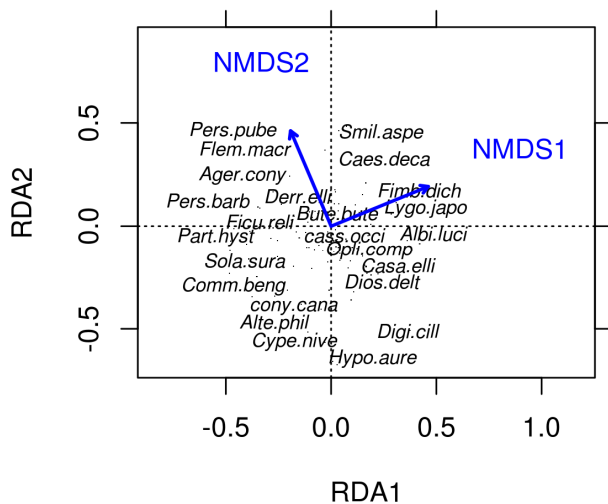


Figure 8. Species and environment biplot after redundancy analysis (RDA). Species abbreviation as in Appendix 1.

Discussion

Succession is a universal and ubiquitous phenomenon which varies with space and properties. Budhi-Rapti river that passes through Chitwan National Park and its buffer zone shifted frequently in different directions at different periods thus creating unique floodplain area. The floodplain has been colonized by different plant species. Plant species of different life forms have found colonized in a definite pattern which characterized the habitat as well as environment created either by themselves or externally imposed. Hence, our findings stated that the area has been experiencing a deterministic and convergent type of succession. This culminates into matured sal (*Shorea robusta*) forest at the end of the successional gradient.

The floodplain showed herb species richness comparatively higher than other life forms. The probable explanation of this result could be that the studied temporal gradient is still younger. The environment created by soil of the study sites, *i.e.* the sand dunes formed nearby the river bank is relatively younger. Younger sand dunes would have lesser humus but high amount of mineral content which primarily favors the growth and establishment of short growing plants. Generally, these characters are good match for herbs which exhibit a

short life span and are generally opportunistic (*i.e.*, *r*-selected) species (Jones and Henry 2003). Likewise, trees and shrubs are found colonized at matured sand dunes.

Measurement of succession gradient is almost impossible at short duration of observation. However, gradient is universal and succession is no exception (Huston 1994). Measurement of succession is a matter of big challenge. Introduction of ordination in community ecology (Matthews 1978; Vetaas 1994; Aikio *et al.* 2000; Mesquita *et al.* 2001; Caccianiga *et al.* 2006; Baniya *et al.* 2009) and implementation of non-metric multidimensional scaling (NMDS) (Matthews 1978; Aikio *et al.* 2000; Mesquita *et al.* 2001; Caccianiga *et al.* 2006) greatly helped to overcome this shortcoming. The NMDS utilizes, via *vegan*, a free community ecology analysis package (Oksanen *et al.* 2015). During NMDS, samples and their species are presented on the basis of their abundance without bias. Sample score values of the NMDS first axis is always highly correlated with main gradient of study, which is succession in this case. Adoption of NMDS1 as succession gradient has been justified by this study.

As the value of NMDS1 increases, abundance of climax-stage-loving species also increases. The second axis may represent the soil moisture gradient as having higher abundance of moisture-loving species and alluvial-soil-loving species towards this NMDS2 gradient. These two axes were taken as major environmental variables to explain species richness, its derivatives and their composition during succession. High occurrence of those indicator species around successional and moisture gradients also matched with similar earlier results such as Olf *et al.* (1993).

The total species richness stood as the strongest response variable in this study that showed statistically significant ($p \leq 0.05$) but negative linear relationship with both NMDS1 and NMDS2. Tree, herb, shrub, orchid, climber and fern richness indicated their major share to the total species richness. Herb and shrub species richness were found decreasing with increasing NMDS1 scores. However, tree species richness was found insignificantly related with both NMDS axes. High richness of tree species at late stage of succession may facilitate higher canopy cover (Turner *et al.* 1998), high carbon biomass, high water table but low soil moisture (Olf *et al.* 1993). Conversely, more diversity and species richness were found towards the beginning of NMDS1 or plots nearby river bank (early succession).

During this floodland succession, herbs and shrubs species may indicate the early-stage species with their higher richness at lower range of NMDS1. Similarly, at the later stages, tree species are also increasing. Due to higher canopy cover at climax stage there would be less herb and shrub species (Turner *et al.* 1998). Due to high soil moisture at the beginning of this study, or at the early succession stages, there were higher

number of invasive species (direct observation from the field). Thus, the decreasing pattern of total species richness with NMDS1 is justified. This is matched with justification given by Aikio *et al.* (2000), Mesquita *et al.* (2001), and Caccianiga *et al.* (2006) in their study.

The observed successional changes in the species composition may also be associated with the seed dispersal pattern of respective species which may be facilitated by wild herbivores (Duncan and Chapman 1999). Plant species of short life cycle or ruderals may increase richness after germination if seeds got chance to drop off from their fur while visiting water sources. That may result high diversity at early stage habitats due to less competition for light and moisture resources compared to late successional habitats.

Total species richness is significantly correlated with herb species richness, i.e. the total species richness is largely governed by the number of herb species in the study site. Junk and Piedade (1997) found similar finding of higher number of terrestrial herbaceous species nearby Amazon floodplain. The result of convergent pattern of total species richness is in agreement with a number of previous studies (Lichter 1998; Fukami *et al.* 2005; Sluis 2002) though the driving factor in this study may be different than the previous studies. Decrease in total species richness can also be explained by various factors that may change during successional stage (represented by NMDS1). Soil biophysical properties – soil characteristics (organic matter content, nutrient cycling; Carson and Barrett 1988), microbial activity (Ohtonen *et al.* 1999), pH and moisture (Olf *et al.* 1993); aboveground vegetation – litter fall, canopy cover and disturbance (Turner *et al.* 1998) may have resulted this model. However, the pattern of species richness for each life form differs on increasing gradient. For instance, in case of herbaceous species, there is significant decrease in richness with increasing NMDS1. Changes in soil characteristics from sandy to clay or alluvial and decrease in soil nutrients (Vitousek and Reiners 1975; Tilman 1985; Mitsch *et al.* 2005) on one hand, and increased canopy cover of tree species on the other represent the changes in the habitat condition during succession (Prach and Pyšek 2001; Wiegleb and Felinks 2001).

Various factors, like seed dispersal, animal movement and grazing may contribute high herb species richness nearby the river bank. Similar condition prevails in case of shrubs. In contrast, fern richness and tree richness increased with increasing distance from the river bank not significantly. Shady and moist condition of forest floor provide suitable site for ferns to flourish (Yarranton *et al.* 1974; Chapin *et al.* 1994). Furthermore, disturbance, like fire has little influence on the regeneration of pteridophytes (Turner *et al.* 1998; Walker *et al.* 2010). Likewise, the increased tree species richness may have facilitated by high nutrient input, high microbial activity

and efficient intake of nutrient by trees. Frequent outbreak of fire, high canopy cover and litter fall also limit the growth and development of shrubs and herbs underneath the forest (Olf *et al.* 1993; Fukami *et al.* 2005). Consequently, habitat of increased tree species richness assisted the higher number of climbers in the respective habitat, resulting an increasing pattern of climber species richness with NMDS1. Herb species such as *Persicaria barbata*, *Parthenium hysterophorus*, and *Ageratum conyzoides* having high abundance towards the positive end of NMDS2 but negative end of NMDS1 represented the early successional species. Likewise, high abundance of tree species such as *Albizia lucidior*, *Ficus religios*, *Shorea robusta* (not seen in figure axes) towards positive end of NMDS1 axis represent the late successional species. This study clearly supported the earlier findings of convergence pattern of floodplain succession.

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Appendix 1. Plant species recorded in the sample plots, their abbreviated form (used in ordination), lifeform, frequency and NMDS1 score value.

S.N.	Scientific name	Short form	Family	Life form	Frequency %	NMDS1
1	<i>Achyranthes aspera</i> L.	Achy aspe	Amaranthaceae	Herb	5	-0.800
2	<i>Adiantum capillus-veneris</i> L.	Adia capi	Pteridaceae	Fern	39	0.156
3	<i>Aegle marmelos</i> (L.) Corr.	Aegl marm	Rutaceae	Tree	1	-0.963
4	<i>Ageratina adenophora</i> (Spreng.) R.M. King & H. Rob.	Ager aden	Asteraceae	Herb	5	-0.406
5	<i>Ageratum conyzoides</i> (L.) L.	Ager cony	Asteraceae	Herb	44	-0.116
6	<i>Albizia lucidior</i> (Steud.) I.C. Nielsen	Albi luci	Fabaceae	Tree	28	0.290
7	<i>Albizia procera</i> (Roxb.) Benth.	Albi proc	Fabaceae	Tree	6	0.153
8	<i>Alstonia scholaris</i> (L.) R. Br.	Alst scho	Apocynaceae	Tree	78	0.138
9	<i>Alternanthera philoxeroides</i> (Mart.) Griseb	Alte phil	Amaranthaceae	Herb	27	-0.209
10	<i>Alternanthera sessilis</i> (L.) R. Br. ex DC.	Alte ses	Amaranthaceae	Herb	31	0.125
11	<i>Amaranthus viridis</i> L.	Amar viri	Amaranthaceae	Herb	2	-0.041
12	<i>Anisomeles indica</i> (L.) Kuntze	Anis indi	Lamiaceae	Herb	3	-0.931
13	<i>Anogeissus latifolius</i> (Roxb. ex DC.) Bedd.	Anog lati	Combretaceae	Tree	1	0.124
14	<i>Anthocephalus chinensis</i> (Lam.) Walp	Anth chin	Rubiaceae	Tree	1	0.302
15	<i>Antidesma bunius</i> (L.) Spreng.	Anti buni	Euphorbiaceae	Tree	2	0.234
16	<i>Apluda mutica</i> L.	Aplu muti	Poaceae	Shrub	11	-0.290
17	<i>Ariopsis peltata</i> Nimmo	Ario pelt	Araceae	Herb	8	-0.039
18	<i>Artemisia indica</i> Willd.	Arte indi	Asteraceae	Shrub	4	-0.451
19	<i>Artocarpus lakoocha</i> Roxb.	Arto lako	Moraceae	Tree	1	-0.071
20	<i>Asparagus officinalis</i> L.	Aspa offi	Liliaceae	Shrub	2	0.306
21	<i>Bauhinia purpurea</i> L.	Bauh purp	Fabaceae	Tree	2	0.606
22	<i>Bombax ceiba</i> L.	Bomb ceib	Bombacaceae	Tree	1	0.131
23	<i>Brachiaria kurzii</i> (Hook. f.) A. Camus	Bras kurz	Fabaceae	Herb	19	-0.115
24	<i>Brassaiopsis glomerulata</i> (Blume) Regel	Bras glom	Araliaceae	Tree	2	1.022
25	<i>Breynia retusa</i> (Dennst.) Alston	Brey retu	Euphorbiaceae	Shrub	12	0.370
26	<i>Bulbophyllum</i> sp.	Bulb spp	Orchidaceae	Orchid	11	-0.657
27	<i>Butea buteiformis</i> (Voigt) Mabb.	Bute bute	Fabaceae	Shrub	2	0.193
28	<i>Caesalpinia decapetala</i> (Roth) Alston	Caes deca	Fabaceae	Climber	14	0.381
29	<i>Calamus acanthospathus</i> Griff.	Cala acan	Arecaeae	Shrub	5	-0.053
30	<i>Callicarpa macrophylla</i> Vahl	Call macr	Verbenaceae	Shrub	48	-0.035
31	<i>Carex filicina</i> Nees.	Care fili	Cyperaceae	Herb	17	0.341
32	<i>Carex inanis</i> Kunth	Care inan	Cyperaceae	Herb	3	-0.433
33	<i>Carex nivalis</i> Boot	Care niva	Cyperaceae	Herb	7	0.135
34	<i>Carex</i> sp.	Care spp	Cyperaceae	Herb	9	0.137
35	<i>Careya arborea</i> Roxb.	Care arbo	Lecythidaceae	Tree	7	0.041
36	<i>Casearia elliptica</i> Willd.	Casa elli	Flacourtiaceae	Shrub	27	0.135
37	<i>Cassia occidentalis</i> L.	Cass occi	Fabaceae	Shrub	1	0.282
38	<i>Cassia tora</i> L.	Cass tora	Fabaceae	Herb	25	-0.453
39	<i>Centella asiatica</i> (L.) Urban	Cent asia	Apiaceae	Herb	9	-0.187
40	<i>Cestrum nocturnum</i> L.	Cest noct	Solanaceae	Shrub	6	-0.365
41	<i>Chromolaena odorata</i> (L.) R.M. King & H. Rob.	Chro odor	Asteraceae	Shrub	79	-0.021
42	<i>Chrysopogon aciculatus</i> (Retz.) Trin	Chry acic	Poaceae	Herb	8	-0.393
43	<i>Cissus repens</i> Lam.	Ciss repe	Vitaceae	Climber	29	0.125
44	<i>Citrullus colocynthis</i> Schrad.	Citr colo	Cucurbitaceae	Climber	33	0.116
45	<i>Cleistocalyx operculatus</i> (Roxb.) Merr. & L.M. Perry	Clei oper	Myrtaceae	Tree	77	0.011

S.N.	Scientific name	Short form	Family	Life form	Frequency %	NMDS1
46	<i>Clerodendrum viscosum</i> Vent.	Cler visc	Verbenaceae	Shrub	134	0.031
47	<i>Colebrookea oppositifolia</i> Sm.	Cole oppo	Lamiaceae	Shrub	16	0.001
48	<i>Colocasia esculenta</i> (L.) Schott.	Colo escu	Araceae	Herb	44	-0.099
49	<i>Commelina benghalensis</i> L.	Comm beng	Commelinaceae	Herb	19	-0.420
50	<i>Conyza canadensis</i> (L.) Crog.	Cony cana	Asteraceae	Herb	23	-0.232
51	<i>Conyza leucantha</i> (D. Don) Ludlow & Raven	Cony leuc	Asteraceae	Herb	5	-0.252
52	<i>Corchorus aestuans</i> L.	Corc aest	Tiliaceae	Herb	65	0.031
53	<i>Corchorus capsularis</i> L.	Corc caps	Tiliaceae	Herb	87	0.031
54	<i>Cordia dichotoma</i> Forster	Cord dich	Cordiaceae	Tree	1	-0.019
55	<i>Costus speciosus</i> (Koenig.) Sm.	Cost spec	Zingiberaceae	Herb	84	0.001
56	<i>Curcuma domestica</i> Valet.	Curc dome	Zingiberaceae	Herb	4	0.219
57	<i>Cyanotis vaga</i> (Lour.) J.A & J.H. Schult	Cyan vaga	Commelinaceae	Herb	58	-0.081
58	<i>Cynodon dactylon</i> (L.) Pers	Cyno dact	Poaceae	Herb	10	-0.682
59	<i>Cyperus compressus</i> L.	Cype comp	Cyperaceae	Herb	35	-0.104
60	<i>Cyperus niveus</i> Retz.	Cype nive	Cyperaceae	Herb	55	-0.158
61	<i>Cyperus rotundus</i> L.	Cype rotu	Cyperaceae	Herb	42	-0.153
62	<i>Dalbergia sissoo</i> DC.	Dalb siss	Fabaceae	Tree	11	-0.942
63	<i>Derris acuminata</i> Benth.	Derr elli	Fabaceae	Tree	9	-0.105
64	<i>Desmodium multiflorum</i> DC.	Desm mult	Fabaceae	Shrub	33	-0.084
65	<i>Desmostachya bipinnata</i> (L.) Stapf	Desm bipi	Poaceae	Herb	10	0.008
66	<i>Digitaria ciliaris</i> (Retz.) Koeler	Digi cill	Poaceae	Herb	23	0.124
67	<i>Dillenia pentagyna</i> Roxb.	Dill pent	Dilleniaceae	Tree	88	0.001
68	<i>Dioscorea bulbifera</i> L.	Dios bulb	Dioscoreaceae	Climber	99	0.060
69	<i>Dioscorea deltoidea</i> Wall. ex Griseb.	Dios delt	Dioscoreaceae	Climber	42	0.082
70	<i>Dryopteris cochleata</i> (D. Don) C. Chr.	Dryo coch	Aspidiaceae	Fern	74	-0.021
71	<i>Dysoxylum binectariferum</i> (Roxb.) Hook.f. ex Bedd.	Dyso bine	Meliaceae	Tree	5	0.031
72	<i>Echinochloa colona</i> (L.) Link	Echi cola	Poaceae	Herb	1	-0.019
73	<i>Ehretia acuminata</i> R. Br.	Ehre acum	Cordiaceae	Tree	9	0.173
74	<i>Eleocharis retroflexa</i> (Poir.) Urb.	Eleo retr	Cyperaceae	Herb	2	-0.192
75	<i>Elephantopus scaber</i> L.	Elep scab	Asteraceae	Herb	44	0.063
76	<i>Eleusine indica</i> (L.) Gaertn	Eleu indi	Poaceae	Herb	3	-0.175
77	<i>Eragrostis tenella</i> (L.) Beauvois ex Roem. & Sch	Erag tene	Poaceae	Herb	4	-0.201
78	<i>Ficus hispida</i> L. f.	Ficu hisp	Moraceae	Tree	2	0.554
79	<i>Ficus racemosa</i> L.	Ficu race	Moraceae	Tree	1	-0.800
80	<i>Ficus religiosa</i> L.	Ficu reli	Moraceae	Tree	2	-1.013
81	<i>Fimbristylis dichotoma</i> (L.) Vahl.	Fimb dich	Cyperaceae	Herb	27	0.236
82	<i>Flemingia macrophylla</i> (Willd.) Merrill	Flem macr	Fabaceae	Shrub	15	-0.198
83	<i>Floscopa scandens</i> Lour.	Flos scan	Commelinaceae	Herb	20	0.079
84	<i>Fraxinus floribunda</i> Wall.	Frax flor	Oleaceae	Tree	7	0.149
85	<i>Garuga pinnata</i> Roxb.	Garu pinn	Burseraceae	Tree	5	-0.372
86	<i>Gmelina arborea</i> Roxb.	Gmel arbo	Verbenaceae	Tree	9	0.245
87	<i>Hedychium ellipticum</i> Buch.-Ham. ex Sm.	Hedy elli	Zingiberaceae	Herb	97	0.031
88	<i>Helminthostachys zeylanica</i> L.	Helm zeyl	Ophioglossaceae	Fern	43	-0.147
89	<i>Holarrhena pubescens</i> Wall. ex G. Don	Hola pube	Apocynaceae	Tree	8	-0.334
90	<i>Hypoxis aurea</i> Lour.	Hypo aure	Hypoxidaceae	Herb	44	-0.005
91	<i>Hyptis suaveolens</i> (L.) Poit.	Hypt suav	Lamiaceae	Herb	11	-0.164
92	<i>Imperata cylindrica</i> (L.) Raeusch.	Impe cylin	Poaceae	Herb	49	-0.053
93	<i>Indigofera dosua</i> Buch.-Ham. ex D. Don	Indi dosu	Fabaceae	Shrub	3	0.026

S.N.	Scientific name	Short form	Family	Life form	Frequency %	NMDS1
94	<i>Lactuca sativa</i> L.	Lact sati	Asteraceae	Herb	3	-0.850
95	<i>Lagerstroemia parviflora</i> Roxb.	Lage prav	Lythraceae	Tree	61	0.019
96	<i>Lantana camara</i> L.	Lant cama	Verbenaceae	Shrub	3	-1.063
97	<i>Leea crispa</i> L.	leea cris	Leeaceae	Shrub	2	-1.063
98	<i>Leea macrophylla</i> Roxb. ex Hornem	Leea macr	Leeaceae	Shrub	82	0.084
99	<i>Leucas indica</i> (L.) R. Br. ex Vatke	lecu indi	Lamiaceae	Herb	2	-0.637
100	<i>Litsea monopetala</i> (Roxb.) Pers.	Lits mono	Lauraceae	Tree	112	0.031
101	<i>Lobelia chinensis</i> Lour.	Lobe chine	Lobeliaceae	Herb	40	-0.141
102	<i>Ludwigia hyssopifolia</i> (G. Don) Exell	Ludw hyss	Onagraceae	Herb	2	0.277
103	<i>Lygodium japonicum</i> (Thunb.) Sw.	Lygo japo	Schizaeaceae	Fern	68	0.220
104	<i>Maesa chisia</i> Buch.-Ham. ex D. Don	Maes chis	Myrsinaceae	Tree	80	0.031
105	<i>Mallotus philippensis</i> (Lam.) Muell.	Mall phil	Euphorbiaceae	Tree	82	0.138
106	<i>Marsdenia roylei</i> Wight	Mars royl	Asclepiadaceae	Climber	18	0.003
107	<i>Mentha spicata</i> L.	Ment spic	Lamiaceae	Herb	1	-0.637
108	<i>Mikania micrantha</i> Kunth.	Mika micr	Asteraceae	Climber	60	-0.014
109	<i>Miliusa velutina</i> (Dunal.) Hook.	Mili velu	Meliaceae	Tree	96	0.256
110	<i>Mitragyna pravifolia</i> (Roxb.) Korth.	Mitr prav	Rubiaceae	Tree	3	-0.942
111	<i>Momordica cochinchinensis</i> (Lour.) Spreng.	Momo coch	Cucurbitaceae	Climber	4	-0.800
112	<i>Murraya koenigii</i> (L.) Spreng.	Murr koen	Rutaceae	Tree	78	0.031
113	<i>Oberonia</i> sp.	Ober spp	Orchidaceae	Orchid	6	-0.487
114	<i>Ophioglossum reticulatum</i> L.	Ophio reti	Ophioglossaceae	Fern	66	0.084
115	<i>Oplismenus compositus</i> (L.) P. Beauv	Opli comp	Poaceae	Herb	10	0.296
116	<i>Oxalis corniculata</i> L.	Oxal corn	Oxalidaceae	Herb	21	-0.489
117	<i>Parochetus communis</i> D. Don	Paro comm	Fabaceae	Herb	8	0.171
118	<i>Parthenium hysterophorus</i> L.	Part hyst	Asteraceae	Herb	9	-0.866
119	<i>Paspalum distichum</i> L.	Pasp dist	Poaceae	Herb	33	-0.221
120	<i>Persicaria barbata</i> (L.) Hara	Pers barb	Polygonaceae	Herb	15	-0.450
121	<i>Persicaria pubescens</i> (Blume) H. Hara	Pers pube	Polygonaceae	Herb	20	-0.162
122	<i>Phoenix sylvestris</i> Roxb.	Phoe sylv	Palmae	Tree	33	0.039
123	<i>Phyllanthus amarus</i> Thonn.	Phyl amar	Euphorbiaceae	Herb	5	-0.931
124	<i>Phyllanthus emblica</i> L.	Phyl embl	Euphorbiaceae	Tree	3	0.163
125	<i>Phyllanthus niruri</i> L.	Phyl niru	Euphorbiaceae	Herb	93	-0.003
126	<i>Pilea symmeria</i> Wedd.	Pile symm	Urticaceae	Herb	3	0.428
127	<i>Piper logum</i> L.	Pipe logu	Piperaceae	Climber	15	0.276
128	<i>Pogostemon benghalensis</i> (Burm. f.) Kuntze	Pogo beng	Lamiaceae	Shrub	66	-0.182
129	<i>Pouzolzia hirta</i> Blume ex Hassk	Pouz hirt	Urticaceae	Herb	50	-0.181
130	<i>Premna integrifolia</i> L.	Prem inte	Verbenaceae	Tree	4	0.188
131	<i>Pteris aspericaulis</i> Wall. ex. J. Agardh	Pter aspe	Pteridaceae	Fern	15	0.237
132	<i>Rumex dentatus</i> L.	Rume dent	Polygonaceae	Herb	1	0.302
133	<i>Rumex vesicarius</i> L.	Rume vesi	Polygonaceae	Herb	41	-0.003
134	<i>Saccharum procerum</i> Roxb.	Sacc proc	Poaceae	Herb	11	-0.069
135	<i>Salvia coccinea</i> Buc'hoz ex Etl.	Salv cocc	Lamiaceae	Shrub	16	-0.632
136	<i>Schleichera oleosa</i> (Lour) Oken.	Schl oleo	Sapindaceae	Tree	4	0.027
137	<i>Scutellaria discolor</i> Colebr.	Scut disc	Lamiaceae	Herb	33	0.197
138	<i>Semecarpus anacardium</i> L. f.	Seme anac	Anacardiaceae	Tree	2	0.216
139	<i>Setaria pallidifusca</i> Hubb.	Seta pall	Poaceae	Herb	15	-0.208
140	<i>Shorea robusta</i> Gaertn.	Shor robu	Dipterocarpaceae	Tree	116	0.031
141	<i>Sida acuta</i> Burm. f.	Sida acut	Malvaceae	Tree	17	-0.242
142	<i>Sida rhombifolia</i> L.	sida rhom	Malvaceae	Shrub	18	-0.012

S.N.	Scientific name	Short form	Family	Life form	Frequency %	NMDS1
143	<i>Smilax aspera</i> L.	Smil aspe	Liliaceae	Climber	5	0.558
144	<i>Solanum surattense</i> Burm. f.	Sola sura	Solanaceae	Shrub	11	-0.393
145	<i>Spondias pinnata</i> (L. f.) Kurz.	Spon pinn	Anacardiaceae	Tree	16	0.277
146	<i>Stephania japonica</i> (Thunb.) Miers	Step japo	Menispermaceae	Climber	9	-0.764
147	<i>Strobilanthes atropurpureus</i> Nees	Stro atro	Acanthaceae	Herb	18	-0.249
148	<i>Syzygium cumini</i> (L.) Skeels	Syzy cumi	Myrtaceae	Tree	19	0.099
149	<i>Terminalia alata</i> Heyneex. Roth	Term alat	Combretaceae	Tree	41	0.194
150	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Term bell	Combretaceae	Tree	10	-0.011
151	<i>Terminalia myriocarpa</i> Van Heurck & Müll. Arg.	Term myri	Combretaceae	Tree	2	-1.063
152	<i>Themeda arundinacea</i> (Roxb.) A. Camus	Them arun	Poaceae	Herb	8	-0.376
153	<i>Thespesia lampas</i> (Cav.) Dalz	Thes lamp	Malvaceae	Tree	12	0.366
154	<i>Trewia nudiflora</i> L.	Trew nudi	Euphorbiaceae	Tree	42	0.016
155	<i>Trichosanthes anguina</i> L.	Tric angu	Cucurbitaceae	Climber	10	-0.942
156	<i>Typha angustifolia</i> L.	Typh angu	Typhaceae	Herb	1	-0.637
157	<i>Vanda tessellata</i> (Roxb.) Hook. ex G. Don.	Vand tess	Orchidaceae	orchid	28	-0.289
158	<i>Ziziphus mauritiana</i> Lam.	Zizi maur	Rhamnaceae	Tree	3	-0.433

Appendix 2. Results of generalized linear model.

S. No.	Variable	Residual df	Residual deviance	Deviance	R ²	F-value	Pr(>F)
1	Sppn ~ NMDS1	19	2204.2				
		18	20.01	2184.2	1.0	2038.2	<2.2e ⁻¹⁶ ***
2	Climb_rich~NMDS1	19	34.95				
		18	6.314	28.636	0.8	82.204	3.946 e ⁻⁰⁸ ***
3	Fern_rich~NMDS1	19	17.75				
		18	3.7559	13.994	0.8	67.787	1.624 e ⁻⁰⁷ ***
4	Herb_rich~NMDS1	19	1342.55				
		18	38.41	1304.1	1.0	638.58	1.643 e ⁻¹⁵ ***
5	Orchid_rich~NMDS1	19	19.8				
		18	13.471	6.3289	0.3	10.74	0.0042 **
6	Shrub_rich~NMDS1	19	159.75				
		18	8.229	151.52	0.9	334.8	4.448 e ⁻¹³ ***
7	Tree_rich~NMDS1	19	80.55				
		18	4.655	75.895	0.9	292.85	1.394 e ⁻¹² ***
8	Alt~NMDS1	19	5407.2				
		18	31.2	5376	1.0	3278.3	<2.200 e ⁻¹⁶ ***
9	Sppn~NMDS2	19	2204.2				
		18	28.42	2175.8	1.0	1367	<2.200 e ⁻¹⁶ ***
10	Climb_rich~NMDS2	19	34.95				
		18	6.333	28.617	0.8	81.913	4.051 e ⁻⁰⁸ ***
11	Fern_rich~NMDS2	19	17.75				
		18	4.0383	13.712	0.8	63.097	2.711 e ⁻⁰⁷ ***
12	Herb_rich~NMDS2	19	1342.55				
		18	36.35	1306.2	1.0	626.7	1.937 e ⁻¹⁵ ***
13	Orchid_rich~NMDS2	19	19.8				
		18	25.274	-5.4744	-0.3		ns
14	Shrub_rich~NMDS2	19	159.75				
		18	15.406	144.34	0.9	165.66	1.620 e ⁻¹⁰ ***
15	Tree_rich~NMDS2	19	80.55				
		18	4.518	76.032	0.9	301.33	1.094 e ⁻¹² ***
16	Alt~NMDS2	19	5407.2				
		18	30.4	5376.8	1.0	3366.5	< 2.200 e ⁻¹⁶ ***

Research

Trade and conservation of medicinal and aromatic plants in western Nepal

Dipesh Pyakurel^{1,2*}, Indira Bhattarai (Sharma)¹ and Suresh Kumar Ghimire³

¹Faculty of Agriculture, Agriculture and Forestry University, Nepal

²Faculty of Science, University of Copenhagen, Denmark

³Central Department of Botany, Tribhuvan University, Nepal

Abstract

This paper quantifies the volume and value of medicinal and aromatic plants (MAPs) traded from Baitadi district in western Nepal. Information related to trade, in the Fiscal Year 2014/015, was collected through pre-tested structured questionnaire. Harvesters ($n = 24$) and the entire population of sub-local ($n = 35$) and local ($n = 7$) traders were interviewed to quantify the value and volume of MAP trade from the district. Results showed that 15.2% households were engaged in harvesting of MAPs for trade, contributing 9.5% of the total cash income giving an impression that MAPs provide supplementary source of income. A total of 731.5 tons of MAPs, comprising 17 products (from 17 species) with total value of NRs 31.3 million (US\$ 315,175) was traded from the district. Rittha (*Sapindus mukorossi*), tejpat (*Cinnamomum tamala*) and pakhanved (*Bergenia ciliata*) were traded in higher amounts, representing 356.5, 171.0 and 70.0 tons, respectively. The cumulative value of trade was highest for satuwa (*Paris polyphylla*) with NRs 7.7 million. Nepalgunj is the favoured 'transit' city for the export of MAPs to India accounting for more than 82% of MAPs sourced from Baitadi. Satuwa has been identified as the most vulnerable species mainly due to premature and over-harvesting in response to high price and growing regional demand coupled with slow growth and high habitat specificity that may results in impaired population growth, thus demanding a detailed bio-physical studies. The study finds that strict regulatory mechanism like ban proves to be less effective in species conservation and suggest adopting alternative management strategies.

Key-words: Baitadi, harvesters, income, MAP trade, *Paris polyphylla*, traders, wholesalers.

Introduction

Millions of people around the globe depend on forest resources for cultural needs and to diversify their source of income (Shackleton *et al.* 2011). The dependency is even higher in developing countries where people rely on forest resources especially for food, fuel, fodder, medicine and construction materials to meet subsistence needs and generate cash income (Sunderlin *et al.* 2005). Angelsen *et al.* (2014) reported that 28% of total household income in developing countries come from environmental resources, out of which 77% from the natural forests. Medicinal and aromatic plants (MAPs), a subset of non-timber forest products, are one of the major forest resources offering rural communities to generate cash income (Ruiz Pérez and Byron 1999; Shackleton and Shackleton 2004). The importance of MAPs is even higher in recent years with the expansion of global herbal market, as Vasisht *et al.* (2016) estimated the annual trade of MAP materials to be US\$ 33 billion in 2014.

MAPs from the Himalayan region are harvested to cure various ailments since millennia as described in 4500 years old Rigveda (Malla and Shakya 1999). Ayurvedic system in India, that dates back to 3000 years, sourced medicinal plants from the Himalayan region (Farnsworth and Soejarto 1991).

Nepal's position in the centre of the Himalaya enables it to host thousands of medicinal plants and is one of the major suppliers of MAPs to India since time immemorial. However, the quantification of trade had started few decades ago when Edwards (1996) estimated 10,000 tons of MAPs from more than 100 species was harvested in and from Nepal. Few years later, Olsen (2005); based on 1997/98 survey, estimated the export of 14500 tons of crude MAPs worth US\$ 16 million to India and China. Recently, using the UN COMTRADE data, Ghimire *et al.* (2015) estimated the export of 10770 tons of MAPs worth US\$ 60.09 million from Nepal in 2014. Likewise, several other trade-related studies were conducted in the past few decades that focused on (i) quantifying trade of specific MAP products of a district (e.g., Hertog and Wiersum 2000; Maraseni *et al.* 2006; Koirala *et al.* 2010; Shrestha and Bawa 2013); (ii) listing the traded MAPs of a district without quantification (e.g., Pandit and Thapa 2003; Bista and Webb 2006); or (iii) listing the traded MAPs of Nepal (e.g., Bhattarai and Ghimire 2006). However, there are very few studies (e.g., Olsen 1998; Humagain and Shrestha 2009) that quantifies the traded MAPs at district level in Nepal. The comprehensive district level trade studies help to understand the livelihoods implications, estimate the contribution of forest products

*Corresponding author. e-mail - dipeshpyakurel@gmail.com; phone, +9779841550852

to local economy and to formulate and design local level development and conservation strategies.

The aggregated effect of increasing global demand, rise in price and contribution to household and local income could lead the MAP species towards overexploitation and may cause threats to their sustainability. Further, studies suggested that most of the MAP species traded in and from Nepal are wild harvested (Olsen 2005; Ghimire *et al.* 2015) and are considered as common property resource (Pandit and Thapa 2003). Most of the high-value MAPs are long-lived perennial with slow growth and show high habitat specificity (Ghimire 2008). Premature and over-harvesting by uprooting or cutting of whole plant is one of the serious concerns for the sustainability of such species (Ghimire *et al.* 2008; Deb *et al.* 2015). It has been estimated that 85% of medicinal plants of Nepal are harvested from Mid- and far-western regions of Nepal (GIZ 2011). Far-west Nepal alone contribute about one third of the total traded volume from Nepal (Kunwar *et al.* 2015). This paper aims to (i) quantify the trade of MAPs and identify the most commonly traded species, (ii) estimate the contribution of MAPs to household income, and (iii) assess the local conservation status of traded MAPs of Baitadi district of far-western Nepal.

Materials and Methods

STUDY AREA

The study was conducted in Baitadi district (29°19' to 29°40'N and 80°22' to 80°50'E) of far-western Nepal (now province number 7 under new federal system). The district is bordered by Darchula in the north, Dadeldhura in the south, Bajhang and Doti in the east, and Uttarakhand state of India in the west. Baitadi district has 1,519 km² area but considerable variation in elevation (390 to 2950 m asl) enables the district to have tropical to temperate climate. The area experiences a maximum temperature of 32.4°C and minimum of 0.5°C, with average annual rainfall of 1242.5 mm (UNFCO 2013). The population of the district is 250898 with a total household of 45167. The overall literacy rate is 63% and the human development index is 0.391 (CBS 2012).

Most of the village development committees (VDCs) of the district are accessible to road network. Three major highways of far-western Nepal pass through Baitadi district: (i) Dashrath Chand highway from Dadeldhura to Jhulaghat (89 km falls in Baitadi), (ii) Jaya Prithibi Bahadur Singh Marg (highway) from Khodpe to Chainpur, Bajhang (29.6 km in Baitadi), and (iii) Baitadi-Darchula highway from Satabanjh

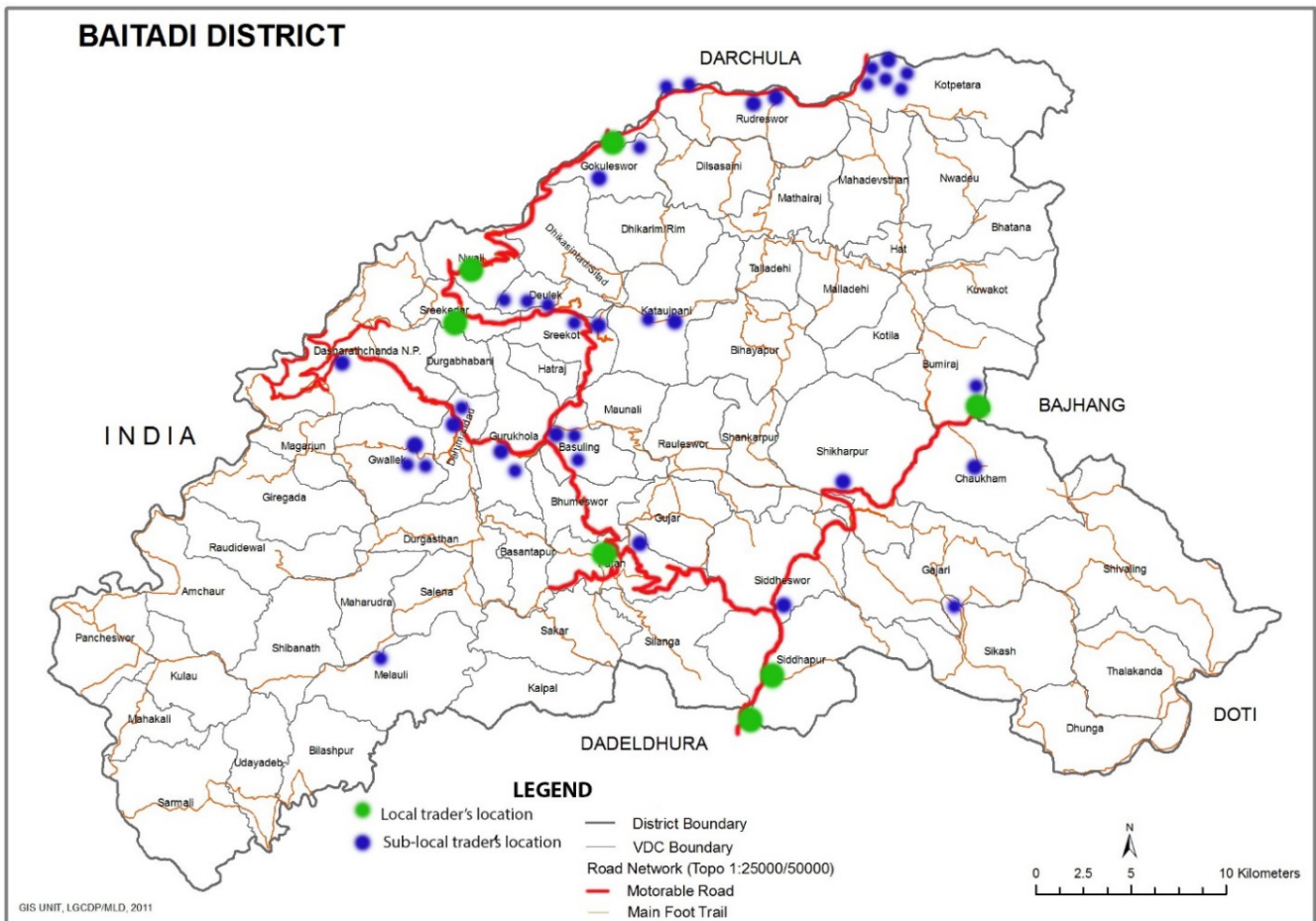


Figure 1. Study district showing the location of local and sub-local traders (map adopted from LGCDP 2014).

to Khalanga, Darchula (53 km in Baitadi). Likewise, there are several earthen roads, *viz.* Gokuleshowr to Kotpetra, Patan to Pancheshwor and seasonal agricultural roads, together making up 240 km of extensive road network within the district (DoLIDAR 2016). Each village or hamlet has minor market centre, whereas major market centres are located at Khodpe, Patan, Dehimandu, Gothalapani, Gokuleshwor, Rudreshwor and Nwali, or along the highways (Figure 1).

Rittha (*Sapindus mukorossi*), kaulo (*Persea odoratissima*), tejpat (*Cinnamomum tamala*), amala (*Phyllanthus emblica*), pakhanved (*Bergenia ciliata*), chutro (*Berberis* spp.), sugandhawal (*Valeriana jatamansi*) and jiwanti (*Dendrobium* sp.) are the major medicinal plants harvested and traded commonly from Baitadi district (DoF 2015). Lichens were one of the major traded MAPs of Baitadi till 2011 (Kala 2003) but the trade was prohibited after Government of Nepal's decision to ban lichen for harvest, use, trade and export in early 2011 (GoN-MFSC 2011).

DATA COLLECTION

Socio-economic and trade data on MAPs for fiscal year 2014/015 (Shrawan 2071 to Ashad 2072) were collected from September to October, 2015 through interviews with harvesters and traders using pre-tested structured questionnaire. Verbal consent, from each respondent, was obtained before interview. Interviews were recorded in Samsung SM-T365 tablet using ODK collect 1.4.10 software (<https://odk-collect.en.uptodown.com/android>) and the data was downloaded in Microsoft excel. The interview was mainly focused on socio-economic (age and experience of respondents and total household income) and trade (number of actors in the district, traded products, including value and volume of trade, advance payments and destination of sale) information. We quantitatively interviewed 24 harvesters, 35 sub-local traders and 7 local traders. We used Olsen and Bhattarai (2005) typology to describe the actors. Harvesters harvest MAPs either from wild or from cultivation and sell to sub-local traders who generally operates from villages. Sub-local traders sell to local traders who operates from district headquarters and major market centres. Local traders transport MAPs out of the district and sell to central wholesalers who mostly reside in Tarai cities or in Kathmandu and finally export to regional wholesalers in India, China and other countries.

The number of harvesters interviewed was less as they were meant to validate the price mentioned by sub-local and local traders. We interviewed the entire population of local traders because we were interested in quantifying the value and volume of trade from the district, and it was local traders who transport MAPs out of the district. Care was taken not to record the products harvested from adjoining districts. We used the information of central wholesalers ($n = 35$, purchasing

MAPs from Baitadi district) to estimate the advance payments and trade destination.

Samples and photographs of traded product were taken, and confused species were taxonomically identified at national herbarium (KATH) in Godawari, Kathmandu. Catalogue of life (Hassler 2017) was referred to validate the scientific name with author citation.

We used rapid vulnerability assessment (RVA) method developed by Cunningham (1996) and adopted by other researchers in Nepal (Ghimire and Aumeeruddy-Thomas 2005; Shrestha and Shrestha 2012) to identify the vulnerability of traded MAPs of the district. However, we excluded all mushrooms and lichen from the analysis due to unavailability of sufficient data on habitat, population size and elevation ranges within Nepal. Similarly, *Dendrobium* sp. was also excluded. Thus we used 13 species traded from the district and 10 predictors of vulnerability with score ranging from 1 to 4: 1 being lowest and 4 being highest vulnerability (Annex 1). Transect walk and empirical observations, interviews and literature were the basis for scoring the vulnerability. Empirical observations and interview gave information on life form, parts used, unit price, trade volume, and habitat specificity and local population size. Likewise, published literature (Polunin and Stainton 1984; Press *et al.* 2000; Watson *et al.* 2011) and online databases (IUCN 2017; Hassler 2017) were referred for global distribution, nativity, habitat specificity, altitudinal span and conservation status.

DATA ANALYSIS

Traded volumes of sub-local and local traders were treated separately to avoid duplication. MAPs that were traded within same actor level (e.g., within local traders) were carefully traded to avoid double counting. Likewise, MAPs that were still in the stock (i.e., not sold due to various reasons) was also deducted for exact quantification of trade from the district. Person correlation coefficients were calculated between age and experience of harvesters, sub-local and local traders. Person correlations and other basic statistics were performed using Stata 12.1 (StataCorp 2011).

Results

CHARACTERISTICS OF HARVESTERS AND TRADERS

In Baitadi district, 6875 households (15.2%) were engaged in harvesting of medicinal plants for trade (traders' reference). There was a positive correlation ($r = 0.463$; $p < 0.001$) between age (mean 39.2 y) and experience (7.9 y) of harvesters. Both male and female household members were engaged in harvesting. Harvesters, in average, harvest two MAP species. Almost 50% harvesters considered themselves to have average wealth. The average household income of harvesters was NRs 135625 (US\$ 1365.8; using the mean conversion rate

Table 1. Characteristics of harvesters ($n = 24$). Data shown are minimum, maximum and average value of socio-economic characteristics*.

Characteristics	Min	Max	Average	SD
Age (year)	15	67	39.2	15.7
Experience in harvest (year)	3	40	7.9	7.1
Total cash income (NRs)	20000	350000	135625.0	68339.1
Cash income from MAPs (NRs, %)	500 (<1%)	43600 (43.6%)	12882.0 (9.5%)	11265.0
Products harvested in average (no.)	1	4	2.1	0.3

*Min – minimum, Max – maximum, SD – standard deviation.

Table 2. Characteristics of sub-local ($n = 35$) and local ($n = 7$) traders*.

Characteristics	Sub-local trader				Local trader			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Age (year)	20	62	41.1	11.5	27	50	39.0	8.7
Experience in trade (year)	2	15	7.3	3.6	2	20	11.5	6.1
Share of MAPs to total income (%)	1	100	19.4	21.0	25	100	67.1	22.7
Advance paid by (%)	0	50	2.3	5.1	0	50	32.2	13.0
Number of products traded in average	1	8	2.8	1.8	2	12	8.7	4.3
MAPs storage time (months)	1	7	2.7	1.3	2	5	4.1	1.1
No of harvesters sold to	40	500	150	98.6	70	500	231	159.4

*Min – minimum, Max – maximum, SD – standard deviation.

of US\$ 1 = NRs 99.3 in 2014/015). Contribution of MAPs to total cash income was 9.5%, that ranged from less than 1% to 43.6% (Table 1) thereby providing a supplementary source of income for harvesters.

Positive correlation existed between age (mean 41.1 y) and experience (7.3 y) of sub-local traders ($r = 0.575$; $p < 0.001$), as well as between age (39 y) and experience (11.5 y) of local traders ($r = 0.742$; $p < 0.001$). Almost 19.5% (in the case of sub-local traders) and 67% (local traders) of their income come from MAPs trade (Table 2). Sub-local traders traded three MAP products in average, the major being rittha (*Sapindus mukorossi*), tejpat (*Cinnamomum tamala*) and pakhanved (*Bergenia ciliata*), whereas local traders, in average, traded nine products. Sub-local and local traders in an average purchased MAPs respectively from 150 and 231 harvesters (Table 2). But local traders in most of the cases purchased from sub-local traders.

TRADE SYSTEM

Harvesters collect the wild crafted ($n = 12$) and cultivated ($n = 5$) MAP species (Table 3) and perform primary processing, like cleansing, drying and packaging. The products were then sold to sub-local traders at minor markets or to local traders at major market centres. Generally, harvesters receive advance in kind (foodstuffs and clothing) from sub-local traders throughout the year therefore they receive very little cash advance (less than 3%) (Table 2). Sub-local traders often have mutual business relationship with harvesters of a specific location. Sub-local traders keep on purchasing MAPs till the volume is enough for a tractor/truck load (depending upon road condition). Sub-local

traders store MAPs for little more than two and half months. They receive 32% advance payment in cash from local traders (Table 2). Once the volume is fulfilled, local traders transport MAPs from minor markets to their warehouse located at major market centres. There were no any local traders in the district headquarters, the possible reason could be the location. The district headquarters is 18.5 km west to the highway and cost of transportation would be high, along with multiple loads and unloads. The usual time of storage was four and half months for local traders after which the products were finally transported to Nepalgunj, Mahendranagar and Kathmandu for export and domestic consumption (Table 3). Local traders received 22% advance from central wholesalers.

VOLUME AND VALUE OF TRADE

We used local traders' data to quantify the value and volume of trade from the district. Total volume of trade was 731.5 tons, from 17 products constituting 17 species (Table 3). *Sapindus mukorossi* was traded in highest volume (356.5 tons), followed by *Cinnamomum tamala* (Tejpat, 171 tons) and *Bergenia ciliata* (70.1 tons). The top five and top 10 products respectively contributed to 93.8% and 99.2% of total traded volume. The trade also constituted government banned lichens, but the trade was in minimal quantity. Furthermore, only sub-local traders mentioned the trade of lichen. Local traders rarely disclosed the unofficial trade as they were aware about the legal provisions.

The cumulative value of trade was NRs 31.3 million (US\$ 315,175). The cumulative value of trade was highest for satuwa (*Paris polyphylla*) with NRs 7.7 million, followed by

Cinnamomum tamala (tejpat, NRs 6.4 million) and *Sapindus mukorossi* (NRs 5.3 million). The top five products contributed to 76.4% of the total trade value, whereas the top 10 products made up 95.3%. Guchi chyau (*Morchella esculenta*) was the most expensive MAP (NRs 12000 per kilogram) harvested and traded from the district, but the production was very low.

More than 82% of traded volume of MAPs harvested from Baitadi were exported to India via Nepalgunj, a city located at the mid-western Tarai of Nepal, making this the most preferred 'transit' city, followed by Mahendranagar (far-western Tarai) and Kathmandu (the capital city, Table 3). Species, like *Paris polyphylla* and *Dendrobium* sp. were exported to various cities of China via Kathmandu, these were mostly airlifted. It has not been long since the trade of *Ganoderma lucidum* has commenced in Nepal. Two local traders from Siddheshwor VDC disclosed the trade of *Ganoderma lucidum* to China via Kathmandu. The most remarkable was the transport of 95 tons of *Sapindus mukorossi* to Kathmandu (Table 3). India was the major destination of *Sapindus mukorossi* for several

years and transporting *Sapindus mukorossi* to Kathmandu signifies that whether it was being domestically processed, or export to other countries via Kathmandu, the actual reason remained unknown.

VULNERABILITY

Out of 17 traded species, seven were listed in conservation/protection categories (Table 4). *Acorus calamus* has been listed as least concern in IUCN red list. *Swertia chirayita* has been listed as vulnerable in IUCN and CAMP (Conservation Assessment Management Plan) list. Though bulk of the supply of *Swertia chirayita* are sourced from cultivation, especially from eastern Nepal, there are no reports of mass scale cultivation in the west. *Asparagus racemosus* and *Paris polyphylla* are vulnerable in CAMP list. All wild orchids (including *Dendrobium* sp.) are in CITES appendix II. Raw export of *Valeriana jatamansi* is banned by the Government of Nepal but valerian oil and marc (residue after extraction of essential oil) obtained from distillation can be exported. We

Table 3. MAPs traded from Baitadi district in FY 2014/015.

Scientific name (family)	Trade name	Volume traded (kg)	Rate (NRs/kg)	Amount (NRs)	Destination cities, with volume (kg) ¹
<i>Acorus calamus</i> L. (Acoraceae)	Bojho	4,150	60.75	252,113	NEP
<i>Asparagus racemosus</i> Willd. (Asparagaceae)	Kurilo	1,000	50	50,000	MAH
<i>Berberis aristata</i> DC. (Berberidaceae)	Chutro	11900	29	345100	NEP (8900), MAH (3000)
<i>Berberis asiatica</i> Roxb. ex DC. (Berberidaceae)					
<i>Bergenia ciliata</i> (Haw.) Sternb. (Saxifragaceae)	Bhiniti, Pakhanved	70,100	25.6	1,794,560	NEP
<i>Cinnamomum tamala</i> (Buch.-Ham.) Th. G.G. Nees (Lauraceae)	Tejpat	171,000	37.16	6,354,360	NEP
	Dalchini	15,550	68.25	1,061,288	NEP (14550), MAH (1000)
<i>Dendrobium</i> sp. (Orchidaceae)	Jiwanti	745	350	260,750	KTM
<i>Everniastrum nepalense</i> (Taylor) Hale ex Sipman (Parmeliaceae)	Jhyau	400	50	20,000	BHA
<i>Ganoderma lucidum</i> (Curtis) P. Karst. (Ganodermataceae)	Livlite chyau	420	3925	1,648,500	NEP (220), KTM (200)
<i>Machilus odoratissima</i> Nees (Lauraceae)	Kaulo	39,500	46.6	1,840,700	NEP (28500), MAH (11000)
<i>Morchella esculenta</i> (L.) Pers. (Morchellaceae)	Guchi chyau	25	12000	300,000	KTM
<i>Paris polyphylla</i> Sm. (Melanthiaceae)	Satuwa	2,085	3700	7,714,500	KTM
<i>Phyllanthus emblica</i> L. (Phyllanthaceae)	Amala	49,500	55.6	2,752,200	NEP
<i>Sapindus mukorossi</i> Gaertn. (Sapindaceae)	Rittha	356,500	14.75	5,258,375	NEP (251500), KTM (95000), MAH (10000)
<i>Swertia chirayita</i> (Roxb.) Buch.-Ham. ex C.B. Clarke (Gentiniaceae)	Tite, Chiraito	2,175	232	504,600	NEP
<i>Valeriana jatamansi</i> Jones (Caprifoliaceae)	Samayo, Sugandhawal	5,450	163.3	889,985	NEP (3950), MAH (1500)
<i>Zanthoxylum armatum</i> DC. (Rutaceae)	Timur	1,000	250	250,000	NEP

¹KTM – Kathmandu; NEP – Nepalgunj; MAH – Mahendranagar; BHA- Bhairahawa.

Table 4. Rapid vulnerability assessment of traded MAPs based on ten predictors (see Appendix 1 for detail explanation of each predictor). Values in parentheses represent RVA scored for a particular predictor.

MAPs	Life form ¹	Parts used ²	Global distribution ³	Elevation span ⁴ (in m)	Trade volume (kg)	Unit price (NRs/kg)	Resource origin ⁵	Conservation/ protection status ⁶	Habitat specificity ⁷	Local population size ⁸	Total RVA score
<i>Paris polyphylla</i>	Ph (2)	Rh (4)	Pan-Himalaya & SE Asia (2)	2260 (2)	2,085 (3)	3700 (4)	WD (4)	CAMP V (2)	HS1 (4)	ES (4)	31
<i>Bergenia ciliata</i>	Ph (2)	Rh (4)	Pan-Himalaya (3)	1600 (3)	70,100 (4)	25.6(2)	WD (4)	NA (1)	HS2 (3)	MLSS (2)	28
<i>Valeriana jatamansi</i>	Ph (2)	Rh (4)	Pan-Himalaya & SE Asia (2)	2800 (1)	5,450 (3)	163.3 (3)	WTC (3)	GoN RE; CAMP V (3)	HS2 (3)	MSSL (3)	27
<i>Machilus odoratissima</i>	Tr (4)	Br (3)	Pan-Himalaya & SE Asia (2)	1900 (2)	39,500 (4)	46.6(2)	WD (4)	NA (1)	HG1 (2)	MLSS (2)	26
<i>Swertia chirayita</i>	An/Bn (1)	Wp (4)	Pan-Himalaya (3)	1800 (2)	2,175 (3)	232 (3)	WTC (3)	IUCN V; CAMP V (3)	HG2 (1)	MSSL (3)	26
<i>Acorus calamus*</i>	Ph (2)	Rh (4)	Holarctic (1)	1150 (3)	4,150 (3)	60.75 (2)	WD (4)	IUCN LC (1)*	HS2 (3)	MLSS (2)	25
<i>Berberis aristata</i>	Sh (3)	Br (3)	Pan-Himalaya (3)	2850 (1)	5950 (3)	29 (2)	WD (4)	NA (1)	HG1 (2)	MLSS (2)	24
<i>Berberis asiatica</i>	Sh (3)	Br (3)	Pan-Himalaya (3)	2500 (1)	5950 (3)	29 (2)	WD (4)	NA (1)	HG1 (2)	MLSS (2)	24
<i>Cinnamomum tamala</i>	Tr (4)	Br/Lf (3)	Pan-Himalaya, E & SE Asia (2)	1900 (2)	1865550 (4)	37-68 (2)	WCC (2)	NA (1)	HG2 (1)	MLSS (2)	23
<i>Phyllanthus emblica</i>	Tr (4)	Fr (2)	Pan-Himalaya, S & SE Asia (2)	1300 (3)	49,500 (4)	55.6(2)	WCC (2)	NA (1)	HG2 (1)	MLSS (2)	23
<i>Sapindus mukorossi</i>	Tr (4)	Fr (2)	Pan-Himalaya, E & SE Asia (2)	800 (4)	356,500 (4)	14.75 (2)	CC (1)	NA (1)	HG2 (1)	MLSS (2)	23
<i>Asparagus racemosus</i>	Sh (3)	Tb (4)	Africa, Asia, Australasia (1)	2100 (2)	1,000 (2)	50 (2)	WCC (2)	CAMP V (2)	HG2 (1)	MLSS (2)	21
<i>Zanthoxylum armatum</i>	Sh (3)	Fr (2)	Pan-Himalaya, S, E & SE Asia (1)	2300 (2)	1,000 (2)	250 (3)	WCC (2)	NA (1)	HG2 (1)	MLSS (2)	19

¹Life form: An/Bn - annual/biennial herb; Ph - perennial herb; Sh - shrub; Tr - tree.

²Parts used: Br - bark; Fr - fruits; Lf - leaves; Rh - rhizomes; Tb - tubers; Wp - whole plants.

³Global distribution: global distribution is based on Press et al. (2000), Watson et al. (2011), Hassler (2017).

⁴Elevation span: elevation span is the difference between maximum and minimum value of elevation records for the species in question drawn from its distribution range in Nepal.

⁵Resource origin: CC - only from cultivation; WCC - wild and small scale commercial cultivation; WTC - wild and trial cultivation; WD - only from wild.

⁶Conservation status: NA - not assigned to any category; CAMP V - Conservation Assessment Management Plan vulnerable (Bhattarai et al. 2002); CITES II - CITES appendix II; GoN B - Government of Nepal ban for collection, transport and trade; GoN RE - Government of Nepal ban for raw export; IUCN V - IUCN vulnerable [*species categorized by IUCN as least concern (IUCN LC) has been considered as non-threatened category].

⁷Habitat specificity: HG1 - habitat generalist 1 (occurring in more than 2 habitats excluding farmlands and wastelands); HG2 - habitat generalist 2 (occurring in more than two habitats including farmlands and wastelands); HS1 - habitat specialist 1 (occurring in single, very specific habitat); HS2 - habitat specialist 2 (occurring in more than one specialized habitat).

⁸Local population size: MLSS (mostly large, somewhere small); MSSL (mostly small, somewhere large); ES (everywhere small).

also recorded the trade of 400 kg of government banned lichen (*Everniastrum nepalensis*) from the district.

We conducted RVA of 13 traded MAP species of Baitadi (excluding 2 species of mushrooms, 1 species of lichen and 1 species of *Dendrobium*) using 10 predictors (Appendix 1, Table 4). *Paris polyphylla* was identified as the most vulnerable species with vulnerability score of 31 (Table 4). There were several reasons for the high vulnerability of this species, but the major ones were (i) destructive harvesting of rhizomes, (ii) high market price, (iii) high habitat specificity (growing in forests with thick crown cover and moist places), and (iv) harvesting only from wild source. More than 2 tons of *Paris polyphylla* with average unit price of NRs 3700 per kg were harvested from wild source in the district (Table 3). Two, out of seven, local traders purchased *Paris polyphylla* and both perceived the declining population due to premature and over harvesting. Usually, harvesting of *Paris polyphylla* should be done after flowering and seed dispersal during October, but it was prematurely harvested due to high price and escalating demand in the region, leading to its declining status in the wild.

Other vulnerable species were *Bergenia ciliata* and *Valeriana jatamansi* with vulnerability score of 28 and 27, respectively; and *Machilus odoratissima* and *Swertia chirayita* each with the score of 26. The least vulnerable species was *Zanthoxylum armatum* with the score of 19. The top eight species in the vulnerable list (Table 4) were mostly harvested from wild, showing that MAPs harvested from wild without adopting any cultivation practice are always at high risk.

Discussion

ECONOMIC CONTRIBUTION AND TRADE

More than 15% households of Baitadi district were engaged in commercial harvesting of MAPs contributing to 9.5% of the total annual household income. Our findings on economic contribution was slightly lower than previous studies: Bista and Webb (2006) estimated 11.7% contribution from NTFPs in Baitadi; Kunwar *et al.* (2013) estimated 20% income from sale of MAPs in Darchula, Baitadi and Dadeldhura; and Olsen and Larsen (2003) estimated 12% contribution of MAPs in higher elevations in Nepal. The contribution of MAPs to the overall income was low because of two main reasons: (i) rather than going for commercial cultivation, communities are adopting small-scale domestication practice by planting few trees (e.g., *Sapindus mukorossi* and *Cinnamomum tamala*) in the backyards or in the edges of agricultural fields providing them with alternative source of income, and (ii) low unit price of traded MAPs of Baitadi with average per unit price of NRs 35.9/kg for top five products that make about 94% of traded volume. But even with the lower price, households are harvesting MAPs for trade because of the extensive road

network in Baitadi that reduces the transportation cost and enables low valued MAPs to find the market. It indicates that though MAPs comprise only a portion of household income, it helps to stabilize household economics by providing supplementary role to the harvesters, as observed by Godoy *et al.* (2000) in the rain forest of Honduras.

Our study quantified the trade of 731.5 tons of MAPs comprising 17 products from Baitadi district in FY 2014/15. Our finding was quite similar to the quantification made by Kala (2003) who mentioned the export of 755 tons (from 16 MAP products) from Baitadi to Pithoragarh (India) for case year 2002. *Sapindus mukorossi*, *Cinnamomum tamala*, *Bergenia ciliata* and *Phyllanthus emblica* were traded in highest quantities in both case years. Among other products, Kala (2003) mentioned the export of 40 tons of lichens, 15 tons of kutki (*Neopicrorhiza scrophulariiflora*) and 10 tons of jatamansi (*Nardostachys jatamansi*). Trade of lichen was legal in 2002, whereas kutki and jatamansi were not found in Baitadi, rather sourced from Darchula. It showed that quantification made by Kala (2003) is overstated by MAPs of Darchula and the trade volume of MAPs sourced specifically from Baitadi district was increased (after deducting the trade volume of lichens, kutki and jatamansi), mostly because of the extensive road network, as stated earlier. There are other studies (ANSAB 2003; Bista and Webb 2006; Kunwar *et al.* 2013) that lists higher number of traded MAPs (range: 22-38 MAPs products) from the district but they lacked quantification. However, the major traded species were similar in all studies.

We recorded the trade of live-lite chyau (*Ganoderma lucidum*) from Baitadi which was not recorded earlier. At the same time, majitho (*Rubia manjith*), halhale (*Rumex nepalensis*) and kakadsinghi (*Pistacia chinensis* subsp. *integerrima*) which were reported previously (e.g., by Kala 2003) were not documented in our study. The appearance and disappearance of MAPs signifies the dynamic nature of trade, probably due new market preferences and declining population of MAPs, but the exact reason is inadequately known.

Till 2002, MAPs of Baitadi and Darchula were exported to India via Julaghat (a small Indian border city west to Jhulaghat of Baitadi, Nepal) but increased political instability in Nepal caused the closure of Indo-Nepal border of Baitadi in 2003 (Bista and Webb 2006). Since then, MAPs of Baitadi and rest of the far-western Nepal are exported to India and China via Nepalese 'transit cities' of Nepalgunj, Mahendranagar and Kathmandu. Nepalgunj has the long history of trading MAPs that dates to centuries. The number of traders and central wholesalers were very high in Nepalgunj in the past because of absence of any regulatory mechanism in the border. Though the import policy of Uttar Pradesh State, Government of India (requirement of transit permit for export to India) dramatically reduced the number of central wholesalers ($n = 12$), significant

volume was still traded from Nepalgunj. For example, Jadibuti Association of Nepal (JABAN) issues the recommendation letter to District Forest Office, Banke (Nepalgunj) for MAPs export and they have recommended to export 4596 tons of MAPs in FY 2014/015 (JABAN 2015), demonstrating that Nepalgunj is still the most favored transit city in Nepal in terms of MAPs export to India. Fourteen out of 17 MAPs were exported to India and only three (*Ganoderma lucidum*, *Paris polyphylla* and *Dendrobium* sp.) were exported to China via Kathmandu.

CONSERVATION

Eleven MAP products from 12 species were wild harvested from community forests or government managed forests. MAPs found in those forest management regimes are considered common property resource and receive intense harvest pressure. However, all MAPs found in a specific area are not equally affected by harvesting pressure (Schippmann *et al.* 2005) as impact of harvest and postharvest recovery is largely determined by availability, market demand, parts harvested and biology/ecology of MAP population (Ghimire *et al.* 2008). *Sapindus mukorossi*, *Cinnamomum tamala*, *Bergenia ciliata*, *Phyllanthus emblica*, *Machilus odoratissima* and *Paris polyphylla* contributed 94.1% of volume and 82.2% of cumulative value of trade from the district, demonstrating high harvest pressure on these MAPs. High market demand of specific MAPs may lead to unsustainable harvesting thereby threatening their survival in wild and affecting the people who depend on these resources.

Fruits of *Sapindus mukorossi* and *Phyllanthus emblica* and leaf of *Cinnamomum tamala* are traded. Harvesting of these plant parts are considered less destructive because they do not directly contribute for reproduction, but intensive harvest of these parts may indirectly affect the reproductive performance (Gaoue and Ticktin 2007). Harvesting of MAPs other than these are considered destructive as removal of roots, bark or whole plants lead to death of plant (Cunningham 1993). Further, species with high economic importance are at risk of overexploitation (Hamilton 2004). *Morchella esculenta*, *Ganoderma lucidum* and *Paris polyphylla* thus are at high risk of overexploitation because of their relatively high trade value. Harvesting of *Morchella esculenta* and *Ganoderma lucidum* involves removal of whole individual and harvesting of *Paris polyphylla* involves the removal of plant and collection of rhizome.

The trade of government banned lichen operates in a small scale using the informal routes, often camouflaged as other products. As per a sub-local trader (ID 100) “we know that lichens are banned for export, but I am purchasing from harvesters, means that there may be more traders who are engaged in trade of lichens”. His statement was true as a

sub-local trader (ID 149) purchased lichen but did not want to disclose the volume. It gives an indication that ban did not work for products that have market demand, rather other regulatory mechanisms (e.g., rotational harvesting, short-term harvest ban in specific areas) should be explored that ensures the sustainability and secure the income for harvesters.

Satuwa (*Paris polyphylla*) was identified as the most vulnerable MAP species of the district and it alone shared 24.6% contribution of cumulative trade value. The price of *Paris polyphylla* increased from NRs 700 per kg in January 2010 (ANSAB 2010) to NRs 3700 per kg in August 2014. This multi-fold increase in price tempted harvesters for premature and over-harvesting from wild. According to a local trader (ID 151) “all satuwa traded in and from Baitadi were harvested wild from Shikharpur and Chaukham VDCs as cultivation of satuwa is not initiated till date”, thereby causing threat to the long-term viability of its populations. The decreasing population of *Paris polyphylla* in wild was well described by another local trader (ID 153) “premature harvesting of MAPs is a major concern. October is the right month for collection, but people start harvesting from August. Five years ago, I used to purchase 40-50 tons of satuwa from Baitadi and Darchula but now I hardly manage to purchase 1-1.5 tons in a year. If situation remains same, I may not be able to purchase satuwa in near future”. The statement indicates the prevailing threat and need of robust plan for MAP conservation. Study by KC *et al.* (2010) reported the unsustainable harvesting of *Paris polyphylla* even though it was harvested only for domestic use in Ghandruk, central Nepal. It was even more surprising that *Paris polyphylla* is not in the conservation list of GoN, CITES Appendix and IUCN red list (www.iucnredlist.org, accessed 13 Dec 2017) despite reports of declining population in the Himalayan region due to the high market demand leading to overexploitation and undocumented trade (Paul *et al.* 2015). The biggest setback to assign the conservation categories may be the unknown population size across the habitat range. Limited global distribution, slow growth rate, habitat degradation, exceptionally high demand and destructive harvesting practice are the major reasons to include *Paris polyphylla* in the global protection list. With these identified threats, a thorough bio-physical study is needed that provides strong basis for Nepal to keep *Paris polyphylla* in any of the national conservation categories.

Conclusions

Nepalese MAPs have been traded for centuries and the demand will continue to rise because of global expansion of herbal market. Nepal must grab this opportunity by initiating commercial cultivation of native traded species (like *Sapindus mukorossi*, *Cinnamomum tamala*, *Zanthoxylum armatum*)

which have established market. Enhanced transportation facilities in the mid-hilly regions makes it ideal place for commercial level cultivation, whereas harvest can be regulated for high-value wild MAPs which are often found in higher elevations. But imposing strict regulation (like ban) seems less effective because species with high trade value are traded one way or another. Therefore, rather than going for strict regulatory mechanism, government must adopt sustainable use and management approach. Though income from MAPs comprise 9.5% of total household income, the trade can help to stabilize the household economics by providing supplementary income. More than 93% of the traded volume is occupied by five MAP species, showing their dominance in trade in one hand and high harvest pressure on the other. Most of the low-value MAPs are still exported to India, whereas few high-value MAPs are exported to China. The study identifies *Paris polyphylla* as the most vulnerable MAP of the district mainly because of large scale collection which may have negative effects on its population given the species biology. Thus, Government of Nepal should conduct integrated trade and bio-physical studies that provides concrete foundation to assign the conservation status for *Paris polyphylla*.

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Annex 1. Predicators and scoring basis for rapid vulnerability assessment of traded MAPs

Predictors	Score (minimum to maximum vulnerability)				Hypothesis
	1	2	3	4	
Life form	Annual or biennial herb	Perennial herb, perennial climbing herb	Shrub, woody climber	Tree	Slow growing trees are more vulnerable than fast growing annual herbs
Parts used	Leaf	Fruit, seed, flower, resin	Bark, stem, wood	Root, rhizome, tuber, whole plant	Harvesting of root and whole plants is more destructive than harvesting leaves or fruits and seeds
Geographical distribution	Pluri-regional	Pan-Himalaya and two other phytogeographical domains within Asia	Pan-Himalaya	Himalayan endemic	Widely distributed species are less vulnerable
Altitudinal span (m)	>2400	>1600 to 2400	>800 to 1600	<800	Species with narrow altitudinal span is more vulnerable
Trade volume (kg)	Up to 100	>100-1000	>1000-10000	>10000	Resources are overharvested to meet the high demand
Per unit price (NRs/kg)	Up to 10	>10-100	>100-1000	>1000	Higher price tempt harvester for premature and overharvesting
Resource origin	Only from cultivation (CC)	Wild and small scale commercial cultivation (WCC)	Wild and trial cultivation (WTC)	Only wild harvested (WD)	Resources only harvested from wild are more vulnerable
Conservation status	Not assigned	In one category	In two categories	In more than two categories	Species assigned in more conservation categories are more vulnerable
Habitat specificity	Habitat generalist 2 (occurring in more than two habitats including farmlands & wastelands)	Habitat generalist 1 (occurring in more than 2 habitats excluding farmlands & wastelands)	Habitat specialist 2 (occurring in more than one specialized habitat)	Habitat specialist 1 (occurring in single, very specific habitat)	Plant that grows in specific habitat are more vulnerable
Local population size	Mostly large	Mostly large, somewhere small	Mostly small, somewhere large	Everywhere small	Species growing in smaller areas with small population are more vulnerable

Research

Cytotoxic activity of crude extracts of *Dendrobium amoenum* and detection of bioactive compounds by GC-MS

Mukti Ram Paudel and Bijaya Pant*

Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Abstract

Dendrobium amoenum is an epiphytic orchid used as tonic because it has many derivatives of phenols. The crude extract of this orchid has been shown to have antioxidant activity. The objectives of this research are to explore the cytotoxic activity of antioxidant-rich crude extract against the human cervical carcinoma and glioblastoma cell lines by MTT assay and to detect the compounds by GC-MS. Methanol (DAM) extract of *D. amoenum* showed high cell growth inhibition percentage against the tested cell lines. DAM extract showed high cytotoxic activity against HeLa cells (IC_{50} – 110.22 $\mu\text{g/ml}$) and least activity against U-251 cells (IC_{50} – 550.55 $\mu\text{g/ml}$). Thirteen compounds were detected and identified in the extract. Based on abundance, four major compounds detected were: (E)-13-docosenoic acid; oleic acid; 11-octadecenoic acid, methyl ester; and hexadecanoic acid, 2,3-dihydroxypropyl ester. The cytotoxic activity of DAM extract is probably due to the presence of these bioactive compounds, confirmation of which needs further investigation. The result also highlighted the potential of this orchid as the source of natural anticancer drug and to explore their isolation, identification and characterization.

Key-words: *Dendrobium amoenum*, extract, GC-MS, HeLa, MTT, U-251.

Introduction

Dendrobium is the second largest epiphytic orchid genus in Nepal comprising 30 species, distributed from tropical to temperate climatic regions (Rajbhandari 2014). Most of the *Dendrobium* species are widely used as traditional medicines (Ng *et al.* 2012; Xu *et al.* 2013). Among them, *Dendrobium amoenum* is mostly used as tonic and also for ornamental purposes (Pant and Raskoti 2013; Rajbhandari 2014). Compounds such as bibenzyl derivatives, phenols, phenanthrenes and sesquiterpenoids have been isolated from this species (Majumder *et al.* 1999; Majumder and Bandyopadhyay 2010; Venkateswarlu *et al.* 2002). Due to the presence of such compounds, the crude extract of this orchid has been shown to exhibit antioxidant activity (Paudel *et al.* 2015).

Elucidating the critical events associated with carcinogenesis provide an opportunity for preventing cancer development by inducing apoptosis, particularly with bioactive agent of traditional medicines (Gali-Muhtasib *et al.* 2015; Lowe and Lin 2000). Apoptosis induction in cancer cells is initially identified by morphological changes, including cell shrinkage, membrane blebbing, chromatin condensation and nuclear fragmentation. Apoptosis, is therefore, an important defense mechanism against cancer (Lowe and Lin 2000; Elmore 2007). Cervical carcinoma and glioblastoma cell lines

were used for the present study. Cervical cancer is common form of cancer in woman's uterus in virtually all developing countries and approximately 12% of cancers in women are due to cervical cancer (Joshi and Mishra 2013; Sherpa *et al.* 2015). Brain tumors, which account for 2% of all cancers, are most common childhood tumor after leukemia, constituting approximately 35% of all childhood malignancies and remain the leading cause of cancer-related deaths in children (Panth 2011). The cytotoxic activity of antioxidant-rich bioactive compounds present in crude extract of this orchid was screened by MTT assay (Mosmann 1983). The objectives of the present research are to explore the cytotoxic activity of crude extract of *D. amoenum* on human cervical carcinoma and glioblastoma cell lines and to detect the bioactive compounds present in it by GC-MS (Gas Chromatography and Mass Spectrometry). The result of this study may help to explore the pharmacological importance of this orchid in formulation of anticancer drug.

Materials and Method

PLANT MATERIAL

Stems of *D. amoenum* were collected from Daman of Makawanpur district, central Nepal at 1600 m asl. The plant species was identified with the help of literature and herbarium specimen. The stems were air-dried in the room temperature.

*Corresponding author. e-mail - bijayapant@gmail.com

EXTRACT PREPARATION

Extract was prepared from the dried stems using soxhlet extractor. Pulverized stem was extracted with hexane, chloroform, acetone, ethanol and methanol successively of their increasing polarity in the ratio of 1:10 (w/v) and extracts were stored at 4°C (Mukherjee *et al.* 2012).

IN VITRO CYTOTOXIC ACTIVITY

Cytotoxic activity of *D. amoenum* crude extract was determined by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay (Mosmann 1983; Sargent and Taylor 1989). The cytotoxic activities of the extract were determined against human cervical carcinoma and Human glioblastoma cell lines. Human glioblastoma cells (U-251) and human cervical carcinoma cells (HeLa) were cultured in RPMI (Roswell Park Memorial Institute) 1640 medium and incubated under 5% CO₂ at 37°C for 48 hours to reach 80% confluence. The cells were harvested by gentle scraping with a cell scraper and resuspended in the medium. From the suspension, 5 × 10³ cells in 100 µl medium was dispensed into each well of 96-well cell culture plate and incubated under the same conditions of cell culture for 48 hours to allow adherence and growth of cells. The supernatants were gently aspirated, and 100 µl of extracts were added over a range of four cytotoxic concentrations (100–800 µg/ml) prepared in medium and incubated for 24 hours. Ten µl of 5 mg/ml MTT was added to every well and the plate was re-incubated for further 4 hours. The formazan crystals formed were dissolved in 100 µl DMSO (dimethyl sulfoxide). The plate was then read on a microplate reader (iMark™, Bio-Rad) at 595 nm. The percentage cell growth inhibition was calculated by using following formula:

$$\% \text{ Cell growth inhibition} = 100 - (A(cs) - A(m)) / (A(cc) - A(m)) \times 100\%$$

where; A(cs) was mean absorbance of treated cells with test samples, A(cc) was mean absorbance of untreated cells, and A(m) was mean absorbance without cells.

A dose-response curve was plotted for each extract to calculate the inhibition of cell growth by 50% (IC₅₀). IC₅₀ of extracts was calculated from the regression equation where abscissa represents the series of concentrations of extract and ordinate the triplicate percentage inhibition of cell growth.

GC-MS ANALYSIS

The analysis of the bioactive chemical constituents of different solvent extracts was done by using GCMS-QP2010 Ultra (Shimadzu Europa GmbH, Germany) with following conditions:

GC condition: column oven temperature 100°C; injection temperature 280°C; column flow rate 0.95 ml/min; pre-column pressure 68.3 kPa; split ratio of 0; high purity helium as the carrier gas.

MS condition: ionization mode EI; ion source temperature 280°C; interface temperature 280°C; detector gain mode relative and detector gain 0.91 kV; mass-charge ratio (m/z) 30 to 600.

Ions detected from the extracts were identified by the molecular formula generated by the use of standards and after through literature search.

STATISTICAL ANALYSIS

Data are expressed as mean of three analyses. IC₅₀ of extracts for cytotoxic activity was calculated by using appropriate linear/non-linear regression equation fitted on percentage cell growth inhibition activity of each extract at various concentrations with F-statistic at $p \leq 0.05$. All the analyses were done using statistical software R, version 3.1.2 (R core team 2016).

Results

CRUDE EXTRACT SHOWED IN VITRO CYTOTOXIC ACTIVITY

The percentage cytotoxic activity of *D. amoenum* is shown in Figure 1 (for HeLa cell line) and Figure 2 (for U-251 cell line). *D. amoenum* methanol (DAM) extract showed the highest percentage cytotoxic activity against the tested cell lines. The percentage of growth inhibition of extract-treated cells was increased with the increasing concentration of the extract. *D. amoenum* methanol (DAM) extract showed highest cytotoxic activity against HeLa cells (IC₅₀ – 110.22 µg/ml) than other extracts. Similarly, *D. amoenum* ethanol (DAE) extract showed moderate cytotoxic activity against HeLa cells (IC₅₀ – 149.14 µg/ml; Figure 1). However, *D. amoenum* methanol (DAM) showed least cytotoxic activity against U-251 cells (IC₅₀ – 550.55 µg/ml; Figure 2). Besides DAM, other extracts did not show any response against U-251 cells. The addition of extract on the cell lines showed apoptosis of cells. It was seen after the addition of MTT on the extract-treated cells that

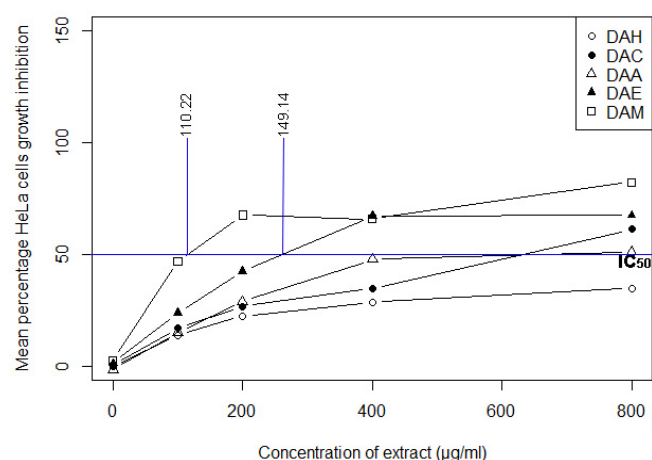


Figure 1. Percentage HeLa cells growth inhibition by crude extracts and their IC₅₀ (DAH – *D. amoenum* hexane, DAC – *D. amoenum* chloroform, DAA – *D. amoenum* acetone, DAE – *D. amoenum* ethanol, DAM – *D. amoenum* methanol).

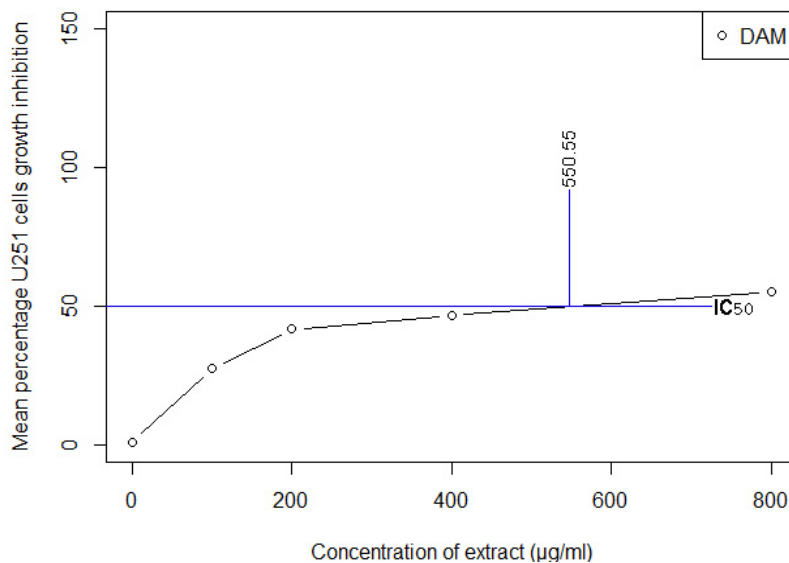


Figure 2. The percentage U-251 cells growth inhibition by DAM extract and its IC₅₀ (DAM – *D. amoenum* methanol).

produced the formazan crystals of HeLa cells (Figure 3) and U-251 cells (Figure 4).

CHEMICAL CONSTITUENTS WERE DETECTED BY GC-MS

Thirteen compounds present in DAM were detected using gas chromatography (GC) and identified through mass spectrometry (MS). The detected and identified compounds

were listed in Table 1 with their retention time (RT), total content in the crude extract (in percentage) and observed mass-charge ratio (m/z). Based on abundance, four major compounds detected were: (E)-13-docosenoic acid (29.95%), oleic acid (26.95%), 11-octadecenoic acid, methyl ester (20.06%) and hexadecanoic acid, 2,3-dihydroxypropyl ester (10.04%) (Table 1).

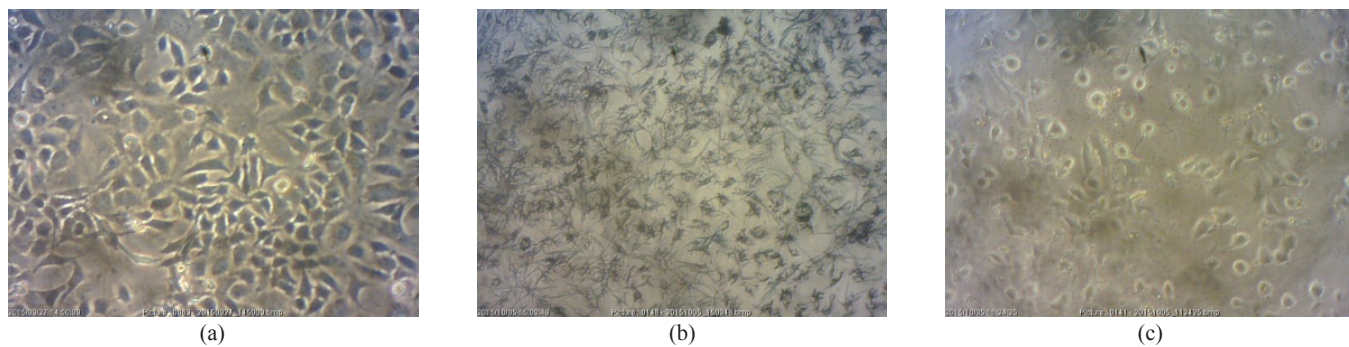


Figure 3. Apoptosis of HeLa cells after addition of methanol extract (200 µg/ml): (a) HeLa cells before addition of extract, (b) formazan crystals in cells treated with the extract after addition of MTT, and (c) dead HeLa cells.

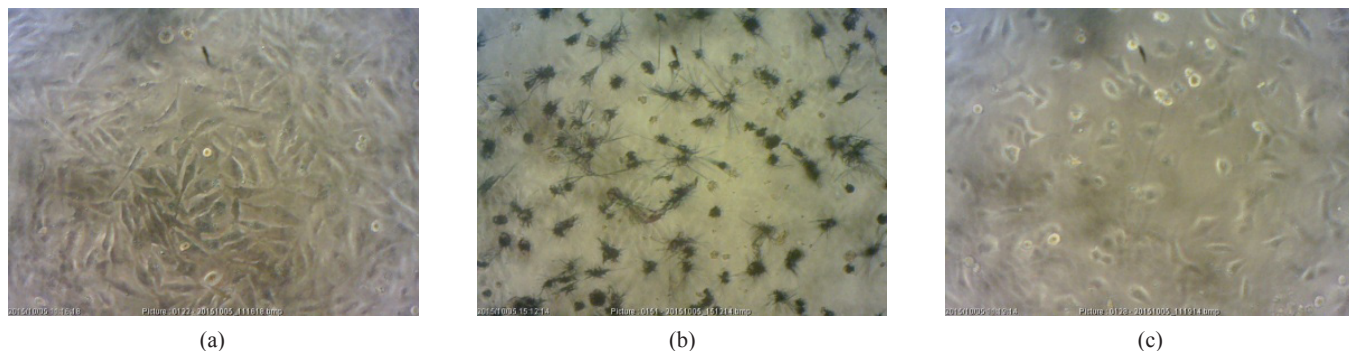


Figure 4. Apoptosis of U-251 cells after addition of methanol extract (800 µg/ml): (a) U-251 cells before addition of extract, (b) formazan crystals in cells treated with the extract after addition of MTT, and (c) dead U-251 cells.

Table 1. Compounds detected and identified by GC-MS analysis of methanol extract (DAM).

S.N.	Compound name	RT (min)	Content %	Mass-charge ratio (m/z)
1	(E)- 13-Docosenoic acid	14.60	29.95	55.05
2	Oleic acid	12.19	26.95	41.05
3	11-Octadecenoic acid, methyl ester	11.54	20.06	55.05
4	Hexadecanoic acid, 2,3-dihydroxypropyl ester	13.15	10.04	43.05
5	n-Hexadecanoic acid	10.87	5.98	43.05
6	2-Tridecenal, (E)-	16.87	1.73	43.05
7	9-Octadecenamide, (Z)-	13.87	1.48	59.10
8	Tetradecanoic acid, 12-methyl-, methyl ester	11.67	0.82	43.10
9	Pentadecanoic acid, 14-methyl-, methyl ester	10.32	0.80	74.10
10	7-Nonenamide	10.95	0.41	59.05
11	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	12.67	0.37	43.10
12	Di- α -L-xylofuranose 1,2':2,1'-dianhydride, 4,4',5,5'-tetra-deoxy-4,4'-bis(methylamino)-	15.16	0.37	55.10
13	Cholesterol 3-O[(2-acetoxy)ethyl]-	17.56	0.37	43.10

Discussion

Cytotoxic activity of the crude extract was observed against the cervical carcinoma and glioblastoma cell lines by MTT assay. The cell growth inhibition effect was strengthened with the increase in crude extract concentrations. The susceptibility of cells to the extract exposure was characterized by IC_{50} values. Considering the IC_{50} values, the methanol extract has better cytotoxic effect than that of other extract types. This indicates that the methanol extract is more potent on the cell lines used. The antitumor and anticancer activities of crude extracts of *Dendrobium* were explained in previous studies (Devi *et al.* 2009; Lam *et al.* 2015; Shrestha *et al.* 2015; Sun *et al.* 2016; Paudel *et al.* 2017). In our previous study, we quantified the total phenolic and flavonoid contents in the crude extract of *D. amoenum* (Paudel *et al.* 2015). In the present study, we further explored and detected 13 compounds from the crude extracts by GC-MS analysis. Compounds, like bibenzyl derivatives, phenols, phenanthrenes and sesquiterpenoids are known to be present in *D. amoenum* (Majumder *et al.* 1999; Venkateswarlu *et al.* 2002; Majumder and Bandyopadhyay 2010). The cytotoxic activity of *D. amoenum* against the cervical carcinoma and glioblastoma cell lines has been therefore attributed to the bioactive compounds present in the crude extracts of this orchid.

Many chemotherapeutic drugs and folk medicinal plants exert their anticancer effect by inducing cell apoptosis (Chen *et al.* 2008; Prasad and Koch 2014; Peng *et al.* 2017). The cervical carcinoma and glioblastoma cells treated with crude extract showed morphological changes, such as reduced size and cell volume, cell shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies, indicating that the extract induced the

apoptosis (Lowe and Lin 2000; Pandey *et al.* 2011; Kim *et al.* 2012; Gali-Muhtasib *et al.* 2015). Studies have shown that phenolic derivatives are responsible for the induction of apoptosis in cancer cells (Prasad and Koch 2014; Chen *et al.* 2015; Milutinoviæ *et al.* 2015).

The cytotoxic activity of DAM extract of *D. amoenum* is due to the presence of several bioactive compounds, confirmation of which needs further investigation. The result also highlighted the potential of this orchid as the source of natural anticancer drug and to explore their isolation, identification and characterization.

Acknowledgments

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Research

Comparative study of macrofungi in different patches of Boshan Community Forest in Kathmandu, Central Nepal

Bhawani Bhandari and Sanjay Kumar Jha*

Central Department of Botany, Tribhuvan University, Kathmandu, Nepal

Abstract

Macrofungi are considered as a group of high-value forest resources worldwide. In this paper, we report species richness and composition of macrofungi in three different forest patches (*Schima-Castanopsis*, *Pinus* and *Alnus* forest) of mid-hill, central Nepal, which were managed under Boshan Community Forest. A Systematic random sampling was applied where 20 rectangular plots of size 10 m x 10 m were laid at 1500 m to 1600 m elevation in each forest type. Species richness and composition of macrofungi were accessed in each forest type. *Schima-Castanopsis* forest was the richest in terms of macrofungal diversity (70 species), followed by *Alnus* forest (64) and *Pinus* forest (56). Polyporaceae was the largest family, followed by Amanitaceae. Agaricales (*Rusulla* and *Amanita*) were dominant in *Schima-wallichii* and *Alnus* forests, whereas Boletales were dominant in *Pine* forest. Macrofungal species richness increased with increasing canopy, soil moisture and soil pH. The species richness, however, had weak relationships with litter cover and disturbance. Based on the present study it can be concluded that the study area is rich in macrofungal diversity. Moist soil followed by litter and decaying wood assist the higher diversity of macrofungi. Species diversity is higher in moist and dense canopy forests (like, *Schima-Castanopsis* and alder) than in open and dry pine forest. Soil moisture, soil pH and tree canopy cover are the most important variables affecting macrofungal diversity.

Key-words: community forest, fungal diversity, mid-hill, species richness.

Introduction

Conservation of biodiversity depends on reliable information about the kinds of organism present, total number of species in each of these group, their genetic diversity, their habitats, distribution pattern, ecology, population size, evolutionary history, and their trends both in time and space. In practice, surveys are targeted towards organism groups that are generally considered spectacular, cute and intelligent. Fungi are one among them, diversity study of which is masked by higher plants and organisms. Thus, macro- and micro-fungi are highly underrepresented in the conservation literature (Heilmann and Vesterholt 2008), but they equally hold large genetic diversity with their potential implication in nature and human welfare.

Macrofungi are a group of fungi which produce mature spore-bearing and morphologically distinct fruiting bodies, which are visible to the unaided eye (Arnolds 1992). They are known to inhabit diverse types of habitat varying in the composition of their tree species and substrates. These habitats have assemblages of varied macrofungal species, some of which are very specific and are facilitated by the presence of trees and other plant species, which provide a functional platform to the fungi (Unterseher and Tal 2006). However, there are some macrofungi that are neutral to the presence or absence of dominant tree species in particular habitat type

(Zhang and Zak 1998). Studies on the specificity of macrofungi in different habitats with higher plants date back to the past century, and since then many qualitative features of such preferences have been observed and analyzed (Arnolds 1992), but how does the aboveground plant diversity correlate with the understory or belowground macrofungal diversity is still a matter of study. It has been well known that macrofungi are the important aspects of the forest ecosystem and biodiversity.

Usually fleshy macrofungi flourish in humid months of the year, whereas shelves fungi, cup fungi or simply dry mushrooms are abundant in dry months too. However, variations in microclimatic condition govern their occurrence, abundance and diversity in particular condition (Kustszegi *et al.* 2015). O’Hanlon and Harrington (2012) stated that compositions of aboveground vegetation also determine the macrofungal diversity. The suitable condition for the occurrence of massive production of carpophores depends upon the humidity, nutritional substrate and the mild atmospheric temperature (Dickinson and Lucas 1979).

The macrofungal species composition and diversity vary with nutrient (particularly nitrogen), moisture, forest type and disturbance (Trudell and Edmonds 2004; Christensen and Heilmann-Clausen 2009; López-Quintero *et al.* 2012; O’Hanlon and Harrington 2012; Pradhan *et al.* 2013). Climatic conditions as well as phyto-geomorphologic features affect

*Corresponding author. e-mail - sk.jha@cdbtu.edu.np

macrofungal fructification (Brunner *et al.* 1992). More information is available on composition and diversity of vascular plants in different forests but ecological study of macrofungi is non-existent except a few studies from West Bengal, India (Pradhan *et al.* 2013; Baral *et al.* 2015). In this paper, we report the effect of forest stand characteristics and environmental factors on macrofungal species composition and richness in mid-hill region, central Nepal.

Materials and methods

RESEARCH SITE

The study was carried out in three different forest patches within Boshan Community Forest in mid-hill region of Kathmandu district, central Nepal (Figure 1). The study was conducted from an elevation of 1500 m to 1600 m above sea level (asl). Meteorological data of the period 2006-2015 obtained from Department of Hydrology and Meteorology, Government of Nepal revealed that the study area is represented by subtropical climate and receives an average of 1488 mm annual rainfall with maximum monthly rainfall of 375 mm occurring during July. The monthly mean temperature is maximum in June (30.27°C) and minimum in January (2.97°C).

STUDY DESIGN

The Boshan Community Forest (BCF) was selected as it has different forest types almost at the same elevation. Three forest types were selected: *Schima wallichii*-*Castanopsis tribuloides* (hereinafter referred to as *Schima-Castanopsis* forest), *Alnus nepalensis* (*Alnus* forest) and *Pinus roxburghii* (pine forest). During preliminary field surveys (in March 2016), elevation of all the three forest types within BCF was obtained using a portable altimeter (and verified with a GPS device) with the help of BCF user group members. In each forest type, rectangular plots each of 10 m × 10 m size were established. The numbers of plot to be sampled were determined on the basis of spatial area of each forest.

FIELD SAMPLING

A detailed sampling of macrofungi diversity was made by applying systematic random method during June/July 2016, where plots were laid in each forest type with a minimal variation in elevation (1500–1600 m asl). A total of 20 plots were laid in each forest types along four transects, maintaining inter-plot distance of at least 20 m (Baral *et al.* 2015).

Presence/absence data of macrofungal species was recorded in each plot. Bio-physical variables, such as tree canopy cover, litter cover and anthropogenic disturbances

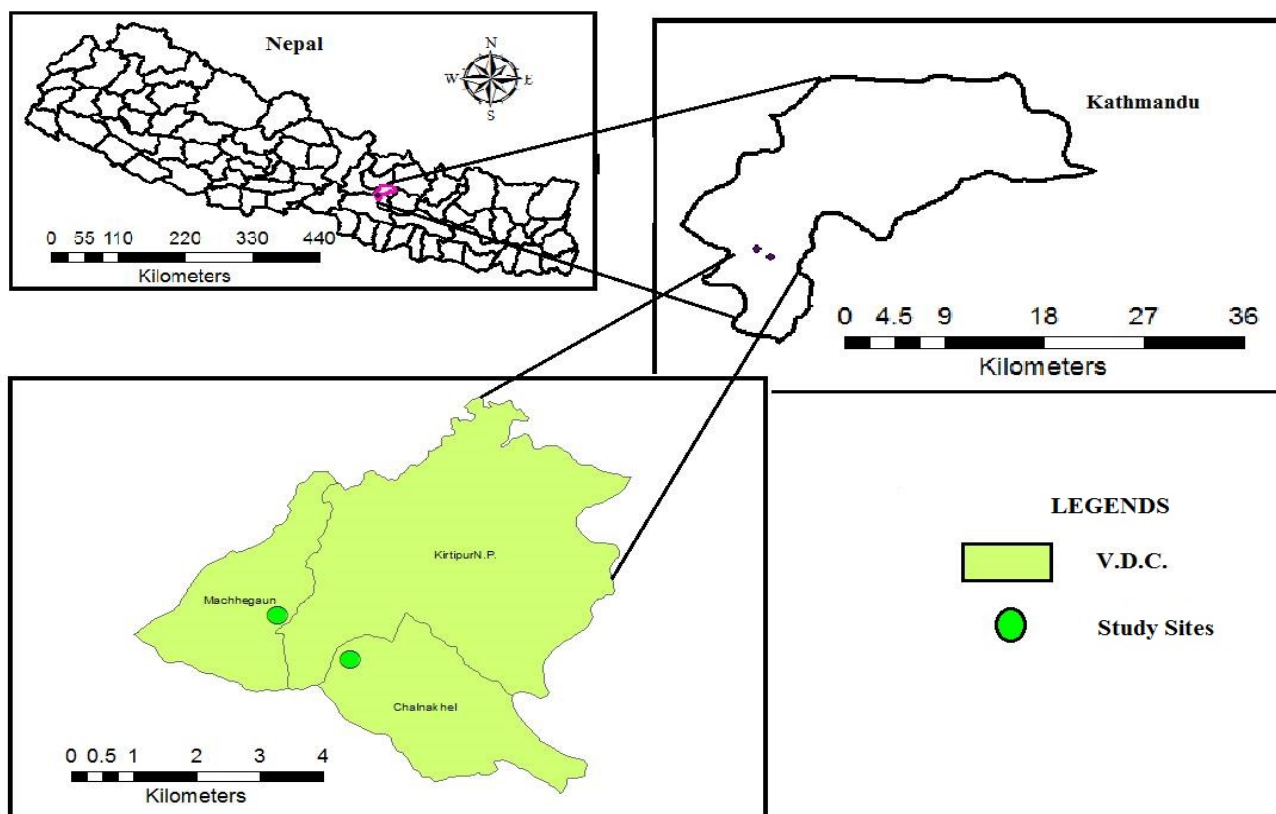


Figure 1. Location map of study area, showing position of Kathmandu district in Nepal: Village Development Committees (VDCs) in district and studied forest in VDCs.

(trampling, grazing and fire) were recorded in each plot. Tree canopy cover and litter cover (in percentage) were estimated visually. For tree canopy cover, observation was made from the middle of each plot. Slope was measured with the help of a clinometer. Soil samples were collected at a depth of 15 cm from four corners and at the middle of each plot using a soil digger. The samples from each plot were mixed thoroughly and from the mixture about 200 g was taken in zipper polythene bag. The samples were air dried in shade for a week and stored in air tight plastic bags until laboratory analysis. Soil pH and moisture were determined following Zobel *et al.* (1987).

Macrofungi were identified *in situ* where possible. Many of the unidentified specimens were preserved (dry and in liquid) and compared with the specimen at National Herbarium (KATH) and Tribhuvan University Central Herbarium (TUCH). Collected samples were studied on the basis of their morphological characters with the help of Myco-key (<http://www.mycokey.com>, www.mushroomexpert.com, www.mycoweb.com, www.mushroomobserver.org), and Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>). Standard identification manual (Marshall 1901; Adhikari 2000; Kumar *et al.* 1990) were also followed for mushroom identification along with the expert consultations.

DATA ANALYSIS

Field data were firstly checked for normality and homogeneity of variance in SPSS Version 20. Macrofungal species richness is defined as the number of infrageneric taxa per plot. Relationships between environmental variables (canopy, soil pH, soil moisture, litter cover and disturbance) and macrofungal species richness were assessed through Pearson correlation analysis. In addition, linear regression was performed in SPSS Version 20 to see the effect of tree canopy, soil moisture and soil pH on macrofungal species richness.

Results

SPECIES RICHNESS AND COMPOSITION

A total of 85 macrofungal taxa were documented, of which 52 were identified up to species level and 24 to generic level. Nine taxa remained unidentified due to immature/over-mature stage. Of the total identified macrofungal (genetic and infrageneric) taxa, 8 belonged to Ascomycota and 68 to Basidiomycota. The identified macrofungi belonged to 26 families. Polyporaceae was the largest family, consisting of 9 species. Amanitaceae and Boletaceae, with 8 species each, represented the second largest families, followed by Agaricaceae and Russulaceae, having 7 and 6 species respectively (Figure 2).

Schima-Castanopsis forest harbored highest macrofungal diversity (total 70 species) in all three substrates studied (Figure 3), followed by *Alnus* forest (64 species) and pine forest (56 species). Agaricales (*Russula* and *Amanita*) were dominant in *Schima-wallichii* and *Alnus* forests, whereas Boletales were dominant in pine forest. As compared to litter and wood, soil was the most important substrate for maintaining macrofungal diversity in all three forest types (Figure 3).

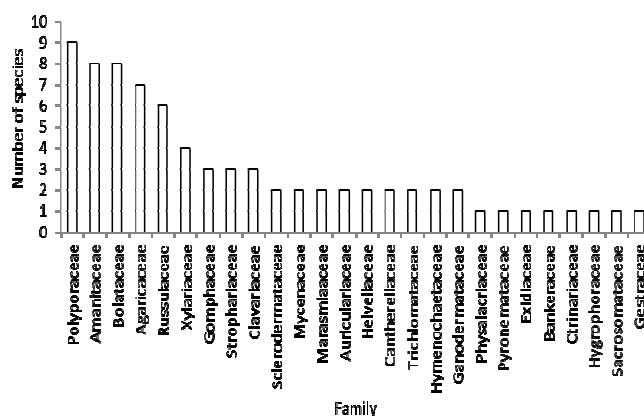


Figure 2. Number of macrofungal species found in study area belonging to their respective families.

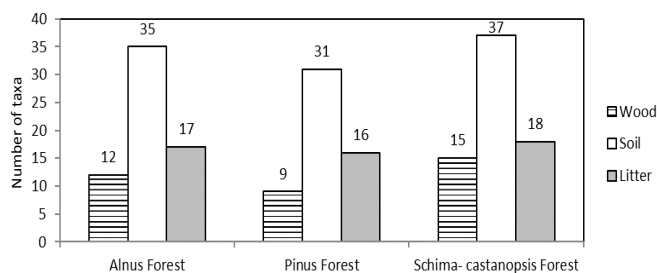


Figure 3. Distribution of macrofungi on the basis of habitat in different forest.

RELATIONSHIPS OF SPECIES RICHNESS WITH ENVIRONMENTAL VARIABLES

Soil pH, soil moisture, tree canopy and litter cover varied from 5.5 to 6.3, 21.0 to 47.7%, 25 to 98%, and 45 to 90%, respectively. Among the environmental variables compared, tree canopy cover, soil pH and soil moisture had the most significant positive relationships with macrofungal species richness (Table 2, Figure 4). Species richness increased with increasing tree canopy, soil pH and soil moisture (Figure 4). Macrofungal species richness showed weak positive relationship with litter cover. Disturbance was negatively correlated with macrofungal species richness, but the result was statistically insignificant (Table 2).

Table 2. Pearson's correlation between environmental variables and species richness of macrofungi.

	Soil pH	Soil moisture	Litter coverage	Tree canopy coverage	Disturbance
Species richness	0.528**	0.598**	0.028	0.523**	-0.061

*Correlation is significant at $p = 0.05$ level; **correlation is significant at $p = 0.01$ level.

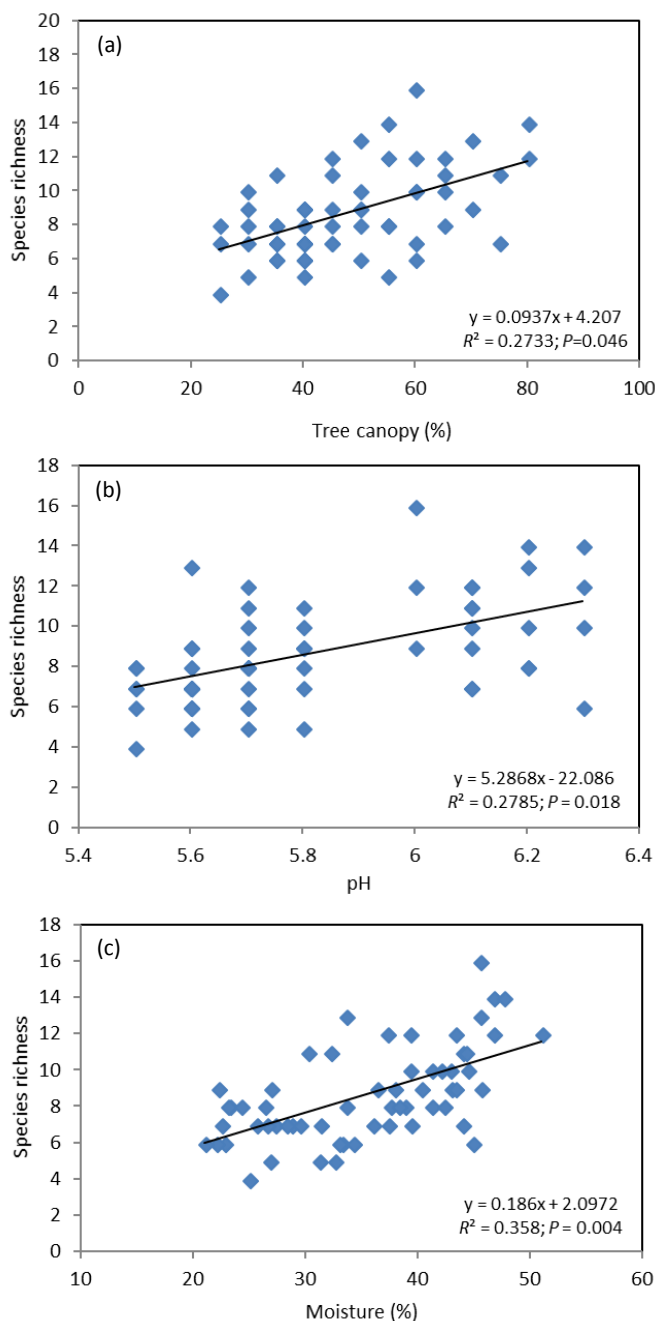


Figure 4. Variation in species richness of macrofungi with (a) tree canopy, (b) soil pH and (c) soil moisture.

Discussion

MACROFUNGAL RICHNESS AND COMPOSITION

The present study has shown that the forest dominated by *Schima-Castanopsis* had the highest macrofungi species richness compared to *Alnus* and pine forests. Of the total

macrofungal taxa collected (85), highest proportion (82.35%) of taxa was contributed by *Schima-Castanopsis* forest, followed by *Alnus* (75.29%) and pine forest (65.88%). The high macrofungal diversity in broad-leaved *Schima-Castanopsis* forest is mainly related to high soil moisture and greater cover of tree species. *Alnus* forest mostly occupy rocky and landslide areas with comparatively less suitable substrate than *Schima-Castanopsis* forest for macrofungal growth. Pine forests usually show comparatively dryness of habitat condition with low soil pH as pine litter have a tendency to acidify the soils (Pandey *et al.* 1995) and this might be the reason why the latter forest type harbored lowest macrofungal diversity among the three forest types compared. Macrofungi were recorded on different substrates, especially on soil, litter and wood. The finding of the study agrees with that conducted in India where macrofungi were reported in various habitats, like wood, litter and moist soil, among others (Nagaraju *et al.* 2014). As compared to litter and wood, soil was the most important substrate for maintaining macrofungal diversity in all three forest types studied. Higher number of macrofungi recorded in the present study on moist soil compared to litter and decaying wood is in agreement with previous study (e.g., Osti 2015). In a community forest in Gorkha, Nepal, Osti (2015) documented 52% of the total macrofungi from forest soil. Higher species diversity in Basidiomycota compared to Ascomycota (the ratio of Ascomycota to Basidiomycota in terms of species composition being 1:8.5) is probably contributed by higher number of mycorrhizal species found on soil as studies have shown that soil moisture and decaying litter facilitate much diverse macrofungi (Mueller 2007).

MACROFUNGAL RICHNESS IN RELATION TO ENVIRONMENTAL VARIABLES

Fungal diversity is closely related to forest structure and composition (Richard *et al.* 2004). In the present study, macrofungi varies with the forest types. The existence of distinctive macrofungal communities related to the dominant tree species of the forest have been confirmed by many other studies (Straatsma and Krisai-Greilhuber 2003; Gates *et al.* 2011; O'Hanlon and Harrington 2011). The formation of specific communities of macrofungi in the present study may be due to host preferences which is similar to the findings of Bills *et al.* (1986), and O'Hanlon and Harrington (2011).

Canopy is another important environmental factor for fungal diversity. Present study revealed highest macrofungal diversity in canopy-rich forests, which coincides with the

observation made by Baral *et al.* (2015) in central Nepal. Sysouphanthong *et al.* (2010) also reported higher macrofungal diversity in forests having higher canopy closure.

Yamanaka (2005) found that many of the saprophytic species of fungi grew well at pH 7 or 8 and the ectomycorrhizal species showed optimum growth at pH 5 or 6. Most species of saprobic fungi consist of wood- and dung-inhabiting species; whereas, the species inhabiting on soil was high in this research (Figure 4). The pH range was found to be 5-6 in most of the plots, so it can be said that pH is the key factor in determining soil fungal community composition. Zang *et al.* (2016) suggest that the pH range of 5 to 6 favors the growth of soil fungi.

The moisture is one of the major environmental factors influencing fruiting in macrofungi. Generally, small-sized species are delicate and fragile with small wiry stipe (Trudell and Edmonds 2004; Christensen and Heilmann-Clausen 2009). Species such as *Coprinus*, *Marasmius* and *Mycena* were found frequently in *Alnus* and *Schima-Castanopsis* forests than in pine forest. These species appeared and disappeared very quickly while the large sized fruit bodies fruited latter a continued period of more than two weeks. This might be due to their small fruiting body and their nature of forming fruiting body at relatively shallow depth thus change of moisture has more effect on them than other group of fungi.

Conclusions

Based on the present study it can be concluded that the study area is rich in macrofungal diversity with species richest families being the Polyporaceae, followed by Amanitaceae, Boletaceae, Agaricaceae and Russulaceae. Moist soil followed by litter and decaying wood assist the higher diversity of macrofungi. Species diversity is higher in moist and dense canopy forests (like, *Schima-Castanopsis* and alder) than in open and dry pine forest. Soil moisture, soil pH and tree canopy cover are the most important variables affecting macrofungal diversity.

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Review

Variation of plant species richness at different spatial scales

Khem Raj Bhattarai^{1,2}

¹Himalayan Resource and Development Centre, Nepal, GPO Box 7426, Kathmandu, Nepal

²Sheridan, Hazel McCallion Campus, 4180 Duke of York Blvd, Mississauga, ON L5B 0G5, Canada

Abstract

It is now realized that the variation in species richness is influenced by spatial and temporal scales. Pattern and scale are a central focus in ecology and biogeography. The species richness relationship depends on the scale of study and their correlated factors. The broad objective of this review is to elucidate how different scales are correlated with different explanatory variables to generate patterns of species richness. Addressing the problem of scale has both fundamental and applied importance in understanding variation in species richness along gradients. The understanding of pattern, its causes, and consequences is central to our understanding of processes such as succession, community development, and the spread and persistence of species. According to the hierarchical theory of species diversity there are mainly three categories of scales: local, landscape and regional. The local species richness or α -diversity is the diversity of individual stands. The β -diversity or species change is turnover between two elevational bands or between two plots or two sites. The regional or γ -diversity is the total richness of whole mountains or study systems and it has a combined influence from α - and β -diversity. The local species richness is affected by both local-scale processes (e.g., internal interactions) and broad-scale processes (e.g., evolutionary). Different explanatory variables according to the scales of study are necessary to explain variation at different spatial scales. Local factors (e.g., disturbance, grazing and tree cover) have been used to detect variation at a local scale. Generally, topographical factors are used to detect variation in species richness at a landscape scale; whereas climate, water-energy dynamics and historical processes are used to detect variation at a regional scale. However, it is not easy to separate strictly one scale from other because there is no clear boundary between them. The study of the whole elevation gradient from tropical to alpine zone or long latitude is a broad-scale study. The intermediate scale is a study on a local mountain, which covers the subtropical to warm temperate zones. To explain patterns of species richness, a pluralistic body of hypotheses, which incorporates historical, biological and climatic factors, is needed. This is depicted by the strong relationship between climate, biological interactions, and historical processes in influencing variation in species richness at different spatial scales.

Key-words: explanatory variables, hard boundaries, productivity, spatial scale, species richness, temporal scale.

Introduction

It has long been observed that species richness varies over a range of temporal and spatial scales (e.g., von Humboldt 1855; Wallace 1878; Simpson 1964; MacArthur and Wilson, 1967; Adams and Woodward 1989; Moore and Keddy 1989; Huston 1994; Brown and Lomolino 1998; Bhattarai 2003; Baniya *et al.* 2010; Gelashe 2017). These observations raise the obvious question in ecology and biogeography: Why are there many species in some places and few in others? These variations have been under intensive investigation by ecologists and biogeographers over the last two centuries (e.g., Dobzhansky 1950; Pianka 1966; Odland and Birks 1999; Whittaker *et al.* 2001; Qian *et al.* 2003; Bhattarai *et al.* 2004a; Pan *et al.* 2016). This is a complex subject of study and many hypotheses have been proposed and discussed to account for this variation. This complexity is reflected by more than 120 hypotheses compiled by Palmer (1994). Early explanations for the variation in species richness primarily considered as historical phenomena (Schluter and Ricklefs 1993). Such explanations were presented before ecology had emerged as a

scientific discipline. The “origin of species” theory of Darwin (1859) revolutionized our understanding of the history of the earth and distributions of its organisms. The views of many naturalists changed and they directed their thinking towards ecological and evolutionary processes to explain the variation in species richness at different spatial and temporal scales.

The rise of community ecology in early 1960s further promoted our understanding and changed traditional thinking on the variation in species richness. Ecologists attempted to explain variation in species richness by ecological interactions (Schluter and Ricklefs 1993). Following the development of theories for population growth and interactions by Lotka (1932), Volterra (1926) and Gause (1934), it was realized that species that closely matched ecologically would compete strongly for resources and hence could not coexist. Hutchinson (1957) developed the concept of the multidimensional ecological niche to conceptualize how environmental conditions could limit the abundance and distribution of organisms. These predictions from community ecology were tested by field experiments to investigate the role of competition

*Corresponding author. e-mail - bhattaraikhemraj@gmail.com

and other interactions in structuring natural populations (e.g., Connell 1961a, 1961b, 1983; Schoener 1983).

Three main fields of ecology – classical biogeography, community ecology, and population ecology – are all concerned in part with comparative species richness. Each of these three approaches has its own viewpoint and approach to interpreting the phenomenon of species richness (Schluter and Ricklefs 1993). Classical biogeographers are more concerned with the relationship of regional faunas and floras (e.g., Cain 1944; Udvardy 1969). Community ecologists emphasize the importance of dissimilarity or ecological distance between community samples (e.g., Goodall 1952). Theoretical population ecologists are more concerned with the role of species interactions (e.g., Hutchinson 1957, 1959). By 1967, MacArthur and Levins had formalized and strengthened the niche concept to explain the coexistence of species within a community. Through the development of community theory, MacArthur (1969) and other ecologists became aware about the importance of all the determinants emphasized in these approaches in influencing species richness along gradients in time and space. They recognized that at the local scale, species richness is likely to be constrained by ecological interactions, whereas species richness at a broad, regional scale may be influenced by historical process and events.

The work of Whittaker (1956, 1960, 1963, 1966, 1967, 1972, 1977) and Whittaker and Niering (1965, 1975) contributed important answers to several ecological issues concerning the distribution and composition of species along ecological gradients. Through the development of the theory of gradient analysis, interpretations of the observed patterns by ecologists and biogeographers were based on underlying biological, climatic, and historical factors. They observed patterns among many organisms in both the marine and the terrestrial realms (Huston 1994). Many explanatory variables, such as biomass (Grime 1973a; Gough *et al.* 1994), elevation (Terborgh 1977), precipitation (Brown and Davidson 1977), disturbance (Connell 1978), dispersal (Shmida and Wilson 1985), geographical range size (Stevens 1989, 1992), hard boundaries (Colwell and Hurtt 1994), species pool (Zobel 1992; Eriksson 1993; Pärtel *et al.* 1996), and water-energy dynamics (O'Brien 1998; Bhattarai 2003) were tested for different groups of animals and plants to find causal relationships. From these studies a common consensus emerged among ecologists and biogeographers, namely that both latitudinal and elevational gradients are governed by the same underlying controls (Brown and Gibson 1983; Begon *et al.* 1990; Rohde 1992; Rahbek 1995). The elevational gradient in species richness is often claimed to mirror the latitudinal gradient (Stevens 1992). Although the elevational gradient in species richness has long been obvious to ecologists and biogeographers, it is not as well documented quantitatively as the latitudinal gradient (Brown and Lomolino 1998).

Elevational gradients can serve as experimental systems to investigate and test several ecological and biogeographical hypotheses (Körner 2000). Therefore, the elevation gradient is now receiving attention from ecologists and biogeographers in an attempt to document the patterns and to find underlying causes (e.g., Odland and Birks 1999; Heaney 2001; Grytnes 2003a). Ecologists and biogeographers have begun to re-evaluate the nature and generality of the elevational gradient in species richness, to clarify its conceptual framework, and to set the agenda for future research (Rahbek 1995, 1997; Lomolino 2001). We are now experiencing a resurgence in mountain research to find a more detailed understanding and to develop more effective strategies for conserving biological diversity. In order to gain a more comprehensive understanding about variation in species richness, Lomolino (2001) has called for a rigorous test of patterns for different groups of taxa and an analysis of climatic variables that are directly associated with a casual explanation for the observed patterns. Such tests and analyses can contribute to the development of a more general theory of species diversity. Consequently, such tests might reduce the many redundant, vague, or un-testable hypotheses and help focus on a reduced subset of hypotheses (Bhattarai 2003; Bhattarai *et al.* 2004a).

It is now realized that the variation in species richness is influenced by spatial and temporal scales (e.g., Palmer and White 1994; Rosenzweig 1995; Gaston 1996; Lomolino 2000). Despite this, there is still a weakness in the ecological literature to relate species to explanatory variables according to particular scales of analysis but all under the general heading of diversity (Whittaker *et al.* 2001). Unambiguous demonstrations of causality can only be attained by testing variables associated with different spatial scales along the gradient.

The Himalayas have the highest mountains with the longest bio-climatic gradient in the world (Bhattarai 2003). Within *ca.* 150-200 km in a north-south transect one can find a gradient from tropical or sub-tropical zones to permanent snow and ice (Dobremez 1976; Bhattarai *et al.* 2004a). Large environmental variation within small geographical areas makes the elevational gradient a unique situation to study species richness. Therefore, Himalayas have become one of the unique place to test many biogeographical hypotheses. The Himalayas have major hotspots of biodiversity with many endemic species (Singh 2001). The biodiversity is vulnerable because of the high human population density, increasing tourism, and global warming (Shrestha and Joshi 1996; Chaudhary 1999). If no conservation measures are undertaken, there is a serious risk of species extinction. Before developing conservation measures, it is essential to understand the processes creating and maintaining the patterns of species richness. If we can understand the factors controlling the spatial patterns, it would be possible to predict how patterns might change over time

under the influence of these controlling factors. Therefore, the detection of patterns and understanding the underlying causal mechanisms behind the patterns are important for the future management and conservation of biodiversity.

Despite years of study at different spatial scales and intense theoretical interests, there are still many unanswered questions in ecology and biogeography. The mechanisms which connect climate and vegetation are poorly understood (Woodward 1987). The variation in species richness along environmental gradients may vary according to plant life-forms due to different eco-physiological processes and climatic tolerances (Bhattarai 2003; Bhattarai and Vetaas 2003). The literature dealing with this issue is sparse. Water-energy dynamics parsimoniously explains variation in woody species richness along the macro-scale climatic gradient, along the latitudinal gradient as well as along the altitudinal gradient (O'Brien 1993; Bhattarai *et al.* 2004a). The same climatic factors may control species richness differently at different ends. This has not, however, been demonstrated clearly. The well-studied hump-shaped relationship between herbaceous species richness and biomass is necessary to understand whether this relationship depends upon the environmental gradient or on internal interactions. Thus, the broad objective of this review is to elucidate how different scales are correlated with different explanatory variables to generate patterns of species richness.

Species Richness and Scale

Scale is the unit of space or time over which signals are integrated to convey a message (Allen and Starr 1982). It gives a rough indication rather than a precise figure (Blöschl and Sivapalan 1995). Pattern and scale are a central focus in ecology and biogeography (Levin 1992) and are closely inter-related (Hutchinson 1953). The species richness relationship depends on the scale of study (Whittaker *et al.* 2001). Addressing the problem of scale has both fundamental and applied importance in understanding variation in species richness along gradients. The understanding of pattern, its causes, and its consequences is central to our understanding of processes such as succession, community development, and the spread and persistence of species (Levin 1992). In the modern ecological literature, awareness of scale has greatly increased (e.g., Levin 1992; Palmer and White 1994; Weiher 1999; Mittelbach *et al.* 2001; Gering and Thomas 2002; Koleff and Gaston 2002; Grytnes 2002).

There are three aspects of scale in ecology: spatial, temporal, and organizational (Levin 1992). Species richness patterns, which are influenced by scale, are associated with the spatial extent and temporal duration (Figure 1). Different evolutionary forces act on these different scales (Levin

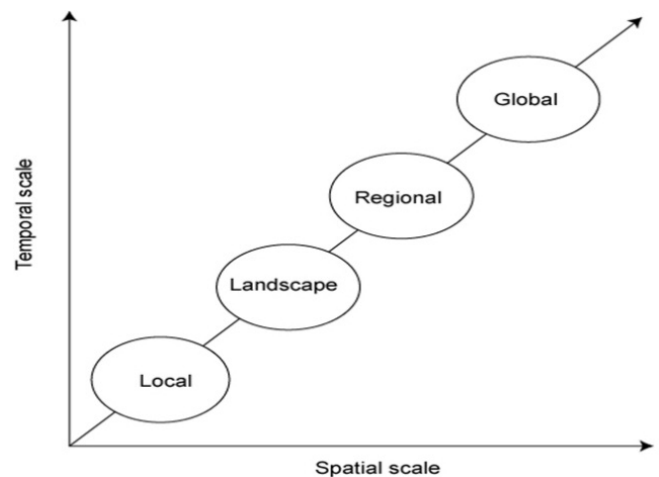


Figure 1. The relationship between spatial scale and temporal scale, and their corresponding patterns

1992). In most ecological studies, the temporal scale is not generally taken into account directly (with the exception of paleoecology) when examining the distribution of species along gradient. In general, systems are organized into a hierarchy of spatial and temporal scales, and hierarchical theory predicts that the dynamics of a system at a lower scale affects the dynamics of systems at higher levels and vice versa (Allen and Starr 1982; O'Neill 1989).

The spatial scale is divided into three components: sample size (size of individual quadrats or grain, or focus), extent of study (geographical distance covered by the study area), and the number of sample units or intensity (Palmer and White 1994). Thus, when saying that patterns are scale-dependent means the degree to which the ecological phenomenon varies as a function of grain, extent, and number of samples. There is a strong relationship between grain size and study area. Small grain size is used to detect variation at a local scale and large grain size is used to detect that at a regional or coarse scale. Mittelbach *et al.* (2001) from a meta-analysis of 171 published studies found a positive relationship ($r = 0.6$) between grain size and study area. Grain size is also related to the size of organisms (e.g., trees or herbs, Bhattarai 2003; for Lichens see Baniya *et al.* 2010). Palmer and White (1994) have demonstrated that variation of species richness in space is influenced by grain, extent, and number of samples. Therefore, it is argued that the selection of appropriate sample size, extent, and intensity is important to detect the relevant patterns along gradients (Bhattarai 2003).

The measurement of species diversity requires a clear and unambiguous term to represent diversity or richness. Different terms have been used to represent diversity (Whittaker *et al.* 2001). The oldest and most fundamental term is species number. As a term, species number proved unpopular, probably because it implies that the number of species in community

can actually be determined. McIntosh (1967) suggested the alternative term of species richness to indicate the number of species in a community. Diversity, in general, has two components: species richness and evenness of the distribution of numbers among species. The term species richness indicates the number of species in a sample (Whittaker *et al.* 2001), and it is perhaps the least ambiguous of all the terms used in diversity research (Peet 1974). Some authors have adopted the term species density for the number of species sampled in a standardized sample unit (e.g., Lomolino 2001) but others have retained the term richness even in a standardized sample plot (e.g., Grytnes 2000). Different indices of diversity have been used by different authors creating considerable confusion in the ecological literature (Hurlbert 1971). However, the use of species richness is the most interpretable, fundamental measurement of community and regional diversity (Gotelli and Colwell 2001). It is a good surrogate as it acts as an integrator of many facets biodiversity patterns, it is applicable to both standardized and non-standardized plots, it is frequently measurable, and it is intuitive to all levels of readers. Gaston and Spicer (1998) called “species richness as a common currency”. Species richness is defined as: the number of species present within smaller unit area at fine scale (e.g., 1×1 m plot, Bhattarai *et al.* 2004b), the number of species present in each 50×20 m plot or meso-scale (Bhattarai 2003) or the number of species present in each 100 m elevation band (Bhattarai *et al.* 2004a).

In addition to the various concepts of diversity, concepts of scale and space also vary. Whittaker (1960) proposed various scale descriptors represented by the Greek letters α , β , γ , δ and ϵ . The δ and ϵ components are not commonly discussed in the literature. According to the hierarchical theory of species diversity (Whittaker *et al.* 2001), there are mainly three categories of scales: local, landscape and regional. The local species richness or α -diversity is the diversity of individual stands, namely 1×1 m plots (Bhattarai *et al.* 2004b), 50×20 m plots (Bhattarai 2003), or in each 100-m elevation band (Bhattarai and Vetaas 2006). The β -diversity or species change is turnover between two elevational bands (e.g., Bhattarai *et al.* 2004a) or between two plots or two sites. The regional or γ -diversity is the total richness of whole mountains or study systems and it has a combined influence from α - and β -diversity (Brown and Lomolino 1998).

Some authors (e.g., Mittelbach *et al.* 2001) have categorized the scale according to geographical distance, local (0-20 km), landscape (20-200), regional (200-4000 km), and continental to global (>4000 km). The measurable spatial heterogeneity in climate can occur over distances of at least 100 km (cf. O'Brien 1998). However, along the elevation gradient, measurable environmental variation can be detected at the 100 m distance between upper and lower elevation band (Bhattarai *et al.* 2004a). Thus, elevated mountains can

be considered as experimental site to various biogeographical hypothesis and principles.

The local species richness is affected by both local-scale processes (e.g., internal interactions) and broad-scale processes (e.g., evolutionary). Different explanatory variables according to the scales of study are necessary to explain variation at different spatial scales (Whittaker *et al.* 2001). Local factors (e.g., disturbance, grazing and tree cover) have been used to detect variation at a local scale (e.g., Vetaas 1997). Generally, topographical factors are used to detect variation in species richness at a landscape scale; whereas climate, water-energy dynamics and historical processes are used to detect variation at a regional scale (O'Brien 1998; Whittaker *et al.* 2001; Pan *et al.* 2016). Thus, if one moves from one scale to another, some information will be retained and some will be lost.

However, it is not easy to separate strictly one scale from other because there is no clear boundary between them. A broad-scale study by one investigator can be a meso-scale study to others and vice versa. The study of the whole elevation gradient from tropical to alpine zone or long latitude is a broad-scale study (Bhattarai 2003; O'Brien 1998). The intermediate scale is a study on a local mountain, which covers the subtropical to warm temperate zones (Bhattarai 2003). The study within a grassland community is a fine-scale study (Bhattarai *et al.* 2004b).

BROAD-SCALE STUDIES AND THEIR EXPLANATORY VARIABLES

Latitudinal and elevation gradients are well known broad-scale species richness patterns. These have been studied for several groups of plant and animals from a variety of habitats and regions (e.g., Richerson and Lum 1980; Currie and Paquin 1987; Wolda 1987; Adams and Woodward 1989; Stevens 1989; Tyron 1989; Currie 1991; Vazquez and Givnish 1998; Kessler 2000; Ohlemüller and Wilson 2000; Brown 2001; Heaney 2001; Md. Nor 2001; Vetaas and Grytnes 2002; Qian *et al.* 2003; Baniya *et al.* 2010). In general, broad-scale explanatory variables cannot detect richness patterns at the finer scales and vice versa.

Climate controls or exerts a strong controlling influence on the distribution of plants in all biomes (Woodward 1987). Climate generates and maintains the richness gradient at a broad-scale (Hawkins *et al.* 2003). Elevation only reflects species richness; climatic factors that co-vary with elevation influence species richness along the gradient. The climatic factors, which may vary with elevation, are temperature, potential evapotranspiration, length of growing season, humidity, air pressure, nutrient availability, ultraviolet radiation, moisture index, and rainfall (Funnell and Parish 2001). These various factors have been particularly emphasized as potentially important factors in explaining much of the variation in species richness at broad-scale studies (e.g.,

Pianka 1966; Richerson and Lum 1980; Currie and Paquin 1987; Currie 1991; O'Brien 1993, 1998; O'Brien *et al.* 1998; Grytnes *et al.* 1999; Odland and Birks 1999; Grytnes 2003a; Baniya *et al.* 2010, Pan *et al.* 2016).

The potential evapotranspiration, length of growing season, mean annual rainfall, number of rainy days, and moisture index are used as explanatory variables. The elevational range size of 100-1500 m is sufficient to encompass a range of climate (e.g., a temperature difference more than 8° C) over which species richness may vary. Along a latitudinal gradient this will be equivalent to more than 1000 km of horizontal distance (cf. Huston 1994). The whole elevation gradient has a temperature difference of more than 25°C which may have different functional effects at the upper and lower ends of the gradient in influencing variation in species richness. Beside climatic factors, other factors that may explain variations in species richness over broad-scales considered are mass effects, hard boundaries, species-pool, species range-size and area.

FINE-SCALE STUDIES AND THEIR EXPLANATORY VARIABLES

One component of species richness patterns that has been the subject of much recent discussion by plant ecologists is the fine-scale patterns. The number of species occurring in plots of a fixed area of small to modest size is fine-scale variation. Ecologists have long been familiar with fine-scale patterns from the middle of the last century, mostly from small experimental plots (see Swingle 1946; Yount 1956). The development of the competitive exclusion principle led to an understanding of how species coexist in a community. One of the first comprehensive explanations for fine-scale species richness was presented by Grime (1973a, 1973b). These are seminal papers that created considerable interest on fine-scale variation in species richness. Fine-scale variations continue to attract the interest of community ecologists and to stimulate further study (e.g., Al-Mufti *et al.* 1977; Moore and Keddy 1989; Guo and Berry 1998; Mittelbach *et al.* 2001; Fox 2003).

Fine-scale pattern is influenced by different processes than in chance broad-scale patterns but productivity has been used to explain variation in richness at both scales (e.g., Mittelbach *et al.* 2001). But the measure of productivity differs between broad-scale and fine-scale studies. Fine-scale studies that have considered relationships with productivity include Grime (1973a, 1973b), Pacala (1993), Rosenzweig and Abramsky (1993), Tilman and Huston (1994), Grace (1999), Gross *et al.* (2000), and Mittelbach *et al.* (2001). Biomass is most often used as a surrogate measure for productivity (e.g., Grime 1973a, 1973b; Moore and Keddy 1989; Oba *et al.* 2001; Rajaniemi 2003). Other variables that are also frequently used to examine fine-scale species richness patterns include plant cover (Grytnes 2000), disturbance (Connell 1978; Huston

1979, 1994; Fox 1981; Vetaas 1997), fire (Auclair *et al.* 1976), mowing (Melman *et al.* 1988), plant size (Oksanen 1996), and species pool (Zobel 1997).

Variables and the Mechanisms Influencing Species Richness

PRODUCTIVITY

Productivity is the rate at which energy flows in an ecosystem (Kj/m²/yr). Therefore, it is not surprising that productivity is correlated with species richness in many situations. Productivity was suggested as an important factor explaining variation in species richness from the mid-1960s (e.g., Connell and Orias 1964). The role of productivity as a determinant of species richness is of fundamental importance in understanding variation in species richness. It has thus been used as an explanatory variable at broad-scales (e.g., Pianka 1966; Currie and Paquin 1987; Currie 1991; Rosenzweig and Abramsky 1993; Huston 1994; Rosenzweig 1995; Hawkins *et al.* 2003; Bhattarai 2003), as well as at fine-scales (e.g., Grime 1973a, 1997; Gross *et al.* 2000; Rajaniemi 2003; Bhattarai *et al.* 2004b).

All ecologists and biogeographers have found a causal relationship between species richness and productivity (e.g., Connell and Orias 1964; Huston 1994; Rosenzweig 1995; Grytnes 2000; Mittelbach *et al.* 2001; Bhattarai 2003). But they have not reached a consensus about the mechanisms that underlie it (Oksanen 1997; Grime 1997). This discrepancy behind the mechanism is associated with different scales of study and the different formulation of productivity and taxonomic groups (e.g., Currie 1991; Wright *et al.* 1993; Rosenzweig 1995; Bhattarai 2003; Bhattarai *et al.* 2004a). The reviews of Grace (1999), Waide *et al.* (1999) and Mittelbach *et al.* (2001) are valuable contributions as they provide a very complete survey of productivity diversity literature. Terrestrial plants are the most commonly studied group (36%), whereas studies of aquatic plants are relatively low (12%) (Mittelbach *et al.* 2001). It is clear that patterns are emerging for both broad and fine-scales for trees, shrubs, woody climbers, ferns, lichens and herbs (e.g., Rosenzweig 1995; Bhattarai 2003; Baniya *et al.* 2010).

Although we define productivity as the rate of energy flow to a system, it is not easy to measure directly; this rate is rarely measured in nature. Instead, productivity is measured indirectly from surrogate variables (Mittelbach *et al.* 2001). Any estimate of productivity is related to scale. There are two common approaches in estimating productivity in natural systems: (i) climatic variables as a surrogate for productivity at broad-scales, and (ii) biomass accumulation as a surrogate for productivity at fine-scales.

(i) *Climatic variables as a surrogate for productivity* – Productivity can be estimated from climate indices (e.g., rainfall, temperature, potential evapotranspiration and actual evapotranspiration), which are surrogates and all correlate with productivity. At a global or continental scale, productivity is most commonly estimated from actual evapotranspiration or potential evapotranspiration (PET) using climatic data (e.g., Currie 1991). At scales smaller than the continent, productivity is estimated from rainfall. But this practice cannot be applied consistently along elevational gradients. Along the elevational gradient, within a small geographical distance, there is a large variation in temperature between the two ends of the gradient, which can influence the overall life-history attributes of plants. Therefore, even at a finer geographical scale, PET can explain a large proportion of the variation in richness data (e.g., Bhattarai 2003).

In early work, ecologists proposed that productivity would increase species richness but it has been found that this does not occur in all situations. It is well known that the productivity-richness relationship is scale dependent as well as being dependent on taxonomic groups (e.g., Bhattarai 2003). Ecologists have reached a new generalization for considering species richness-productivity relationships. Within a region (*ca.* small to medium-size areas), species richness is a unimodal function of productivity; whereas in biogeographical provinces or at a global scale, species richness is a monotonic function of productivity (e.g., Rosenzweig and Abramsky 1993; Mittelbach *et al.* 2001).

(ii) *Biomass as a surrogate of productivity* – The biomass-species richness relationship under the domain of “productivity diversity” is one of the well-studied subjects in ecology, but it is strongly debated (e.g., Rosenzweig 1995; Oksanen 1996; Grime 1997; Rapson *et al.* 1997; Waide *et al.* 1999; Grace 1999; Mittelbach *et al.* 2001). Most authors agree that biomass affects diversity (e.g., Brown 1973; Tilman and Pacala 1993; Grace and Jutila 1999; Mittelbach *et al.* 2001; Bhattarai *et al.* 2004b). However, no general consensus concerning the form of the pattern has emerged from either theoretical considerations or empirical findings (see the review by Waide *et al.* 1999).

Studies on biomass and species richness are especially common for terrestrial plants (e.g., Silvertown 1980; Tilman 1982; Huston 1994; Gough *et al.* 2000). Most studies that have experimentally manipulated productivity find that species richness decreases following an experimental increase in productivity (Gough and Grace 1998 and references therein). In herbaceous communities, the hump-shaped relationship between biomass and species richness is common (e.g., Waide *et al.* 1999). Other types of relationships found are positive linear, negative linear, and no relationship (e.g., Goldberg and Miller 1990; Mittelbach *et al.* 2001).

Grime (1979) proposed a hump-backed model to explain the species richness-biomass or-productivity relationship in herbaceous vegetation. It attempted to summarize the contribution of five different local processes: (i) dominance (competitive exclusion), (ii) environmental stress, (iii) disturbance, (iv) niche differentiation, and (v) colonization.

Huston (1979) proposed a dynamic equilibrium model to explain the species richness-productivity relationship. High productivity leads to high growth rates, which ultimately promote higher competitive displacement and low species richness. At the same time, a moderate frequency of disturbance lowers the rate of competitive displacement and promotes high species richness.

In a highly productive habitat, one or two species monopolies the available resources and exclude less competitive species, thereby creating low species richness. Huston (1994) elaborated this equilibrium theory to fit into a general framework, and proposed that, the population number of species remains constant as a result of a deterministic balance between the competitive abilities of the species. Tilman (1982) argued that equilibrium coexistence could occur when different resources control two competing species. The local heterogeneity in the ratios of resources can permit numerous species to coexist and this will favor a greater number of species at low supply rates of resources. Therefore, a lower equilibrium diversity will be expected under highly productive conditions.

From a meta-analysis, Mittelbach *et al.* (2001) found that when biomass is used as a measure of productivity, the unimodal relationship is dominant (65%). Grace (1999) from a literature survey of biomass-species relationships came to a similar conclusion. Thus, the humped-shaped relationship between species richness and biomass is consistent with many other studies. In considering this relationship, various authors have gone as far as to state that the hump-shaped curve is “true” (e.g., Rosenzweig 1992) or “ubiquitous” (e.g., Huston and DeAngelis 1994).

Moore and Keddy (1989) have demonstrated that the species richness biomass relationship varies according to vegetation types. They found a hump-shaped relationship among vegetation types but no relationship within vegetation types. A number of other authors have reached similar conclusions, that the hump-shaped relationship is detected when studies are conducted over broad productivity ranges (e.g., Rosenzweig 1995; Guo and Berry 1998; Mittelbach *et al.* 2001).

Most of the fine-scale productivity-species richness relationships have been performed in wet and temperate regions (e.g., Gough and Grace 1998; Grace and Jutila 1999). It has been shown that the hump-shaped relationship depends upon internal interactions rather than external environmental

factors. It is thought that environmental factors seem important than internal interactions in arid regions (Noy-Meir 1973). As far as I know, the hump-shaped relationship has not been tested in arid pasture-lands at high elevation.

Mechanisms underlying the hump-backed model

Despite the various studies, the productivity-diversity relationship has often been assumed to be unimodal (e.g., Al-Mufti *et al.* 1977; Rosenzweig and Abramsky 1993; Tilman and Pacala 1993). Rajaniemi (2003) grouped two categories of hypotheses to explain this unimodal relationship: (1) competition for any crucial resource leads to exclusion, and (2) competition for light leads to exclusion. The total competition intensity hypothesis (Grime 1977) and dynamic equilibrium hypothesis (Huston 1979) belong to the first category and argue that any resource leads to faster exclusion. The second set of hypotheses emphasizes higher productivity excluding poor light competitors [e.g., the habitat heterogeneity hypothesis of Tilman and Pacala (1993); the light competition intensity hypothesis of Newman (1973); light competition/random species loss hypothesis of Goldberg and Miller (1990)]. Oksanen (1996, 1997) proposed a no-interaction model, and argued that the hump-shaped relationship between plant species richness and biomass is apparent without considering any environmental variables and thus that it is an “artefact”. He explained the humped-shaped curve as a result of a scaling “artefact” when small sample plots of fixed size are used for plants with variable dimensions. Therefore, important variables would not be the biomass as is assumed but the number of plants per unit quadrat. Since the publication of the no-interaction model, many debates have followed (e.g., Grime 1997; Marañón and García 1997; Rapson *et al.* 1997). There are three assumptions in Oksanen’s (1996) hypothesis for the humped-back model: plants have a fixed size, bigger plants have more biomass, and there is a monotonic relationship between the number of plants and the number of species. Rapson *et al.* (1997) evaluated if the humped-back response is a result of fixed grain size. They found a similar hump-shaped curve with different quadrat sizes and the “no-interaction model” actually received limited support. Marañón and García (1997) discussed weak points in the “no-interaction model” on the grounds that a monoculture of plant density and fixed plant size are unrealistic situations for natural communities.

WATER-ENERGY DYNAMICS

Some authors have treated water-energy dynamics under the rubric of productivity (e.g., Hakwins *et al.* 2003) but it is argued that they are not exactly the same, and they are treated separately here. Water and energy are the basic categories needed to describe the dynamic variability in climate (Mather 1974). Precipitation acts as a measure of the amount of water

actually available to meet the environmental demands of water. Potential evapotranspiration (PET; i.e., energy) is fundamental to water-budget analysis (Holdridge *et al.* 1971). O’Brien (1993, 1998) and O’Brien *et al.* (1998) demonstrated that spatial variation in woody species richness along the macro-scale gradient is explained by a linear function of water and a parabolic function of energy [i.e., species richness = water + (energy)²], which is the water-energy dynamics model (Figure 2). This model parsimoniously explains variation in woody species richness along climatic gradients (Bhattarai 2003). It comprises a resource gradient (water) plus a regulatory gradient (energy), so it is a complex gradient model.

Water-energy dynamics predicts maximum species richness at sites where biological activity or photosynthesis is at a maximum and low species richness where biological activity is at a minimum. Such a relationship is consistent with earlier studies that found aspects of climate to be strongly related to various biological functions and factors, especially productivity, life-form diversity, and vegetation type (O’Brien *et al.* 1998).

This model is applicable to explain spatial patterns in woody species richness elsewhere in the world (O’Brien *et al.* 1998; Bhattarai 2003). Hawkins *et al.* (2003) found that water-energy dynamics could explain spatial variation in richness in 82 of a total 85 cases better than other climatic and non-climatic variables. In nature, optimum energy and maximum moist conditions always promote photosynthesis, which ultimately influences all ecophysiological processes and promotes species richness (Bhattarai 2003). The maximum species richness conditions in nature occur when water availability is high and the energy availability is optimum for plant physiological activities (Figure 2). The optimum function of energy and maximum function of water means that water is actively available to plants when energy conditions are not too high or too low. At very low energy conditions, i.e. high mountaintops or polar regions, water will freeze and occur as snow and ice, and is largely unavailable to plants. Their physiological activities and photosynthesis are at a minimum (Bhattarai 2003).

Similarly, tropical and sub-tropical deserts were characterized by a maximum heat/energy, where water will evaporate so that it is unavailable to plants, thus biological activity is at a minimum. The ambient energy regime determines the physical state of water. This means that water variables are not independent of energy variables. The maximum woody species richness at 600-800 m along the elevational gradient in Himalayas is associated with maximum moisture (mean annual rainfall *ca.* 2200 mm) and optimum energy (1200-1300 mm year⁻¹, Bhattarai 2003). Similarly, Bhattarai *et al.* (2004a) argues that maximum fern species richness at 2000 m along the Himalayan elevational gradient is associated with maximum moisture conditions

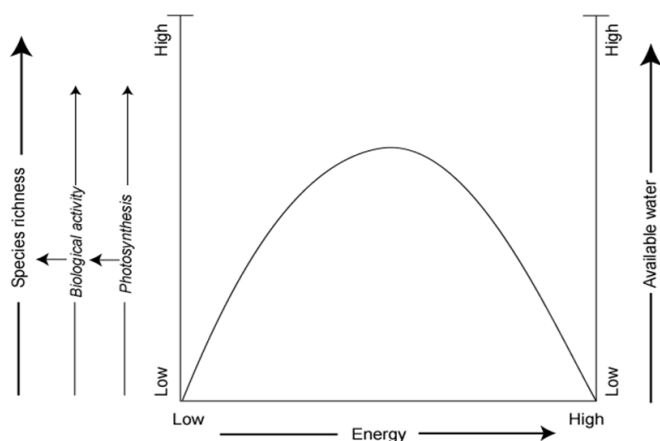


Figure 2. Water-energy dynamics model proposed by O'Brien (1993, 1998) to show the relationship between terrestrial plant species richness and water-energy. Species richness is a function of biological activity, which depends on photosynthesis, which in turn depends on water-energy dynamics (species richness = water + (energy)²). Species richness increases or decreases as a linear function of liquid water (right ordinate) and a parabolic function of energy (abscissa).

(having the highest number of rainy days and cloud zones) and optimum energy conditions. It is found that the optimum energy condition is different for ferns and woody (flowering plants) species. Ferns have low optimum energy requirements compared to flowering plants. This suggests that ferns require more moist conditions (cf. Odland 1993). The higher PET at lower elevations limits water availability, which creates harsh conditions for cool, moisture-loving ferns. Generally, fern richness is constrained by low energy and a short growing season (Bhattarai *et al.* 2004b). Odland (1993) studied phenology of frond development in ferns and found that more than one-month period is needed for its full development. This indicates that at higher elevation shorter growing season constraint for ferns to complete their life cycle, thus, ferns are found hardly up to 4800 m in the Himalayas (Bhattarai *et al.* 2004b). Flowering plants are reported up to 6500 m in Himalayas (Miehe 1989). This suggests that ferns are more sensitive to growing season compared to flowering plants as extensive fern growth does not occur until soil temperatures exceed 7°C.

MASS EFFECTS AND SPECIES POOL

Shmida and Wilson (1985) proposed the concept of mass effects in order to combine historical process, biological interactions, and climatic influences into a broad and potentially more complete understanding of variation in species richness along gradients. It is the establishment of species by propagules at sites where they cannot maintain viable populations. Ecologists have given different names for the phenomenon of mass effects. Brown and Kodric-Brown (1977) and Stevens (1992) called it the "rescue effect", and Pulliam (1988) called it the "source-sink effect". Whatever it is

called, it is the same phenomenon and can influence variation in species richness along the environmental gradients.

Natural communities are never closed systems, exempt from the influences of adjacent areas. Therefore, every community may be influenced by mass effects (Shmida and Wilson 1985). Mass effects influence the spatial variation in species richness at both fine and broad-scales. The mass effects function to increase alpha or local species richness in general, but their influence depends upon the system. If surrounding habitats are highly heterogeneous, there is the probability of establishing new species from surrounding habitats and increasing beta diversity along the gradient (cf. Wilson and Shmida 1984; Shmida and Wilson 1985). If surrounding systems are homogeneous, mass effects will increase alpha-diversity through the expansion of species ranges. This is called neighborhood effects (Shmida and Wilson 1985).

The phenomena of mass effects argue that maximum species richness at mid-elevations along the elevational gradient may be associated with broad-scale mass effects where species have migrated from lower and higher elevations (Bhattarai 2003). Species migrations from the source will increase richness at the target site (mass effects), the source is a species pool, and the target is a sink. Therefore, mass effects and the species pool are linked concepts in ecology. But if we consider the temporal dimension, the two phenomena seem rather different. The species pool has a self-maintaining population in the target site but the mass effect produces temporary populations due to a failure in reproduction. This will be insufficient to balance local mortality (Pulliam 1988). Populations may persist in such habitats by continued immigration from more productive "source" areas nearby.

The mass effects along the elevational gradient may dilute the relationship between species richness and climatic variables. Generally, a weak pattern in herbaceous species richness along the elevational gradient is associated with mass effect (Bhattarai 2003). This may be caused by herbaceous attributes, like faster phase of growth, easier establishment and high dispersal ability (cf. Grime 1977; Bhattarai 2003). Many herbaceous species, particularly grasses, are wind dispersed and this may increase the mass effects and obscure the response to the climatic-elevational gradient (cf. Shmida and Wilson 1985). Therefore, non-significant patterns in herbaceous life-forms along the elevational gradient may be linked to active mass effects caused by attributes of herbaceous plants (Bhattarai 2003). Shmida and Wilson (1985) and Bhattarai (2003) argue that mass effects may explain unexplained patterns in species distributions along gradients. Several authors (e.g., van Horne 1983) have discussed the need to distinguish between sources and sink habitats in field studies for community analysis. The herbaceous species, particularly grasses, are more influenced by local factors compared to

macro-scale climatic factors. Mass effects should be removed when one attempts to examine the casual relationship between species richness and climate variables (MacArthur 1965). Therefore, it can be argued that one should not lump herbaceous species particularly grasses and woody species together to examine the climatic relationships with species richness along climatic gradients.

HARD BOUNDARIES

In spite of the plethora of climatic, ecological, and evolutionary explanations proposed to explain variation in species richness along latitudinal or elevational gradients, the geometry of species ranges in relation to geographical boundaries has been ignored in most of the ecological literature (e.g., Pianka 1966; Terborgh 1977, 1985; Huston 1979; Shmida and Wilson 1985; Currie 1991). If geographical ranges for a group of species are placed at random within the area they jointly occupy, without regard to climate or history, more species will be found near the center of that area than near the edges; such process is called the hard boundary or mid-domain effect (Colwell and Hurtt 1994; Colwell and Lees 2000; Grytnes and Vetaas 2002; Grytnes 2003b). This random, one-dimensional placement of ranges between two boundaries or null model predicts a convex, symmetrical pattern of species richness. The pattern is either parabolic or quasi-parabolic depending upon alternative distributions of range size and midpoints (Colwell and Lees 2000).

Any natural biogeographical barriers that present some degree of resistance to species dispersal are called hard boundaries (Colwell and Hurtt 1994). Mountaintops in the elevational gradient and oceans, and arctic snowline in latitudinal gradients are hard boundaries because they limit the expansion of organisms beyond the boundaries (Colwell and Lees, 2000). Hard boundaries can be physical or climatic like the tree-line, snow-line and desert boundary. Thus, natural boundaries vary in their potential to limit species distributions. Lyons and Willig (1997) introduced the term soft boundaries, which provide some degree of resistance (e.g., orographic barriers) to the expansion of species ranges.

The mid-domain effect or hard boundary is a relatively new hypothesis and has been recently added to the list of species diversity hypotheses. Quantitatively, it explains a surprisingly high proportion of the geographical variation in species for diverse groups of organisms (Colwell and Lees 2000). Although the mid-domain effect can explain variation in species richness, it has been criticized as a theory of species diversity (e.g., Hawkins and Diniz-Filho 2002). They argued that such models ignore the fact that terrestrial species are distributed in two dimensions. The geometric models must explain longitudinal as well as latitudinal gradients (see Bokma and Mönkkönen 2000). The mid-domain effect has been tested by generating two-dimensional models in all cases but the fit of

data to the models was poor (e.g., Bokma *et al.* 2001; Hawkins and Diniz-Filho 2002; Diniz-Filho *et al.* 2003).

Researchers (e.g., Bhattarai and Vetaas 2006) observed wider elevation ranges at mid-elevations and narrow elevation ranges at either end of the gradient and suggested that these may be caused by the presence of a mid-domain effect. When the gradient ends from both directions are discarded to avoid any hard-boundary effects, the hump-shaped pattern between species richness and elevation does not disappear. This suggests that the hump-shaped patterns might be primarily controlled by climate. If one considered the hard boundary as a primary factor in controlling variation in species along the elevation gradient it may underestimate the importance of climate variables (Hawkins *et al.* 2003). Hawkins and Diniz-Filho (2002) argued that the mid-domain effect cannot explain the species richness gradient, so it should therefore be eliminated from the theory of species diversity. But works based on simulations and on analytical null models by several authors (e.g., Colwell and Hurtt 1994; Willig and Lyons 1998; Colwell and Lees 2000; Grytnes and Vetaas 2002) have demonstrated that the mid-domain effect is often essential to explain variation in species richness in diverse groups of organisms. Therefore, it is argued that it needs to be verified further by future studies before making any final conclusions about the acceptance or rejection of the mid-domain hypothesis.

RAPAPORT'S RULE

Rapaport (1975, 1982) proposed that geographical ranges of species are larger at higher latitudes than at the equator. Stevens (1989) found a similar pattern from his observation on trees, mammals, birds, marine mollusks, fish, and reptiles in North America and named this pattern Rapaport's rule. He claimed that this rule can explain the variation in many different kinds of organisms at broad-scales. This rule became popular as "latitudinal Rapaport's rule". Stevens (1992) extended "latitudinal Rapaport's rule" to an "elevation Rapaport's rule", stating that species richness decreases with increasing elevation due to the increasing altitudinal range of species. Following its publication, it was used as potential theory to explain the variation of species along both altitudinal and latitudinal gradients. But this rule has been debated in many studies (e.g., Rohde *et al.* 1993; Colwell and Hurtt 1994; Rohde 1996) and has become a topic of intense debate and discussion within biogeography (e.g., Gaston *et al.*, 1998; Fleishman, *et al.*, 1998). Bhattarai and Vetaas (2006) test how tree species richness along the elevational gradient is explained by Rapaport's elevation rule. They observed hump-shaped patterns between tree species richness and elevation and the elevation range sizes are larger at the middle of the gradient and decline at both ends, contrary to Steven's prediction.

AREA

The relationship between species richness and area has a long history in ecology (e.g., de Candolle 1855). Ecologists noticed it before any other diversity patterns. Indeed, it is one of the most general and best-documented patterns in nature for a wide variety of taxa and ecosystems (Brown and Lomolino 1998; Lomolino 2000). Regardless of the taxonomic group or type of ecosystem being considered, large areas have more species than small areas (Gleason 1922; Preston 1962; Williams 1964; Lomolino 2000). The species-area relationship truly deserves the status of a rule, and that we can confidently use it as a universal tool for understanding and conserving biological diversity. Rosenzweig (1995) comments that “you will find more species if you sample a larger area”. The larger areas have more species due to the availability of more individuals, more habitats, and more biogeographical provinces (Williams 1964; Rosenzweig 1995, Bhattarai 2003). Therefore, area is an important variable in explaining variation in species richness at both broad and finer scales.

The influence of area on variation in species richness along the elevational gradient and latitudinal gradient has rarely been considered (e.g., Md. Nor 2001; Heaney 2001). Ecological studies that do not take into account the effect of area may detect spurious patterns (Rahbek 1997). It is very important to account the effect of area to find real patterns. One of the study where area is accounted is made by Bhattarai *et al.* (2004b) prior to considering variation in species richness along elevational gradients. Contrary to predictions from the species-area relationship, Bhattarai and Vetaas (2006) did not observe maximum species richness at the largest areas available along the elevation gradient. The areas often decrease with increasing elevation because of the generally steeper terrain towards the higher peaks (Körner 2000, 2002; Lomolino 2001). The reduction in area may influence species richness, which is well known from island biogeography, where small islands support fewer species than larger islands (Körner 2002 and references therein). This area-based explanation seems reasonable at first sight but only when applied to gamma diversity or total richness, not to species richness along the elevation gradients (Lomolino 2001).

Conclusions

To explain patterns of species richness, many hypotheses are proposed but most of them are complementary rather than exclusive. It is not possible to capture the many disparate phenomena of species richness at only one scale of analysis and or to express the patterns in few simple formulae. Therefore, a pluralistic body of hypotheses, which incorporates historical, biological and climatic factors, is needed to explain the observed variations in species richness. This is depicted by the strong relationship between climate, biological interactions,

and historical processes in influencing variation in species richness at different spatial scales.

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