Variation in leaf biomass and fruit output of *Juniperus indica* along an elevation gradient in north-central Nepal

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Biomass and reproductive output are important functional traits that influence aspects of plant performance. Measurements of these attributes by harvesting plant parts are often destructive and impractical. Therefore, non-destructive methods, based on allometric relationships, have been recommended for measuring plant biomass and reproductive output, particularly in the ecosystems where plant harvesting is not very practical or feasible. Here, we assessed the variation in the traits related to vegetative and reproductive performance (including plant height, trunk diameter, canopy area, leaf biomass and number of fruits set) among populations of *Juniperus indica* distributed along an elevation gradient in Manang district of the north-central Nepal, and finally determined the allometric relationships addressing the leaf biomass and the fruit output. The distribution range of *J. indica* was divided into lower- (3,350–3,580m), mid- (3,650–3,880m) and higher- (3,950–4,250m) elevation classes where we made 54 sample plots of 10m × 10m size. In each plot, we recorded the number of individuals of *J. indica* classifying into seedling, juvenile and mature classes, and measured their vegetative traits and fruit output. Trunk diameter, leaf dry-weight and fruits set parameters spatially varied within the same elevation class. The individuals at the lower-elevation were larger in vegetative size with larger- trunk, height and canopy area, and produced higher leaf biomass and greater number of fruits as compared to those produced by the individuals situated at the mid- and higher-elevations. The regression analysis showed the strongest relationship between the canopy area and the leaf biomass. Thus, the use of outer canopy dimension is found to be the best option for estimation of leaf biomass of *J. indica* using non-destructive method.

Key words: fruit output, leaf biomass, non-destructive method, Manang

Plant biomass and reproductive output are important functional traits that determine plant growth, aspects of individual performance and competitive ability. There are two methods available for estimating plant biomass- i) destructive method and ii) non-destructive method. The destructive method, also known as the harvest method is the most commonly used method for measuring plant biomass and reproductive output. It is the most direct and accurate method, and involves plant harvesting in the known area, and measuring the fresh or oven-dried weight of the different plant parts for biomass estimation (Tackenberg, 2007; Vashum and Jayakumar, 2012). Measurements of these attributes by harvesting plant partsare often destructive, and time-as well as resource-consuming, and requirea large number of samples, which is impractical for rare and threatened

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species. Therefore, non-destructive methods, based on allometric relationships, have been recommended for measuring plant biomass in such ecosystems where plant harvesting is not very practical or feasible (Tackenberg, 2007; Vashum and Jayakumar, 2012). Non-destructive measurements of plant allometric attributes (e.g., height, canopy dimensions and stem diameter) have long been used to estimate plant biomass (Mason and Hutchings, 1967; Peek, 1970; Ludwig *et al*., 1975) and reproductive output (Haymes and Fox, 2012; Otárola *et al*., 2013). Recent interests in quantifying ecosystem carbon stocks, and potential uses of bio-energy have shown the need of implementing non-destructive methods to estimate total above ground biomass (Ansley *et al*., 2012). Studies indicate that canopy area and/or stem diameter can provide the best regression fit for above ground biomass prediction in several tree species including *Juniperus* (Mason and Hutchings, 1967; Ansley *et al*., 2012).

Juniperus indica is an important component of sub-alpine forest of Manang district, north-central Nepal (Ghimire *et al.*, 2008a). *Juniperus* forest in the Nepal Himalayas is under threat due to high anthropogenic pressure (e.g., destructive practices, such as over-harvesting of leaves for incense and slash-burning to harvest its wood) as well as harsh climatic conditions. Studies carried out in other parts of the world have shown that the principal ecological problems in junipers are related to low production of viable seeds (Juan *et al.*, 2003; Otto *et al*., 2010). Juan *et al.* (2003) assessed viability in *J. oxycedrus* which showed difficulties in seed germination because of harsh cold climate. In some species of *Juniperus*, low reproductive success is due to low amount of pollen that reaches female individuals resulting in less number of fruits set (Juan *et al*., 2003).

Most of the works on Himalayan junipers are confined to essential oil variation in leaf(e.g., Adams and Chaudhary, 1996; Adams *et al.*,1998), taxonomic determination (e.g., Adams *et al.*, 2009), and ethnobotany (e.g., Bhattarai *et al.*, 2006). Ethnobotanical study of junipers (*J. indica, J. squamata* and *J. communis*) in Manang by Bhattarai *et al.* (2006) revealed that the local community and traditional Tibetan traditional practitioner had been using almost all parts for different purposes. Fruits, leaves, stem and barks of *Juniperus* spp. are used in traditional medicine to cure kidney, skin and lymph disorders, fever, cough and cold, sores, wounds, and paralysis of limbs (Bhattarai *et al.*, 2006; Ghimire *et al.*, 2008b); leaves are also burnt for incense by followers of Buddhism. The plant is also used as fencing material and for carving different household items (Bhattarai *et al.*, 2006). Dried leaves are sold locally for incense, and essential oil obtained from steam distillation of fresh leaves is traded internationally for its use in medicines and cosmetics (Ghimire *et al.*, 2008b; Gurung, 2010). Leaves are harvested throughout the year while fruits during July to August. These all activities put heavy pressure on *Juniperus* stands in the forest ecosystem.

In this study, we sampled *J. indica* along an elevation gradient in Manang district situated in the north-central Nepal, and examined the relationship among the traits associated with vegetative and reproductive performances. We assessed the variations in the traits related to vegetative and reproductive performance (including plant height, trunk diameter, canopy area, leaf biomass and number of fruits set) among the populations distributed along the three elevation gradients. Finally, we determined the relationship among the allometric traits with leaf biomass and fruit output.

Materials and methods

Study species

J. indica is native to high-altitude Himalaya, occurring from the northern Indus Valley in Kashmir to western Yunnan in China and it occurs throughout Nepal at elevations ranging from 3,300m to 4,500m above sea level (Press *et al.*, 2000). The plant is found on open and rocky alpine slopes in drier areas; sometimes forming forests at lower elevations. The plant occurs as dwarf woody-shrub at higher elevations exceeding 4,200m and as tree growing at lower elevations of range 3,300–4,000m above sea level (Ghimire *et al.*, 2008b). The leaves are dark grey-green, dimorphic, mature plants having mostly scale-like leaves which are decussate or sometimes in whorls of 3, closely appressed, 1-3 mm long; while young plants have mostly needle-like leaves, which are borne in whorls of 3 and are 5-8mm long. Needle-like leaves are also found on shaded shoots of adult plants. The plant is dioecious with male (pollen) and female (seed) cones on separate plants. The pollen cones are sub-globose or ovoid, 2–3mm long; seed cones are ovoid, berry-like, 6–10 mm long, glossy black when ripe, and contain a single seed. The cones are seen in April to May, and mature in October to December. The seeds are mostly dispersed by birds, which eat the cones (Ghimire *et al.*, 2008b).

Study area

The study area is located in Manang district of the north-central part of Nepal (Fig.1). It lies within the broad U-shaped Trans-Himalayan valley dissected by the Marshyangdi River, and is extended up to the north of the Annapurna Mountain Range (up to 7,000m asl). The northern part of the Manang Valley, therefore receives very low annual monsoonal precipitation of 450mm, whereas the precipitation at southern region (Chame, Manang, at 2,680m asl) remains to be over 1,000mm (Miehe *et al.*, 2001; Baniya *et al.*, 2009). Similarly, the mean annual temperature remains 6.2^0 Celsius in the northern Trans-Himalayan valley while 11.0^0 Celsius in the southern region in Manang district. The moisture is found to be decreasing from east to west in the Upper Manang Valley, and the south-facing slopes are much drier than those facing north (Bhattarai *et al.*, 2004; Ghimire *et al.*, 2008a).

Vegetation in the Manang Valley at elevations above 3000m supports the luxuriant stands of *Pinus wallichiana*, *Betula utilis* and *Abies spectabilis* on the north-facing slopes, and some patches of *P. wallichiana* on the dry south-facing slopes (Baniya *et al.*, 2009). *J. indica* and *Rosa sericea* with other shrubs are dominant on the dry south-facing slopes. At the lower elevations, the vegetation mainly comprises *Juniperus squamata*, *Lonicera obovata* and *Caragana gerardiana*.

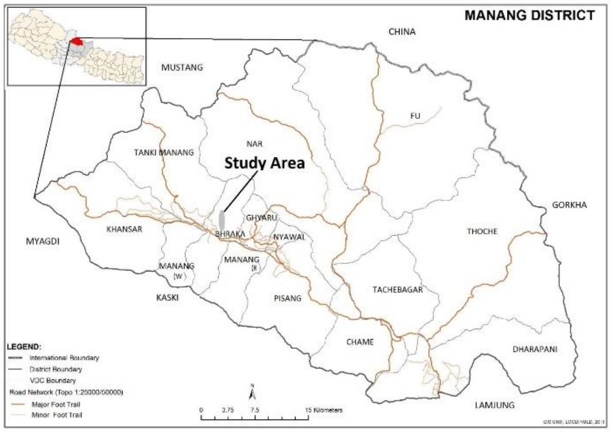


Fig.1: Map of the study area.

Methods

The sampling of *J. indica* population was carried out in September 2011 during fruiting season in the north-eastern part of the Manang Valley. A systematic sampling approach was used. The study was started from Bhraka Village (3,350m asl) almost at the bottom of the valley to the Ice Lake (4,250m asl). The whole of the distribution range was divided into lower- (3,350–3,580m), mid- (3,650–3,880m) and higher- (3,950–4,250m) elevation classes so as to cover the wider range of distribution of *J. indica*, heterogeneous environmental conditions and diverse vegetation types. In each elevation class, three horizontal transects were laid at 75–100m elevation intervals. In each transect, six plots of size 10m × 10m were sampled at 50–100m length intervals, totaling 54 plots from all transects and elevation classes. Each plot (100m^2)was further divided into 4 subplots of 5m × 5m size.

In each subplot, individuals of *J. indica* of different size (maturity) classes were recorded separately. The size classes were recognized corresponding to their growth stages following Schemske *et al.* (1994). The size classes were broadly defined according to the plant height or trunk diameter as seedlings (height <0.1m and trunk diameter <1cm), juveniles (height 0.1–1.0m, trunk diameter <1cm) and mature (height usually >1m, trunk diameter >1cm and also bearing reproductive structure). The mature individuals were recorded for their height (ground level to the top of the canopy), trunk diameter, canopy area and number of fruits. The trunk diameters of mature>1–3m tall individuals were measured at 25cm aboveground, whereas the trunk diameters of mature>3m tall individuals were measured at 137cm aboveground. The crown cover was directly measured in terms of the canopy area occupied by each adult individual using Measuring Tape. In each plot, a mature individual was randomly selected, and its leaves were collected within an area of 0.0625m^2 by randomly placing a small quadrat (0.25m × 0.25m) on the crown surface. All the leaves within the selected quadrats were collected from the crown base to the tip across the vertical profile. The leaves were packed in paper bags, and fresh weight was taken with the help of a Spring Balance (error of ±2.5gm).In the field, all the leaf samples, collected from each plot, were packed in cotton bags, and dried in shade. After returning to the laboratory, the samples were oven dried at 60^0 C for 72 hours, and dry weight was recorded with the help of a Digital Weighing Machine (error ±1.5gm). We could collect the samples from only 46 plots due to the absence of mature individuals in the rest 8 plots. The number of fruits per tree was counted only from one mature individual present within each sub-plot. We could measure the number of fruits from only 90 plants, one each from 90 sub-plots as the mature individuals were completely absent in the remaining sub-plots as in the 8 plots. The latitude, longitude and altitude of each plot were recorded with the help of Global Positioning System (GPS) device. The aspect and slope of each plot were recorded with the help of Compass and Clinometer, respectively.

The variations in the vegetative and reproductive traits among the three elevation classes was tested using one-way ANOVA. The relationships between the vegetative and reproductive traits were analyzed by calculating Pearson Correlation Coefficients. The traits exhibiting statistically significant correlations were further analyzed through linear regression analysis to evaluate the strength of relationships and derive allometric equations. Particularly, we focused on significant allometric traits to predict the leaf biomass (vegetative trait) and the number of fruits (reproductive trait).To meet the statistical assumptions of normality and homogeneity of variance while fitting linear regression, the trunk diameter, the plant height, the leaf dry weight and the number of fruits were log transformed, and the canopy area was square-root transformed. All the statistical analyses were performed using the SPSS 17.0 Software.

Results and discussion

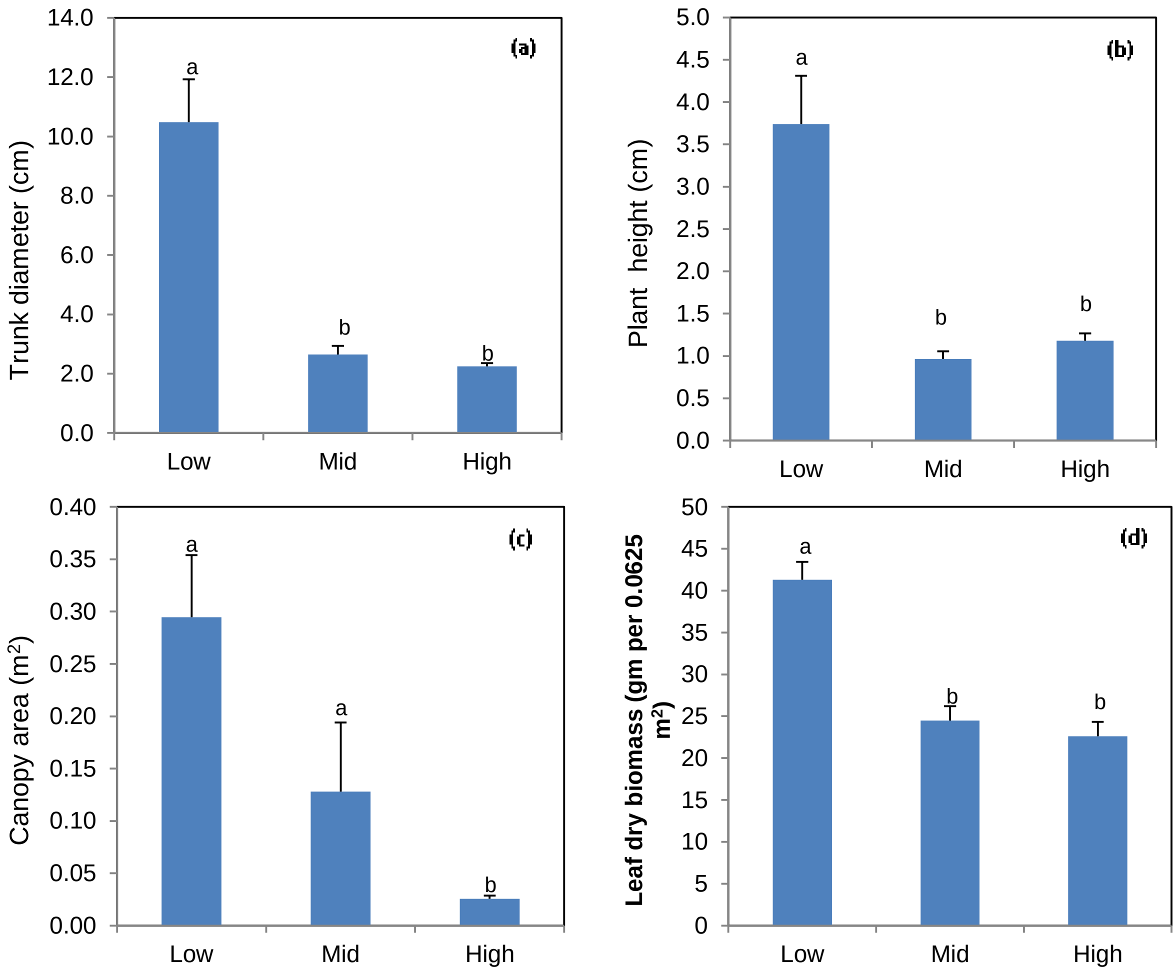
Variation in vegetative and reproductive traits

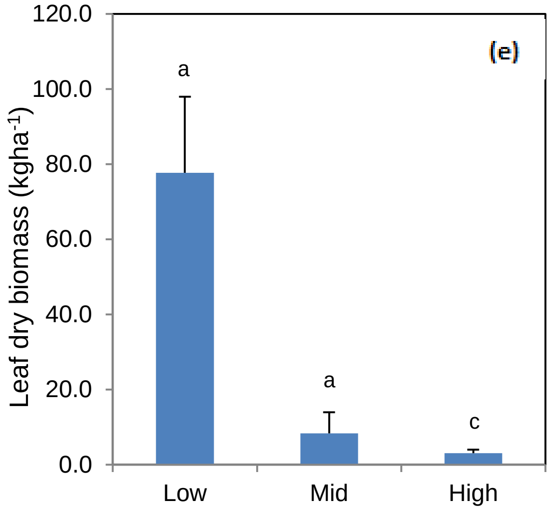
The Mean, Standard Error (SE) and the range values of the vegetative and the reproductive traits of *J. indica* recorded in the Ice Lake Area of the Upper Manang Valley are given in Table 1. Most of the individuals were of moderate to small size with the height, trunk diameter and canopy area ranging from 0.5m to 25.0m, 0.95cm to 60.48cm and $0.01m^2$ to $2.01m^2$, respectively. The mean leaf dry weight per $0.0625m^2$ canopy was found to be 0.031kg, ranging from 0.013kg to 0.058kg; the mean dry leaf biomass was calculated to be 28.98kgper hectare. The mean number of fruits per plant ranged from 10 to1040 (mean 202.9).

Table 1: Mean, SE and range values of the vegetative and the reproductive traits of *J. indica* recorded in the Ice Lake Area of the Upper Manang Valley

Traits	N	Mean	SE	Minimum	Maximum
Plant height (m)	134	2.62	0.26	0.50	25.00
Trunk diameter (cm)	134	5.82	0.71	0.95	60.48
Canopy area (m^2)	134	0.16	0.03	0.01	2.01
Leaf dry weight (g) per $0.0625m^2$ canopy	46	31.12	1.65	12.50	58.10
Leaf dry weight (g) per plant	46	153.26	47.27	1.22	1801.51
Leaf dry biomass (kg ha^{-1})	46	28.98	8.37	0.18	202.20
Number of fruits per plant	90	202.90	21.64	10.00	1040.00

J. indica showed higher values of all its vegetative and reproductive traits at the lower-elevation than at the mid- and the higher-elevations (Fig. 2). The individuals of *J. indica* at the lower-elevation were found to be larger in size, with larger trunk, height, canopy area, and produced higher leaf biomass and greater number of fruits as compared to those from the mid- and the higher-elevations (Fig. 2).





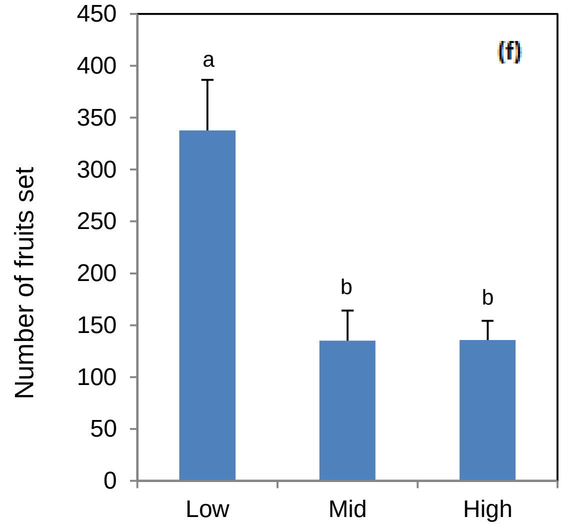


Fig. 2: Variations in (a) trunk diameter, (b) plant height, (c) canopy area, (d) leaf dry biomass (gm per 0.0625 m^2), (e) leaf dry biomass (kgha^{-1}), and (f) number of fruits set of *J. indica* at the three elevation classes (low, mid and high) around the Ice Lake in the Upper Manang Valley. The means with different letters represent significant difference in the traits among the three elevation classes at <0.05 level of significance based on the One-way ANOVA.

Correlation among the traits

The Pearson Correlation Analysis revealed significant relationships among a numbers of traits of *J. indica* (Table 2). Significant correlations (p=<0.01) were observed between- the trunk diameter and the plant height (r = 0.58), the trunk diameter and the canopy area (r = 0.34), the trunk diameter and the leaf dry weight (r = 0.54), the canopy area and the leaf dry weight (r = 0.93), the trunk diameter and the number of fruits (r = 0.44), and the plant height and the number of fruits (r = 0.34).

Table 2: The Pearson correlation coefficients among the vegetative and reproductive traits of *J. indica*.

Traits	Abbreviation	TrDiam	Ht	CanAr	DrWtPl	NoFr
Trunk diameter	TrDiam	1 (n = 131)				
Plant height	Ht	0.580* (n = 129)	1 (n = 129)			
Canopy area	CanAr	0.344* (n = 124)	-0.074 (n = 122)	1 (n = 127)		
Leaf dry weight per plant	DrWtPl	0.538* (n = 53)	0.252 (n = 52)	0.932* (n = 51)	1 (n = 56)	
Number of fruits per plant	NoFr	0.439* (n = 87)	0.339* (n = 87)	0.100 (n = 82)	0.332 (n = 33)	1 (n = 88)

Note: Statistically significant correlations (p=<0.05) are denoted by asterisk (*); other correlations being insignificant.

Regression analysis for statistically significant traits

The allometric traits exhibiting statistically significant correlations with leaf dry weight and fruit production were further analyzed using simple linear regression analysis to evaluate the strength of relationships and derive allometric equations (Fig. 3). The relationship between the plant height and the leaf dry weight and between the canopy area and the number of fruits were not further analyzed as their strength of relationship was very low (Table 2). Of the three allometric measurements (trunk diameter, plant height and canopy area), the canopy area was found to be the strongest variable to explain the variability of the total leaf biomass (Fig. 3b).

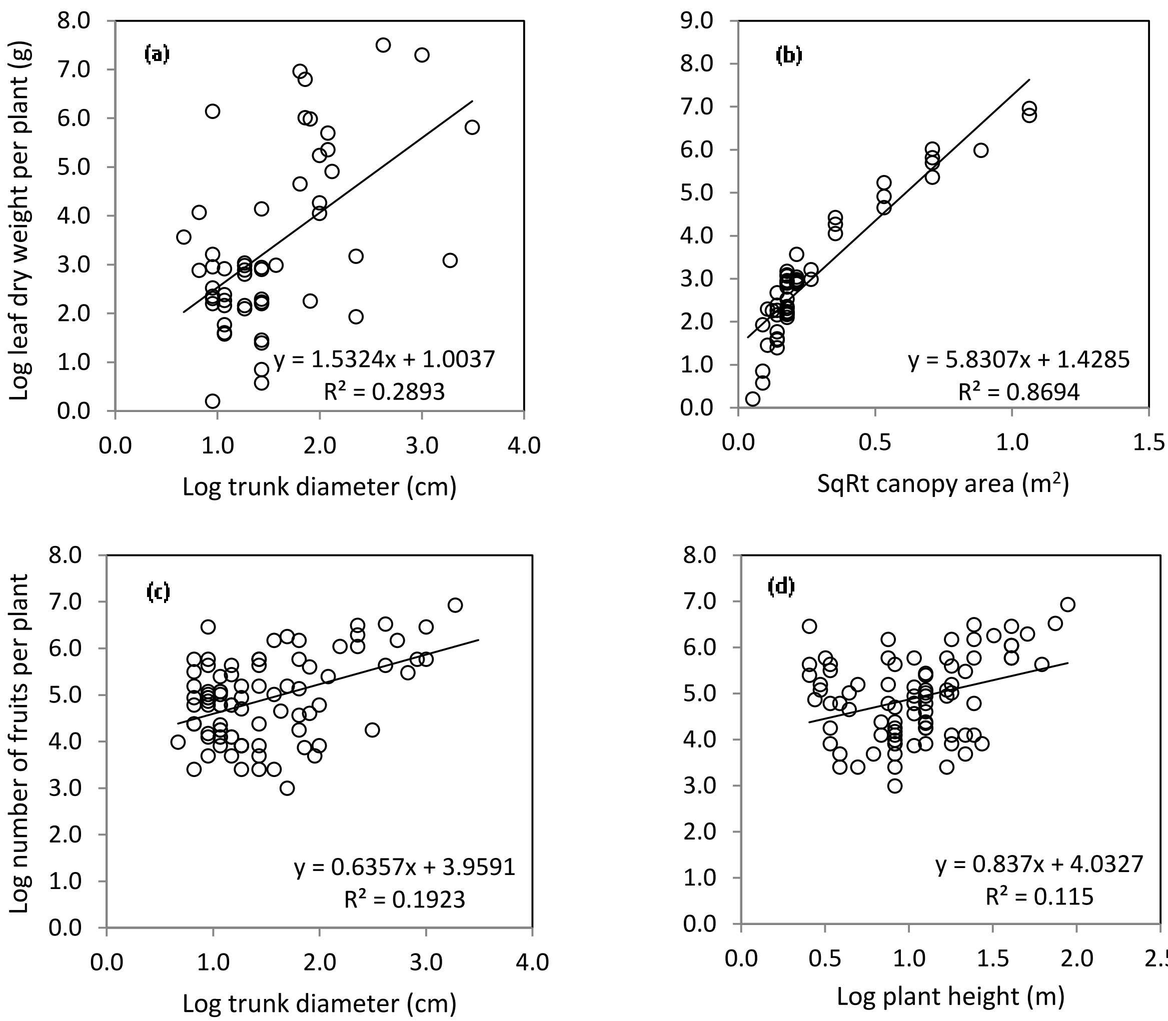


Fig. 3: Relationships between (a) the trunk diameter and the leaf dry weight, (b) the canopy area and the leaf dry weight, (c) the trunk diameter and the no. of fruits, and (d) the plant height and the no. of fruits. Fitted line based on linear regression model.

The canopy area of *J. indica* in the present study varied from $0.01m^{-2}$ to $2.01m^{-2}$ per tree which is far less than the findings of Ghimire and Devkota (2008) who measured the canopy area of *J. indica* of the Kangchenjunga Conservation Area, East Nepal to be $7.98\pm0.32m^2$ (mean±SE, range: 0.01–$7.98m^2$). This might be because of the difference in the micro-climate between the more mesic condition of the Eastern Himalaya and the xeric microclimate of the Manang Valley. Of the three allometric measurements (trunk diameter, plant height and canopy area), the canopy area was found to be the strongest variable ($r^2 = 0.869$) for predicting the total leaf biomass. The Average canopy area and, thus, the mean leaf dry weight per plant tended to be high at the lower-elevation, which decreased gradually towards the mid- and the higher- elevations. Subedi (2016) also reported highest leaf biomass in *J. squamata* at the low elevation in the Manang Valley. Linear negative relationship between biomass and elevation has been reported for several woody plant speices (e.g., Rastetter *et al.*, 2004). Plants growing along an elevation gradient exhibit reduction in radial and vertical growth of their stem towards higher elevations mostly caused by corresponding decline in temperature and nutrient availability, and delay in start of seasonal growth (Körner *et al.*, 1983; Klinka *et al*., 1996).

Among the other allometric variables, the trunk diameter was found to be less strong for predicting total leaf biomass ($r^2 = 0.289$). Plant height was found to be even less effective predictor of leaf biomass, which is similar to the findings of Ansley *et al.* (2012). The reason might be due to the suppression and release from suppression in vertical growth at different time periods and space, corresponding to light intensity, moisture and temperature. Measurements of basal trunk diameter and canopy height are difficult in bushy-juniper because of very compact canopy with a high density of under-growth stems that restrict access to the core base of the stems. Thus, the use of individual outer canopy dimensions may be the best option for non-destructively estimating leaf biomass of *J. indica* as reported in other species (Ansley *et al.*, 2012).

None of the allometric traits considered in this study showed strong power for predicting fruit-output. Nevertheless, larger plants [with greater trunk diameter ($r^2 = 0.192$) and height ($r^2 = 0.115$)] produced more fruits as compared to the smaller ones (Table 2). As the individuals at the lower elevation are generally larger in size, they produced greater number of fruits than those in the mid- and higher-elevations. The less number of fruits produced per plant in the mid- and high-elevations may also be due to the limited pollination success as reported by Juan *et al.*(2003) as a result of the wider spatial distance between the male and the female plants. However, further study is needed to support this statement in the local scenario.

Conclusion

Vegetative and reproductive traits are found to be the most important characteristics to differentiate the populations of *J. indica* that are influenced differently by the variation in elevation as the individuals at the lower-elevation were larger in vegetative size, with larger trunk, height, canopy area, and produced higher leaf biomass and greater number of fruits as compared to the individuals at the mid- and higher-elevations in this study. Trunk diameter, leaf dry-weight and fruits set parameters spatially varied within the same elevation class. The use of outer canopy area was also found to be the best option for non-destructively estimating the leaf biomass of *J. indica*. This technique can be, therefore, used for estimating the leaf biomass of *J. indica* for different purposes, e.g., for its bio-energy estimation as well as for quantification of its carbon stock.

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References

Adams, R. P. and Chaudhary, R. P. 1996. Leaf essential oil of *Juniperus indica* Bertol. from Nepal. *Journal of Essential Oil Research* **8:** 677–680.

Adams, R. P., Chaudhary, R. P., Pandey, R. N. and Singh, L. 2009. *Juniperus recurva* var. *uncinata*, the hooked branchlet juniper, a new variety from Nepal. *Phytologia* **91(3):** 361–366.

Adams, R. P., Thapa, R. K., Agrawal, S. G., Kapahi, B. K., Srivastava, T. N. and Chaudhary, R. P. 1998. The leaf essential oil of *Juniperus recurva* Buch. Ham. ex D. Don from India and Nepal compared with *J. recurva* var. *squamata* (D. Don) Parl. *Journal of Essential Oil Research* **10:** 21–24.

Ansley, R. J., Mirik, M., Surber, B. W. and Park, S. C. 2012. Canopy area and aboveground mass of individual redberry juniper (*Juniperu spinchotii*) trees. *Society for Range Management* **65 (2):** 189–195.

Baniya, C. B., Solhøy, T. and Vetaas, O. R. 2009. Temporal changes in species diversity and composition in abandoned fields in a trans-Himalayan landscape, Nepal. *Plant Ecology* **201:** 383–399.

Bhattarai, K. R., Vetaas, O. R. and Grytnes, J. A. 2004. Relationship between plant species richness and biomass in an arid sub-alpine grassland of the central Himalayas, Nepal. *Folia Geobotanica* **39:** 57–71.

Bhattarai, S., Chaudhary, R. P. and Taylor, R. S. L. 2006. Ethnobotany of wild junipers (*Juniperus* species) in Manang district, central Nepal. *Scientific World* **4 (4):** 109–112.

Ghimire, B. K., Lekhak, H. D., Chaudhary, R. P. and Vetaas, O. R. 2008a. Vegetation analysis along an altitudinal gradient of *Juniperus indica* forest in southern Manang Valley, Nepal. *International Journal of Ecology and Development* **9:** 20–29.

Ghimire, S. K. and Devkota, B. 2008. Non-Timber Forest Product (NTFP) Management Action Plan: Kumbhakarna Conservation Community Forest, Kanchenjunga Conservation Area, Lelep – 9, Ghunsa, Taplejung. WWF Nepal (in Nepali).

Ghimire, S. K., Sapkota I. B., Oli, B. R. and Parajuli, R. R. 2008b. **Non-Timber Forest Products of Nepal Himalaya: Database of Some Important Species found in the Mountain Protected Areas and Surrounding Regions.**WWF Nepal Program, Kathmandu, Nepal.

Gurung, K. 2010. **Essential Oils Sector Study in Nepal: A Detailed Study of Anthopogon, Juniper and Wintergreen Essential Oils.** GTZ, Lalitpur, Nepal.

Haymes, K. L. and Fox, G. A. 2012. Variation among individuals in cone production in *Pinus palustris* (Pinaceae). *American Journal of Botany* **99 (4):** 640–645.

Juan, R., Pastor, J., Fernandez, I. and Diosdado, J. C. 2003. Relationships between cone traits and seed viability in *Juniperus oxycedrus* L. subsp. *macrocarpa* (Sm.) Ball (Cupressaceae). *Acta Biologica Cracoviensia Series Botanica* **45 (2):** 69–78.

Klinka, K., Wang, Q., Carter, R. E. and Cher, H. Y. H. 1996. Height growth-elevation relationships in subalpine forests of interior British Columbia. *The Forestry Chronicle* **72 (2):** 193–198.

Körner, C. and Chochrane, P. 1983. Influence of leaf physiognomy on leaf temperature on clear midsummer days in the Snowy Mountains, south-eastern Australia. *Acta Oecologia* **4:** 117–124.

Ludwig, J. A., Reynolds, J. F. and Whitson, P. D. 1975. Size–biomass relationships of several Chihuahuan desert shrubs. *The American Midland Naturalist* **94**: 451–461.

Mason, L. R. and Hutchings, S. S. 1967. Estimating foliage yields on Utah juniper from measurements of crown diameter. *Journal of Range Management* **20:** 161–166.

Miehe, G., Winiger, M., Böhner, J. and Zhang, Y. 2001. The climate diagram map of High Asia: purpose and concepts. *Erdkunde* **55:** 94–95.

Otárola, M. F., Sazima, M. and Solferini, V. N. 2013. Tree size and its relationship with flowering phenology and reproductive output in Wild Nutmeg trees. *Ecology and Evolution* **3 (10):** 3536–3544.

Otto, R., Krüsi, B. O., Delgado, J. D., Fernández-Palacios, J. M., García-Del-Rey, E. and Arévalo, J. R. 2010. Regeneration niche of the Canarian juniper: the role of adults, shrubs and environmental conditions. *Annals of Forest Science* **67 (7):** 709.

Peek, J. M. 1970. Relation of canopy area and volume to production of three woody species. *Ecology* **51**: 1098–1101.

Press, J. R., Shrestha, K. K. and Sutton, D. A. 2000. **Annotated Checklist of the Flowering Plants of Nepal**. The Natural History Museum, London.

Rastetter, E. B., Kwiatkowski, B. L, Dizes, S. L. and Hobbie, J. E. 2004. The role of down-slope water and nutrient fluxes in the response of arctic hill slopes to climate change. *Biogeochemistry* **69:** 37–62.

Schemske, D. W., Husband, B. C., Ruckelshaus, M. H., Goodwillie, C., Parker, I. M. and Bishop, J. G. 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* **75:** 584–606.

Subedi, P. U. 2016. Population Structure and Plant Performance of *Juniperus squamata* Buch.-Ham. ex D. Don along an Elevation Gradient in Manang, Nepal. M.Sc. Thesis, Central Department of Botany, Tribhuvan University, Kritipur, Nepal.

Tackenberg, O. 2007. A new method for non-destructive measurement of biomass, growth rates, vertical biomass distribution and dry matter content based on digital image analysis. *Annals of Botany* **99:** 777–783.

Vashum, K. T. and Jayakumar, S. 2012. Methods to estimate above-ground biomass and carbon stock in natural forests - A review. *Journal of Ecosystem and Ecography* **2:** 4 http://dx.doi.org/10.4172/2157–7625.1000116 accessed on Dec 27, 2016.