Research Article

Plant Species Richness, Structure and Life Form Respond to an Altitudinal Gradient in Central Bhutan, Eastern Himalayas.

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A team in the field carrying out survey in the eastern Himalaya

Abstract

Understanding how species richness changes with altitude is important for conservation management. In Central Bhutan, an altitudinal vegetation transect from 2985 m to 3790 m along an eastern spur on Kiki Phu, in Bumthang, was surveyed for plant species richness in autumn (November) 2014. Species richness, life form diversity, canopy cover and ground cover were measured every 100 m of altitude using two 25 m x 25 m quadrats (north and south). For the dominant tree species, height and diameter at breast height (DBH) were measured and compared against altitude. In total 114 species of vascular plant species were found, with a significant decrease in species richness occurring as altitude increased, from 42 species to 124 species, with altitude 3157 m showing a slight increase against this trend. Life form diversity varied significantly with altitude with forbs in particular decreasing in species richness. Maximum canopy cover, tree height and DBH of dominant species occurred at the mid elevations of 3255 m – 3461 m, and these were significantly different on the southern side of the ridge, where canopies were open

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and trees shorter. Mid altitude patterns may be related to moisture regimes and grazing with dampening effects on canopy cover, tree height and life forms particularly on the southern sides of the ridge. A Principal Coordinates Analysis showed species composition clustered with altitude. These results establish baseline floristic data for autumn which may be important for monitoring change over time and space to assess impacts of anthropogenic pressure and climate change.

Keywords: altitude, Bhutan, dominant species, elevation gradients, impact, species richness, vegetation.

Introduction

The biogeographical patterns of species and communities along environmental gradients have intrigued ecologists and biogeographers for over two centuries (e.g., Humboldt and Bonpland, 1805). Discovering that vegetation diversity and structure changes with altitude, the pioneers of biogeography also reported that plant diversity decreases non-linearly from the lowest to the highest elevations (Lomolino *et al*. 2006). Preferential sampling at some elevations, may have influenced these findings however (Carpenter 2005), and since then, several researchers have identified other trends and relationships between species richness and elevation — from linear (Hamilton 1975; Stevens 1992), to bimodal (Tanaka & Sato 2014) to unimodal associations (Bhattarai & Vetaas 2003; Lieberman et al. 1996; Odland & Birks 1999; Toledo-Garibaldi & Williams-Linera 2014; Vetaas & Grytnes 2002; Wangda & Ohsawa 2006a).

Many researchers in the Himalayas have sought to describe these patterns, and almost half (Carpenter 2005) have found a unimodal distribution, with maximum species richness generally occurring below an altitude of 2000 m (Acharya *et al*. 2011; Behera & Kushwaha 2007; Bhattarai & Vetaas 2003; Bhattarai *et al*. 2004; Carpenter 2005; Vetaas & Grytnes 2002), or c. 2500 m (Chawla *et al*. 2008; Grau *et al*. 2007), between 1760 m and 2820 m in the dry valley near Bajo, Bhutan (Wangda & Ohsawa 2006a) or between 3500 – 4000 m in Ladakh (Namgail *et al*. 2012). High unimodal mid-elevation diversity has several causal explanations including optimal climatic variables, water availability (Carpenter 2005) and proximity to adjacent source communities (Bhattarai & Vetaas 2003). The harshness of the high-altitude climate, along with the smaller area and greater isolation due to the shapes of mountains are thought to be important influences on the lower diversity at higher altitudes (Lomolino *et al*. 2006). Other Himalayan researchers have found increasing species richness at higher altitudes (Shimono *et al*. 2010), or a decreasing pattern (Roy & Behera 2005) or a reversal of the unimodal pattern, with a decrease in species richness in the middle altitude ranges (Jamtsho & Sridith 2013).

In general, altitudinal patterns of biodiversity remain relatively poorly understood, partly hindered by the heterogeneous conditions that characterize montane environments and their subsequent impacts on soil, microclimate and water–energy dynamics (Desalegn & Beierkuhnlein 2010). Yet an understanding of biogeographical patterns and variation in species richness, and the trend of endemic species richness increasing with altitude, probably due to speciation from isolation, (Lomolino 2001; Vetaas & Grytnes 2002), is critical to any future conservation strategy. Developing a theoretical framework for understanding these patterns is an imperative, and of interest in this paper is the drive to understand these processes to better inform conservation and management.

The eastern Himalayan bioregion comprises a major biodiversity hotspot that is under increasing threat from human population growth and changing climates (MoA 2009). Bhutan, which has protected 51.32% of the country (MoA 2009), has a significant number of people living in protected areas (Wangchuk 2007) and a focus for research in Bhutan is on the management of protected areas. This strategy is dependent upon a robust database of species' occurrences and the influence of topographical relief on biodiversity. The aim of our study was to contribute to this database and to provide baseline data for longer term studies. Our objectives were to characterise plant species' richness and community composition over an altitudinal gradient from grassland at 2985 m to the treeline at 3790 m. The changes in plant life form and structure, and possible reasons for these patterns are examined.

Methods

Study Location

Field work was undertaken along an eastern spur of the Kiki Phu ridgeline, Bumthang, Central Bhutan (Fig. 1). The valley is geologically dominated by the Greater Himalayan Sequence that consists of predominantly high grade metamorphic rocks with intermittent outliers of metasedimentary rock (Grujic *et al*. 2002). The resultant soil types vary from soils poor in organic matter, weak podzols, brown soils and non-volcanic andosols and alpine turf soils (Baillie *et al*. 2004). All these soil types are regarded as highly erodible (Baillie *et al*. 2004). The area experiences warm wet summers and cool dry winters. Rainfall is highest between the months of June and August with an average total rainfall of around 765mm (DSU 2012). The area studied falls into the ecofloristic zones of cool temperate forests that range from 2100 - 4200 masl and alpine, that range from 3500 - 4600+ masl in elevation (Tharchen 2013). Temperatures throughout the year are consistently mild. Annual average temperatures range from 14° C maximum, to -6° C minimum during the coldest month of January. During the warmest month of August temperatures range from a maximum of 23°C to minimum overnight temperatures of 14° C (DSU 2012).

Field studies were conducted either side of the Tharpaling Monastery trail (4.3 km) along a ridge of the Kiki Phu, west from Lamai Gompa (27° 32' 44.28" N, 90° 43'29.48"). The trail runs east to west along an eastern spur (Fig. 1). The area is used for grazing by cattle and yak, with timber gathering by herders and monks (R. Singye, personal communication). Studies using camera traps set along the ridge in 2014 have recorded human, cattle, and yak movement up to 3800 m (K. Vernes, R. Rajaratnam, T. Sangay, R. Singye, unpublished data). The study site is on crown land at the Ugen Wangchuck Institute for Conservation and Environment (UWICE) and is protected forest under the *Forest and Conservation Act of 1995.*

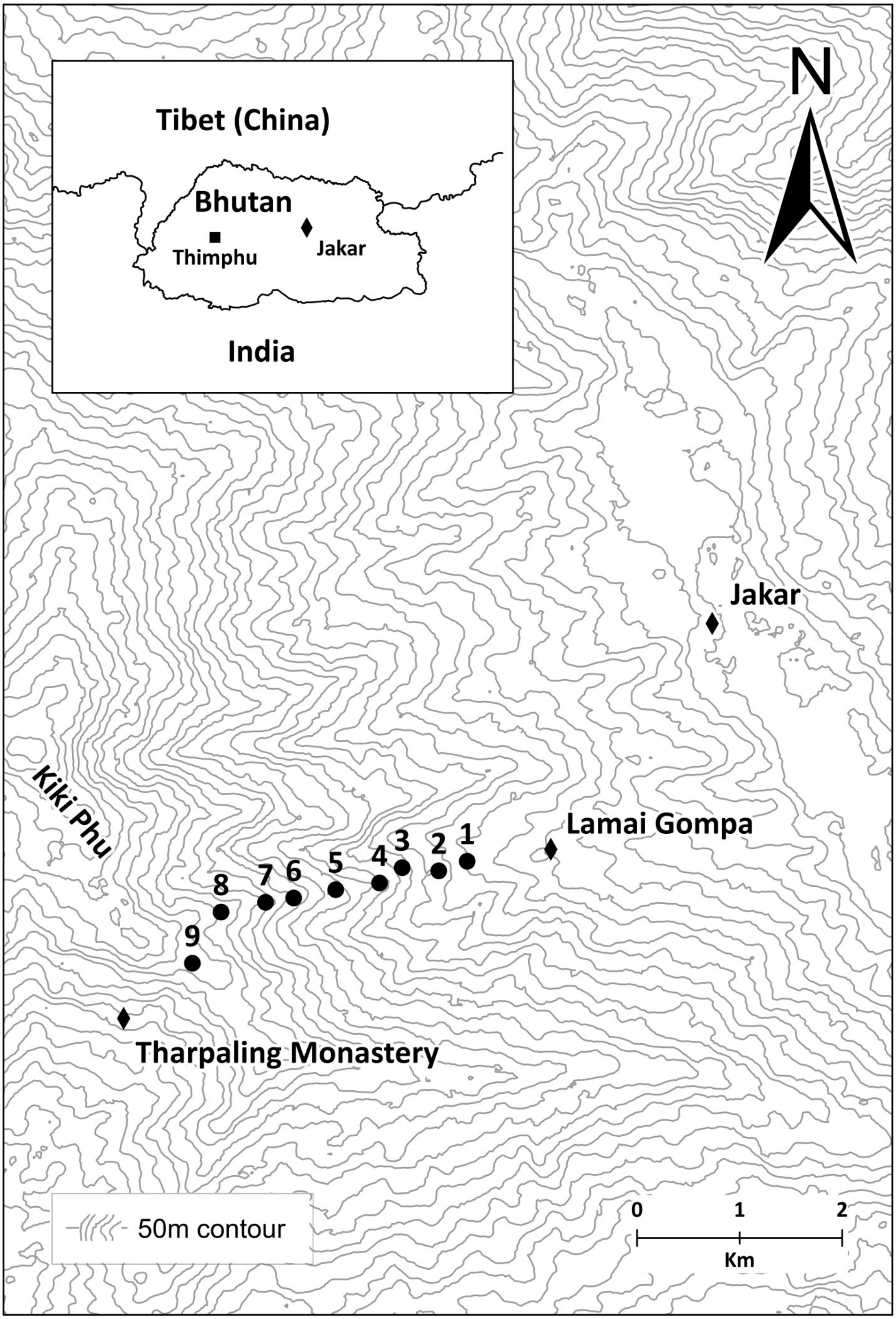

FIGURE 2 Location of the study site in central Bhutan (insert) with details of the quadrat locations adjacent to the track to Tharpaling Monastery, west of Lamai Gompa, Bumthang, Bhutan.

Field Sampling

Data collection occurred on the 21 - 22 November 2014. The first site was located above Lamai Gompa at 2985 m asl. Two 25 m x 25 m quadrats were positioned 2 metres either side (north and south) of the track to capture aspect differences and to avoid the bias of edge species. At each site GPS readings were obtained using a Garmin Etrex, WGS84 datum. The subsequent eight sites were located each c. 100 m apart up slope (Table 1, Appendix 1).

To ensure standardised sampling, six to seven people spent 10 minutes each collecting a sample for identification of every plant species encountered in the quadrat. Plants that were not identifiable in field were collected and tagged for identification against reference material held in the laboratory. Canopy cover was recorded with a photo from a digital camera or smartphone taken skywards from the centre of each quadrat and then ImageJ V1.48 software was used to process each image into a binary (black or white) output, separating clear sky from canopy. Percentage canopy cover was determined by quantifying black (canopy) and white (clear sky) pixels for each photo. The dominant species in each quadrat was determined visually, and then five specimens of it were chosen (closest to each corner, and to the middle), from which the circumference at breast height (1.2 m) was measured and height was estimated. Tree circumference was later converted to diameter at breast height (DBH) using diameter = circumference/π. In the lower corner of each quadrat, closest to the trail, a 2 m x 2 m quadrat was marked and the percentage cover of the types of ground cover (moss, grass, herbs, bare earth, leaf litter, bamboo, snow) was estimated. For sites on the northern side of the ridge, the 2 m x 2 m quadrat was in the south-eastern corner and for sites on the southern side the quadrant was situated in the north-eastern corner. Overnight snowfall occurred in sites six to nine and snow cover was added as a ground cover variable in the 2 m x 2 m quadrats. Specimens were identified using publications (Grierson & Long 1983 - 2001; Noltie 1994 & 2000) and local knowledge. A voucher collection was not obtained due to time constraints and some species were specified as "unknown", or were identified to family only. Species were classified by life form (trees, shrubs (including the bamboo, *Arundinaria racemosa*), ferns, vines, graminoids (grasses and sedges), or forbs (herbs excluding graminoids, ferns and grasses, Appendix 2)

Table 1 Summary of the altitudes, GPS co-ordinates, and description of the plant communities, for nine sites, each with a 25mx 25m quadrat, on the north side and south side of the track to Tharpaling Monastery, on an eastern spur of the Kiki Phu ridgeline, west of Lamai Gompa, Bumthang, Bhutan, November 2014.

Altitude(m)	Site	GPS (North)	GPS (East)	Aspect	Description of Vegetation Community
2985	1	27°32.672'	90°43.8'	South	Open blue pine woodland with swertia dominated grassland
				North	Open blue pine woodland with understorey of swertia
3077	2	27°32.622'	90 °42.899'	South	Blue pine forest with understorey of bracken

				North	Open blue pine forest with understorey of bracken, and daphne
3157	3	27º32.638’	90 º42.706’	South	Blue pine forest with understorey of *Rosa sericea*
				North	Blue pine forest with understorey of *Rosa sericea,* bracken and bamboo
3255	4	27º32.558’	90 º42.587’	South	Mixed blue pine and hemlock forest with understorey of bamboo
				North	Mixed blue pine and hemlock forest
3363	5	27º32.523’	90 º42.357’	South	Hemlock Forest with understorey of bamboo and rhododendron
				North	Hemlock forest
3461	6	27º32.479’	90 º42.135’	South	Mixed conifer woodland with understorey of bamboo and moss
				North	Mixed conifer woodland with understorey of bamboo and rhododendron
3579	7	27º32.456’	90 º41.987’	South	Blue pine and spruce forest with understorey of bamboo
				North	Mixed conifer open forest with understorey of bamboo and rhododendron
3690	8	27º32.405’	90 º41.753’	South	Open woodland adjacent to a mixed conifer forest with bamboo understorey
				North	Fir forest with an understorey of rhododendron and bamboo
3790	9	27º32.136’	90 º41.602’	South	Alpine grassland dominated by *Poa* sp., with occasional rhododendron shrubs
				North	Birch and rhododendron thicket with spruce emergent

Data Analysis

Species richness against altitude was investigated using linear regression with data pooled from both quadrats at each site. Life form data were pooled within site into three groups: (1) forbs, (2) trees and shrubs and (3) ferns, vines and graminoids and analysed using chi-square goodness of fit test to determine if lifeform was independent of altitude. A Wilcoxin matched pairs test was used to compare canopy cover within altitudes between south and north quadrats. The influence of the factors aspect and altitude, on the height of dominant trees, was analysed using a multifactor analysis of variance with homoscedastic variances and DBH as a covariate. A type III sums of squares was used so that the contribution of each factor was measured having removed the effects of the other factors, as we suspected that the interaction between aspect and altitude would be significant. A linear regression model was applied to tree height with DBH. The percentage ground cover data from the 2m x 2m quadrats was graphed and analysed descriptively. Principle Coordinates Analysis (PCoA), was used to visualise site similarities in species composition. Analyses were undertaken using Statgraphics Centurion, Minitab 17 or Excel add-ons (Peakall & Smouse 2006, 2012).

Results

A total of 114 species of plants were collected over the 18 quadrats between 2985 m and 3790 m on Kiki Phu, consisting of 11 tree species, 30 shrubs, 47 forbs, 15 graminoids, 6 ferns and 5 species of vine (Fig. 2, Appendix 2). Exotic species included *Trifolium repens* and *Impatiens bracteata* (with the possibility that nine unidentified graminoids may have been exotic). The four most dominant plant families (species richness) were from the Asteraceae, Ericaceae, Poaceae and Rosaceae (Appendix 2). Plants in the Asteraceae, Poaceae and Rosaceae were found in every site, and species in the Ericaceae were found at all sites with the exception of the two lowest altitude sites. The combined species richness of the north and south quadrats at each site show a total species richness of 42 at 2985 m, declining non-linearly to 24 species at 3790 m (Table 2). A statistically significant relationship was found between site and the species richness of life forms ($\chi^2 = 26.700, P = 0.045, N = 2, 16$), indicating that the proportion of species of trees and shrubs, versus forbs, and other life forms, changes with site (Fig. 3). Forbs in particular decrease in species richness at higher altitude sites and in proportion to other lifeforms and an abrupt disappearance of climbing species occurred above site 7 (3579 m), corresponding to the snow line.

TABLE 2 Summary of plant species richness (number of species) for trees, ferns, vines shrubs, graminoids, forbs and total, identified in two 25mx 25m quadrats, at 9 sites at altitudes from 2985m to 3790 m, adjacent to the Tharpaling Monastery track, Kiki Phu west of Lamai Gompa, Bumthang, Bhutan, November 2014.

Quadrat	Altitude (m)	Trees	Ferns	Vines	Shrubs	Graminoids	Forbs	Total
1	**2985**	1	1	1	9	9	21	42
2	**3077**	3	1	3	12	1	16	36
3	**3157**	3	2	4	13	3	15	40
4	**3255**	4	2	5	12	0	8	31
5	**3363**	3	4	2	10	0	4	23
6	**3471**	6	1	3	9	1	6	26
7	**3579**	7	1	0	10	1	7	26
8	**3690**	8	2	0	9	3	4	26
9	**3790**	4	1	0	10	3	6	24

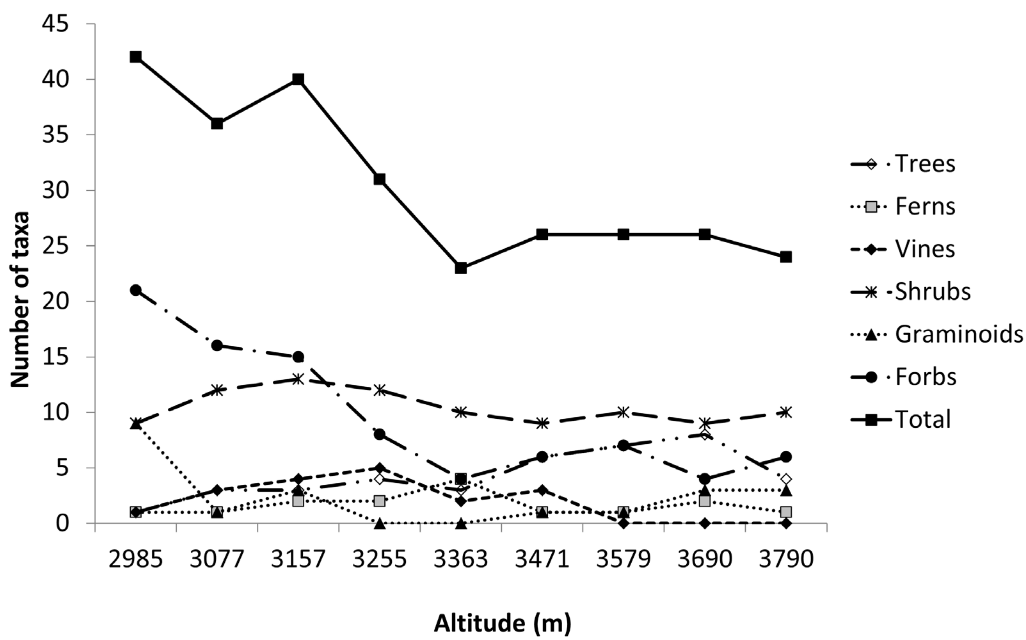

FIGURE 3 Species richness of life forms from site 1 (2985 m) to site 9 (3790 m) for the combined north and south quadrats at each of the nine altitude elevations for the transect on Kiki Phu, adjacent to the track to Tharpaling Monastery, west of Lamai Gompa, Bumthang .November, 2014

Linear regression revealed a significant negative relationship between altitude and species richness suggesting that 72% of the variation in species richness can be attributed to altitudinal position (*$y = 104 - 0.0218001x$, $R^2 = 72.08$, $P = 0.004$*, **Fig. 3**).

The percentage canopy cover showed a non-linear relationship with altitude, with the maximum canopy cover at both 3255 m and 3461 m, and the southern sites of the ridge in general having a significantly less-dense canopy cover (*$W+ = 40$, $W- = 5$, $N = 9$, $P < 0.001$*, Fig. 4).

The dominant species from 2985 m to 3471 m were blue pine (*Pinus wallichiana*), with hemlock (*Tsuga dumosa*) appearing at 3255 m, and fir (*Abies densa*), birch (*Betula utilis*) and shrubby rhododendrons (*Rhododendron campylocarpum, R. falconeri, R. lepidotum, and R. wightii)* dominating at 3790 m, where the treeline began (Table 3). Heights of the dominant trees varied significantly with aspect and altitude (aspect $F_{1, 68} = 171.6$, $P < 0.001$, altitude $F_{8, 68} = 9.79$, $P < 0.001$, interaction $F_{8, 68} = 171.6$, P = 0.07*ns*, Fig. 5)

Table 3 Dominant species for the north and south quadrats for nine sites, at altitudes from 2985m (site 1) to 3790m (site 9), adjacent to the track to Tharpaling Monastery, Kiki Phu west of the UWICE, Bumthang, Bhutan, November 2014. Shaded cells are where the taxa were dominant on both (no lettering), or the north or the south sides of the ridge.

		Altitude (masl)								
Family	**Species**	**2985**	**3077**	**3157**	**3255**	**3363**	**3471**	**3579**	**3690**	**3790**
Betulaceae	*Betula utilis*									North
Ericaceae	*Rhododendron* spp. *lepidotum*									South
Pinaceae	*Abies densa*								■	
	Tsuga dumosa				North	■		North		
	Pinus wallichiana	■	■	■	South		■	South		

With trees in the north side quadrats on average taller than trees in the south with a unimodal change in tree height with altitude (Fig. 5). Diameter at breast height also varied with aspect and altitude (aspect $F_{1, 69} = 12.91$, $P < 0.001$, altitude $F_{8, 69} = 5.65$, $P < 0.001$, interaction $F_{8, 68} = 1.35$, P = 0.23 *ns*) and DBH was a moderate predictor of tree height (*$y = 605.165 + 25.8065x$, $R^2 = 55.74$, $P < 0.001$*).

The percentage ground cover in each of the quadrats showed higher grass cover at the lowest (2985 m) and highest (3790 m) altitudes with snow appearing at site 7 (3579 m). Leaf litter was prominent in sites 2-5 (3077 – 3363 m), and moss became a feature in higher altitude sites 5-8 (3363 – 3690 m) (Fig. 6).

A Principal Co-ordinates Analysis of the 114 species for 18 sites from nine altitudinal positions showed a clustering of quadrats according to altitude (Fig. 7), with 42% of the variability between sites able to be explained by the first three axes or dissimilarities between sites. Quadrats in the same clusters have more similar individual species of plants present than those quadrats further apart. In general it can be seen that the sites most similar were at nearby altitudes, and that north and south sites at the same altitude tended to have similar species.

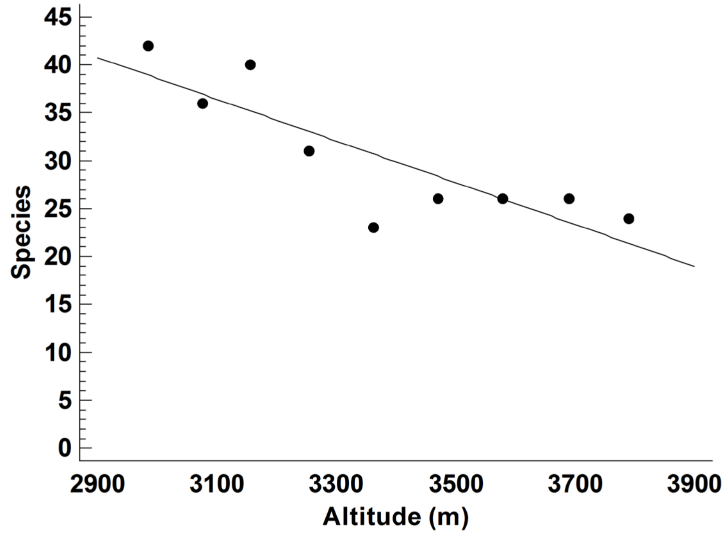

FIGURE 4 Linear regression of the total species richness (number of species) for the combined north and south quadrats at each of the nine altitude elevations (see Table 1 for descriptions) for the transect on Kiki Phu, adjacent to the track to Tharpaling Monastery, , west of Lamai Gompa, Bumthang, Bhutan, November 2014. y = 104 - 0.0218001x, R2= 72.08, P = 0.004,

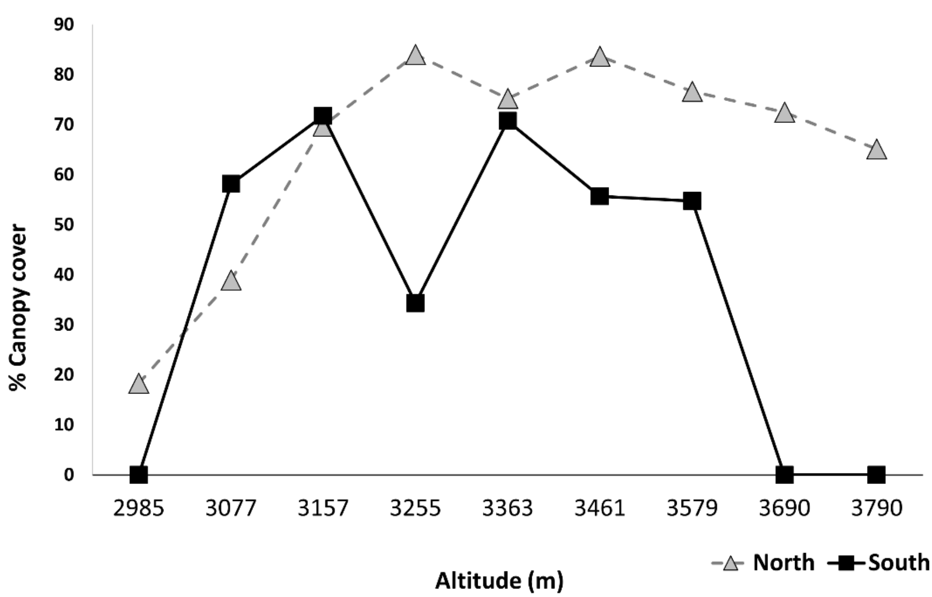

FIGURE 5 Percentage canopy cover for the north and south quadrats for nine sites, at altitudes 2985 m (site 1) to 3790 m (site 9) on the Kiki Phu, adjacent to the track to Tharpaling Monastery, west of Lamai Gompa, Bumthang, Bhutan, November 2014.

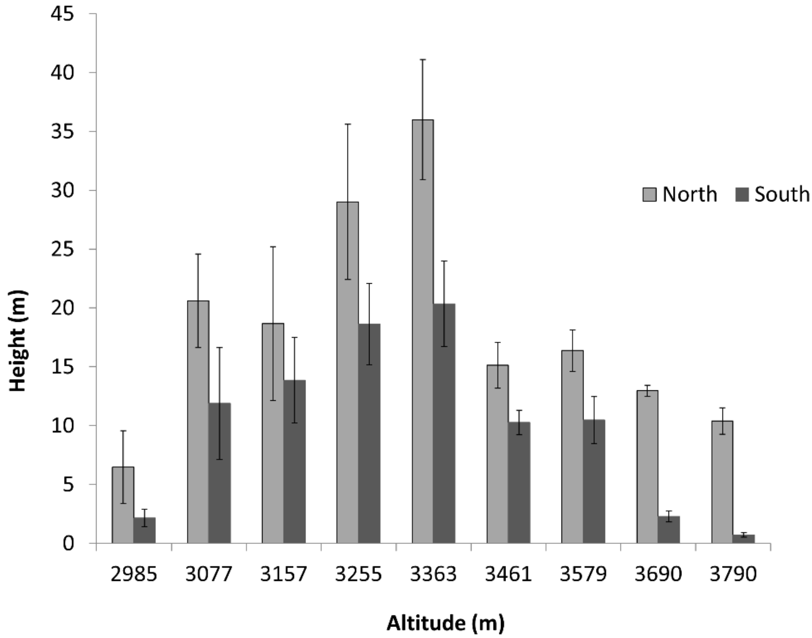

FIGURE 6 Mean tree height ± s.e. for the dominant tree species in each site for the north and south quadrats for nie sites, at altitudes from 2985 m (site 1) to 3790 m (site 9) on the Kiki Phu, adjacent to the track to Tharpaling Monastery, west of Lamai Gompa, Bumthang, Bhutan. November, 2014

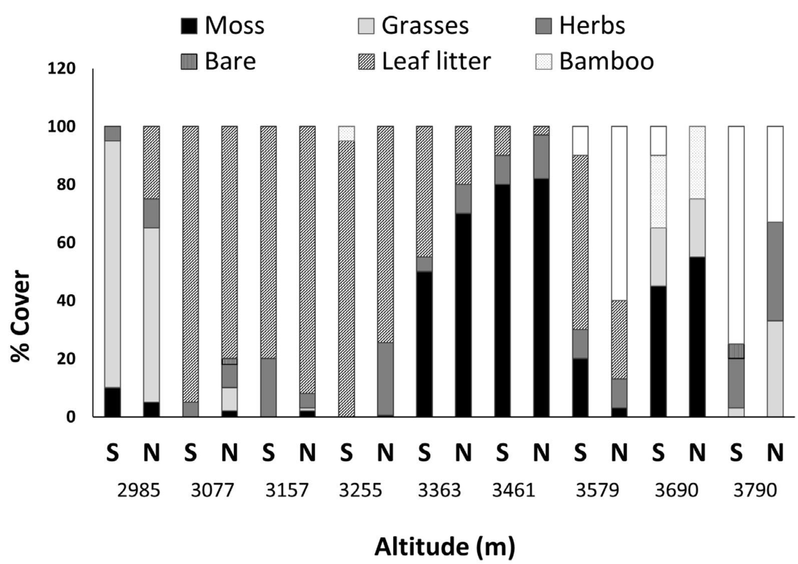

FIGURE 7 Principle Co-ordinates Analysis of the vegetation Gradient for the north (N) and south (S) quadrats for nine sites, 18 quadrats, at altitudes from 2985 m (site 1) to 3790 m (site 9) on the Kiki Phu, adjacent to the track to Tharpaling Monastery, west of Lamai Gompa, Bumthang, Bhutan. November, 2014

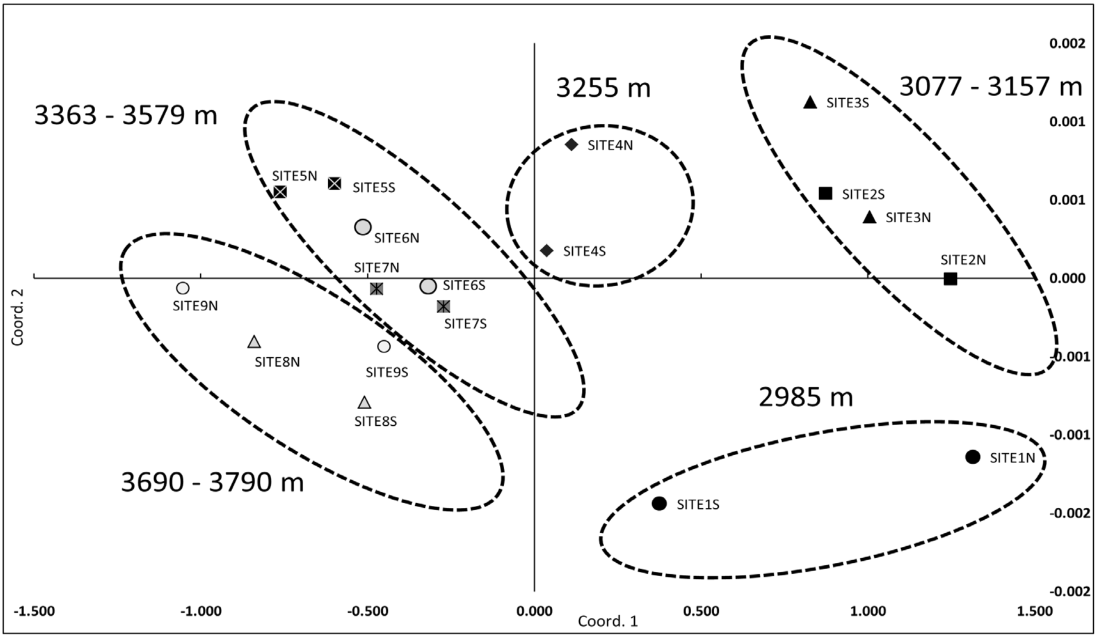

FIGURE 8 Percentage ground cover in 2 metre x 2 metre plots for the north (N) and south (S) quadrats for nine sites, at altitudes from 2985 m (site 1) to 3790 m (site 9) on the Kiki Phu, adjacent to the track to Tharpaling Monastery, west of Lamai Gompa, Bumthang Bhutan, November 2014. Cover categories are moss, grasses, herbs, bare soil, bamboo and snow

Discussions

On an eastern spur of Kiki Phu a non-linear decrease in plant species' richness was observed between the altitudes of 2985 m and 3790 m, with a total of 114 species being collected. Studies supporting unimodal distributions of species' richness have found the peaks of species' richness generally below 2500 (see above). We would need to survey beyond 2500 m altitude in the Bumthang Valley to test for this effect.

Life form changed over the transect, with forb species-richness decreasing with altitude, and grasses being present at the lower altitude (2985 m) and at the tree-line in the upper quadrats. The maximum species richness occurred at the lowest site. Graminoids and forbs were highest in species richness in the lower altitudes with a slight increase at site 9 (3790 m asl) in line with reduced canopy cover and higher light levels. Shrub species richness was relatively consistent throughout with slightly higher numbers in the lower altitudes. Tree species richness rises from lower altitude to higher altitudes corresponding with changes in the vegetation communities to "mixed conifer" (Table 1). The exception to this was at 3363 m where *Tsuga dumosa,* dominated and at 3790 m where alpine grassland was encountered on the southern quadrat site.

Throughout Bhutan, xeric upper slopes and conditions support blue pine (*Pinus wallichiana*) communities whereas hemlock (*Tsuga dumosa*) is found on slopes where high soil moisture levels are maintained through high rainfall and humidity (Wangda & Ohsawa 2006a). The increased ferns numbers and presence of moss ground cover in our 2 m x 2 m quadrats at hemlock dominated sites (3363 m) support the hypothesis that these sites have a higher moisture levels.

At the mid elevations of sites 4 and 5 (3255 m to 3363 m), height, DBH, and canopy cover were at their maximum (as well as site 6 for canopy cover). This is also reflected in the percentage ground cover, with high levels of leaf litter present in the mid altitudes, and grasses and bamboos having a stronger presence in the lower and higher sites. Hence, whilst there is decreasing species richness over the elevation, there is a peak in canopy cover, height and DBH at the mid elevations.

A similar study in Bhutan in a dry valley near Bajo, over an elevation from 1250 m to 3550 m, also measured environmental gradients at each site, and found the wettest sites were those at 3185 m in the conifer zone (Wangda & Ohsawa 2006a). It was at this zone, that the maximum heights of dominants were found (47 m), and that this correlated with soil moisture (Wangda & Ohsawa 2006a). Moreover, the maxima for DBH also correlated to some extent with soil moisture; overall the authors suggest that soil moisture is a strong determinate of forest type, followed by temperature which decreases with altitude (Wangda & Ohsawa 2006a). It is possible that these determinants are also at play on Kiki Phu, with the evidence of moisture-loving mosses at sites 5 and 6 supporting this, as well as local knowledge of the mid-altitude hemlock (*Tsuga*) forests being wetter (R. Singye, personal observation). Measuring climatic data along the ridge would be informative in testing this hypothesis.

The heights of dominant species, the canopy cover, and to some extent the DBH were greater on the north side of the ridge compared to the south side. To elucidate why this might be, further data on slope and aspect, and environmental variables such as energy input and precipitation would need to be collected.

For Kiki Phu, grazing and forest harvesting may be pressures which have impacted on the upper and lower sites in particular, as well as the southern sites in general, and investigations of resource use could be used to inform management decisions and programs to protect vegetation as well as people's livelihoods (Jamtsho & Sridith 2013; Wangda & Ohsawa 2006b).

As with many Himalayan studies, issues of topography and steepness of sites made it challenging to ensure no sampling bias. Time limitations prevented the formation of a voucher collection, and this is essential to verify species identification. The *Clerodendrum* sp. collected may be a new species; as well, some species, such as *Lyonia ovalfolia*, *Imperata clyindrica* and *Rhododendron falconeri* were found at altitudes higher than recorded in the standard "Flora" (Grierson & Long 1983 - 2001), and these are issues that have occurred in other studies (Shimono *et al*. 2010), highlighting the gaps still to be filled in knowledge of the flora of the region.

Several previous studies of altitudinal changes have used herbaria data rather than field sampling, (Acharya *et al*. 2011; Bhattarai *et al*. 2004; Vetaas & Grytnes 2002) which some suggest has led to inaccuracies due to less rigorous altitude readings, and interpolation of species presence between given altitude zones (Carpenter 2005; Lee *et al*. 2013). This study sought to address this issue by using in-field sampling for data collection. As well, to reduce spurious results due to sampling effort (Bhattarai & Vetaas 2003; Carpenter 2005; Lomolino *et al*. 2006) consideration was given to standardised sampling effort.

The PCoA suggested a graded change in species composition, and thus similarity groupings of sites over the transect, and consideration could be given to using this technique to look at similarities between sites over time, and space, to assess the impacts of anthropogenic pressure, regeneration attempts, invasive species, and in particular, climate change which is predicted to be a major impact over the next century (Salick *et al*. 2014; Shimono *et al*. 2010), making biodiversity assessment now, and in the future, an imperative.

Our ability to detect species however was limited by the season with diversity and richness likely to be higher in spring and summer (Bhattarai & Vetaas 2003). It is intended to follow up this study with a similar project in spring to determine if more species are present overall, and if seasonal factors influence species richness data.

In conclusion, this study in autumn 2014, found a non-linear decrease in species richness on the western slope of Kiki Phu from 2985 m to 3790 m, with a total of 114 species collected. The maximum heights of dominant species and maximum canopy cover occurred at a mid-altitude range of 3255 m to 3363 m, with DBH correlated with heights. It is possible that moisture regimes may be supporting this mid altitude growth. The northern and southern sides of the ridge showed differences, with the southern canopies being more open, and of lower height, and this may be related to aspect or slope, but could also be due to grazing and anthropogenic influences. Further data is needed to clarify this. Techniques used in this study are of relevance in setting baseline biodiversity records, and in monitoring seasonal influences and other differences over time and space, and in particular, the impact of global climate change.

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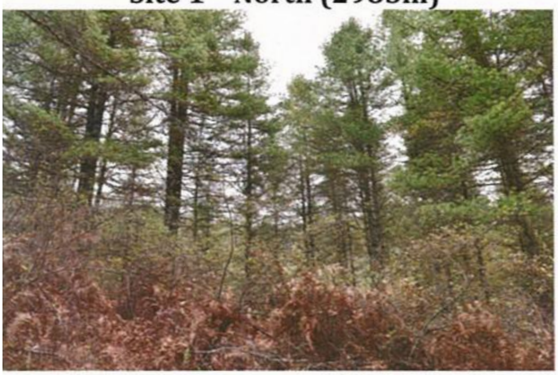
Appendix 1. The nine sampling sites, north and south quadrats, along Kiki Phu, west of Lamai Gompa on the track to Tharpaling Monastery, Bumthang, Bhutan, November 2014.

Site 1 – North (2985m)

Site 1 – South (2985m)



Site 2 – North (3077m)

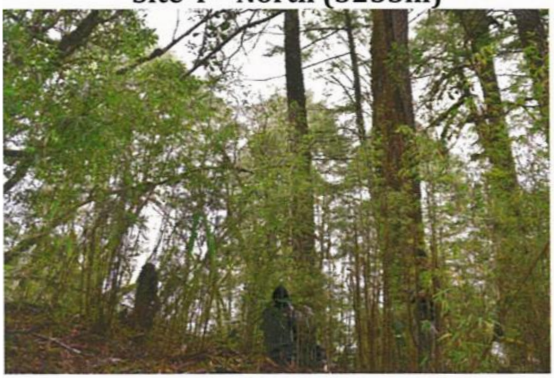
Site 2 – South (3077m)

Site 3 – North (3157m)

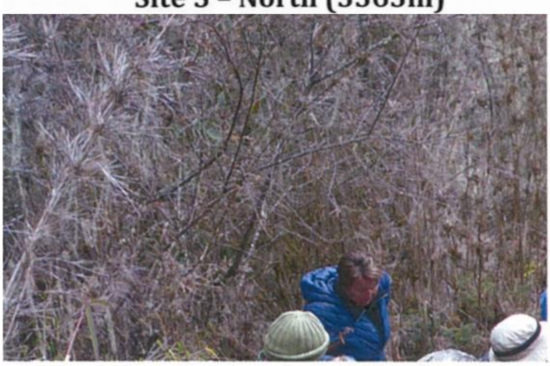
Site 3 – South (3157m)

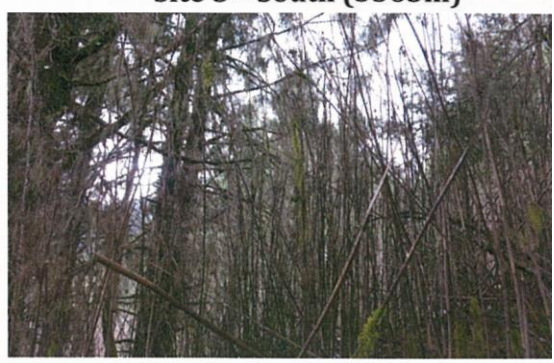

Site 4 – North (3255m)


Site 4 – South (3255m)


Site 5 – North (3363m)


Site 5 – South (3363m)


Site 6 – North (3461m)


Site 6 – South (3461m)


Site 7 – North (3579m)


Site 7 – South (3579m)


Site 8 – North (3690m)


Site 8 – South (3690m)


Site 9 – North (3790m)


Site 9 – South (3790m)

APPENDIX 2. Plant species and life forms (presence +) found at nine altitudes (2985-3790 metres) on Kiki Phu, west of Lamai Gompa on the track to Tharpaling Monastery, Bumthang, Bhutan, November 2014.

Family	**Species**	**Life form**	**2985**	**3077**	**3157**	**3255**	**3363**	**3471**	**3579**	**3690**	**3790**
Aceraceae	*Acer* sp. 1	Tree						+		+	
Apiaceae	*Apiaceae* sp. 1	Forb	+	+					+		
(Umbelliferae)	*Selinum tenuifolium*	Forb			+				+		
Asparagaceae	*Liriope* sp. 1	Forb		+							
	Ainsliaea aptera	Forb					+				
	Anaphalis nepalensis	Forb	+	+	+	+		+		+	+
	Artemisia dubia	Shrub	+	+	+						
	Aster falconii	Forb	+							+	
	Aster sp. 2	Forb	+								
	Asteraceae sp. 1	Forb				+					
	Cirsium sp. 1	Forb	+		+						
Asteraceae	*Gnaphalium affine*	Forb	+			+					
(Compositae)	*Inula grandiflora*	Forb			+						
	Ligularia fischeri	Shrubs	+								
	Pseudognaphalium hypoleucum	Forb						+	+	+	+
	Saussurea sp. 1	Forb									+
	Senecio laetus	Forb	+	+							
	Senecio scandens	Vine	+	+	+	+					
	Senecio sp. 1	Forb	+								
	Senecio sp. 2	Forb							+		

Balsaminaceae	*Impatiens bracteata*	Forb		+	+	+					
Berberidaceae	*Berberis asiatica*	Shrub	+	+	+	+		+	+	+	+
	Berberis praecipua	Shrub		+	+	+		+	+	+	+
Betulaceae	*Betula utilis*	Tree					+		+	+	+
Caprifoliaceae	*Lonicera* sp. 1	Vine		+	+	+	+	+			
Carophyllaceae	*Cerastium glomertum*	Forb			+						
	Silene sp. 1	Forbs	+								
Cupressaceae	*Juniperus recurva*	Tree								+	
	Juniperus squamata	Shrub						+		+	
Cyperaceae	Cyperaceae sp. 1	Graminoid	+								
	Cyperaceae sp. 2	Graminoid	+								
Dennstaedtiaceae	*Pteridium* sp. 1	Fern	+	+	+	+			+		
Elaeagnaceae	*Elaeagnus parvifolia*	Shrub	+	+	+						
Ericaceae	*Gaultheria nummularioides*	Shrub	+	+	+			+	+		
	Lyonia ovalifolia	Tree							+	+	+
	Rhododendron anthopogon	Shrub									+
	Rhododendron arboreum	Tree		+	+	+		+			
	Rhododendron barbatum	Shrub					+				
	Rhododendron camelliiflorum	Shrub					+				
	Rhododendron campanulatum	Shrub							+		
	Rhododendron campylocarpum	Shrub									+
	Rhododendron falconeri	Shrub					+			+	+
	Rhododendron grande	Tree					+				

	Rhododendron lepidotum	Shrub						+	+	+	+
	Rhododendron thompsonii	Shrub						+	+	+	
	Rhododendron triflorum	Shrub			+	+					
	Rhododendron sp. 1	Shrub				+	+				+
	Rhododendron wightii	Shrub									+
	Vaccinium nummularia	Shrub			+	+	+				
Euphorbiaceae	*Euphorbia griffithii*	Forb		+							
Fabaceae	*Piptanthus nepalensis*	Shrub		+		+					
	Trifolium repens	Forb	+								
Fagaceae	*Quercus* sp. 1	Tree							+		
Fern	*Angiopteris* sp. 1	Fern			+	+	+	+			+
Fern	Pteridophyta sp.3	Fern					+				
Fern	Pteridophyta sp.4 ('pinnate')	Fern								+	
Fern	Pteridophyta sp.2 ('lacey')	Fern					+			+	
Fern	Pteridophyta sp.1	Fern					+				
Gentianaceae	*Swertia* sp. 1	Forb	+								
	Swertia bimaculata	Forb	+	+							
Geraniaceae	*Geranium* sp. 1	Forb		+	+						
Hypericaceae	*Hypericum hookerianum*	Shrub				+					
Lamiaceae (Labiatae)	*Clerodendrum* sp. 1	Shrub			+		+				
	Clinopodium umbrosum	Forb		+							
	Elsholtzia strobilifera	Forb	+								
	Origanum sp. 1	Forb		+							
	Prunella vulgaris	Forb	+								

Lycopodiaceae	*Lycopodium* sp. 1	Forb			+			+			
Orchidaceae	Orchid spp.	Forb	+	+	+	+			+		
Philadelphaceae	*Philadelphus* sp. 1	Shrub		+							
Pinaceae	*Abies densa*	Tree						+	+	+	+
	Picea spinulosa	Tree				+		+	+	+	+
	Pinus wallichiana	Tree	+	+	+	+		+	+	+	
	Tsuga dumosa	Tree		+	+	+	+	+	+	+	
Poaceae (Bambusa)	*Arundinaria racemosa*	Shrub	+	+	+	+	+	+	+	+	+
Poaceae	*Festuca* sp. 1	Graminoid								+	
	Echinochloa sp. 1?	Graminoid	+		+						
	Poaceae sp. 2	Graminoid	+								
	Poaceae sp. 2b	Graminoid	+								
	Poaceae sp. 3	Graminoid	+								
	Poaceae sp. 3b	Graminoid	+								
	Poaceae sp. 4	Graminoid	+	+	+				+		
	Poaceae sp. 5	Graminoid	+								
	Poaceae sp. 6	Graminoid			+						
	Poaceae sp. 7	Graminoid									+
	Imperata cylindrica	Graminoid						+		+	
	Panicum sp. 1	Graminoid									+
	Poa sp. 1	Graminoid								+	+
Primulaceae	*Primula glomerata*	Forb				+			+		
	Primula sp. 1	Forb	+	+							

Ranunculaceae	*Clematis* sp. 1	Vine		+	+	+	+	+			
	Ranunculaceae sp. 1	Forb						+			
Rosaceae	*Cotoneaster microphyllus*	Shrub	+	+	+				+	+	
	Cotoneaster sherriffii	Shrub		+	+						
	Duchesnea indica	Forb		+	+	+	+	+	+		+
	Potentilla astrosanguinea	Forb	+							+	+
	Potentilla sp. 1	Forb									+
	Rosa sericea	Shrub	+	+	+	+	+	+	+		+
	Rubus hypargyrus	Shrub	+								
	Spiraea bella	Shrub		+	+	+		+	+	+	
Rubiaceae	*Galium aparine*	Forb						+			
	Rubia hispidicaulis	Vine			+	+		+			
Rutaceae	*Skimmia laureola*	Shrub	+			+	+				
Saxifragaceae	*Astilibe rivularis*	Forb				+					
	Bergenia sp. 1	Forb		+							
Scrophulariaceae	*Hemiphragma heterophyllum*	Forb	+		+						
Smilacaceae	*Smilax ferox*	Vine				+					
Thymelaeaceae	*Daphne bholua*	Shrub		+	+	+	+				
Violaceae	*Viola biflora*	Forb					+				
Unknown	Unknown sp. 3	Forb	+								
	Unknown sp. 4	Forb		+	+		+				
	Unknown sp. 5	Forb			+						
	Unknown sp. 6	Forb			+						
	Unknown sp. 1	Forb	+								

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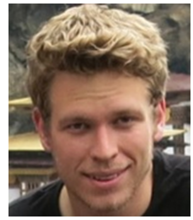

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