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Regeneration of *Pinus wallichiana* AB Jackson in a trans-Himalayan dry valley of north-central Nepal

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We studied the elevational pattern of forest composition and regeneration of the subalpine conifer tree species *Pinus wallichiana* in Manang, a trans-Himalayan dry valley in north-central Nepal. Thirty-five quadrats (10 m × 10 m) were laid between 3300 and 4000 masl on both north- and south-facing slopes. We measured diameter at breast height (DBH) of each mature individual of all tree species (DBH ≥10 cm), and recorded the number of seedlings (DBH <10 cm, height <30 cm) and saplings (DBH <10 cm, height >30 cm). We also measured soil moisture and soil pH, estimated canopy cover, and recorded slope and altitude in each quadrat. For all species together and for several species individually, tree density, seedling density, sapling density and tree basal area were found to decrease with elevation on both north and south aspects. This trend is largely explained by the progressively harsher environment at higher elevations. The north-facing slopes in our study area have denser forests than the south-facing slopes, the density of all size classes (seedling, sapling and mature plants) and basal area being greater on the northern aspects. These aspect-wide differences are attributable to the stark difference in soil moisture between northern and southern aspects, which is in turn due to the difference in insolation. Irrespective of elevation and aspect, all the forests studied are regenerating, as indicated by inverse J-shaped density-diameter curves. The elevational pattern of seedling and sapling abundance is explained only by elevation. Whereas other variables (*e.g.*, canopy) are considered to have an important influence on seed germination and seedling establishment, they turn out not to be significant predictors of density of seedlings and saplings. This failure to identify a relationship is probably due to our use of non-parametric test (tree regression analysis) that we used to establish the relationship between density and its potential explanatory variables or due to our selection of 1 standard error rule yielding sub-optimal models for regression trees.

Key words: density-diameter curve, regeneration, seedling, sapling, altitude, canopy, Manang Valley

Pinus wallichiana AB Jackson (Himalayan Blue Pine) is native to the Himalaya, Karakoram, and Hindu Kush mountains. It has an extensive distribution and grows all along the Himalayas in an almost continuous range from eastern Afghanistan and Pakistan through India, Nepal, Bhutan, Myanmar, and China at elevations between 1800 and 4300 masl (Critchfield and Little 1966, Polunin and Stainton 1997). The plant is generally found in valleys and foothills, sometimes in pure stands but often in association with other conifers including *Cedrus deodara*, *Abies pindrow*, *Picea smithiana* and *Juniperus indica* [*Juniperus excelsa* subsp. *polycarpos*], and with broadleaved species including *Quercus semecarpifolia*, *Betula utilis*, *Acer* and *Ilex* species (Earle 2009). *Pinus wallichiana* forests grow in drier areas susceptible to fire where the plant grows as an early successional species (Numata 1966, Stainton 1972, Ohsawa et al. 1986). In wet areas, the plant grows in secondary forests (Polunin and Stainton 1997). *P. wallichiana* is an important source of timber and fuel for villagers in mountain valleys and is also important in protecting the upper parts of mountain watersheds (Ives and Messerli 1989, Stainton 1972).

Regeneration of tree stands depends on a combination of factors controlling seed availability, germination, seedling growth and establishment (Greene et al. 1999, Dovciak et al. 2003). Whereas environmental conditions play an important role in establishment and distribution of seedlings (Bonnet et al. 2005), regeneration of dominant trees in dry valleys is influenced even by small-scale human impacts. Under such impacts, the typical inverse J-shaped DBH (diameter at breast height) class distribution observed among forest species, where frequency of individuals in larger size classes falls systematically and progressively, resulting in a non-linear relationship between frequency and size class, generally gives

Table 1. Description of study area

Aspect	Transect	Locality	Elevation (masl)	Dominant tree species	Number of quadrats sampled
North	1	Above Humde	3300–3500	*Pinus wallichiana* *Juniperus indica*	10
		Above Humde	3500–3800	*Pinus wallichiana* *Abies spectabilis*	15
	2	Above Gangapurna lake	3800–4000	*Betula utilis* *Pinus wallichiana*	10
South	3	Ngawal side	3300–3500	*Pinus wallichiana* *Juniperus indica*	10
		Ngawal side	3500–3800	*Juniperus indica*	15
	4	Above Braga	3800–4000	*Juniperus indica*	10

way to a sporadic and/or unimodal distribution (Wangda and Ohsawa 2006a). Inverse J-shaped distribution is indicative of a forest in a state of regeneration (Kimmins 1987). A shift from inverse J-shape to unimodal or multiple-peaked distribution is the result of substantial changes in the state and pattern of forest regeneration, suggesting that the forest is in trouble. Such a shift is generally caused by both anthropogenic and natural causes (climatic or biotic), as reported for *P. roxberghii* forest on dry valley slopes along the Punatsangchu river in west-central Bhutan (Wangda and Ohsawa 2006a). Several studies have focused on the altitudinal and latitudinal forest zones of the Himalayas in relation to climate (Saxena and Singh 1984, Ohsawa 1987, Tang and Ohsawa 1997, Wangda and Ohsawa 2006a). However, little is known about the comparative ecology and regeneration dynamics of the forest along the typical dry valley slopes, which are particularly important because the steep environmental gradients are associated with rapid change in forest types (Wangda and Ohsawa 2006b).

Regeneration and seedling distribution in conifer forests have been shown to be influenced by both large-scale disturbances such as wildfire and forest clearing (Turner et al. 1998, Bonnet et al. 2005) and also small-scale disturbances such as animal grazing (wild and domestic), lightening and disease (Bonnet et al. 2005). Most studies on regeneration of *Pinus* species have focused on such disturbances (e.g., Spanos 1994, Spanos and Spanos 1996, Spanos et al. 2000, Scott et al. 2000, Spanos et al. 2001, Bonnet et al. 2005, Darabant et al. 2007). A few studies investigated the effect of physical factors, such as aspect and elevation (Wangda and Ohsawa 2006a, Mong and Vetaas 2006), on forest regeneration. In this study, we examine forest composition and regeneration patterns of *P. wallichiana* along an altitudinal gradient on slopes of the dry Manang Valley in north-central Nepal, with particular attention to the differentiation of slopes with northerly and southerly exposure. Principle objectives of this research were (a) to determine the community structure of *P. wallichiana* forest, (b) to understand the regeneration pattern of *P. wallichiana*, and (c) to identify environmental factors that affect regeneration of *P. wallichiana*.

Materials and methods

Study area The study area lies in Manang Valley (also called Nyeshang) of Manang District in north-central Nepal. This trans-Himalayan Valley (28° 37' – 28° 39' N, 83° 59' – 84° 08' E) is located in the northern part of the Annapurna Conservation Area. The valley is traversed by the Marsyangdi River and is surrounded by the high peaks (>6000 masl) of the Annapurna range to the south, Manasalu to the east, Peri, Himlung and Choya to the north, and Damodar and Mukti-nath to the west. The climate of the valley is dry, characteristic of the trans-Himalayan region. It is a rain shadow area with a mean annual precipitation of 444 mm (at 3420 masl) and mean annual temperature of 6.2°C (Miehe et al. 2001). The area is covered by snow from November to March. Snow melt water is the main source of soil moisture in forested areas (Shrestha et al. 2007). The south-facing slope (south aspect) is substantially drier than the north-facing slope (north aspect) (Bhattarai et al. 2004). Vegetation composition on the north-erly and southerly aspects is quite distinct. *P. wallichiana, Abies spectabilis, Betula utilis, Juniperus indica* and *Salix* species are the common tree species on north facing slopes, while on the southern slope *B. utilis* and *A. spectabilis* are found only in moist gullies. The natural forest in the valley extends from 3000 to 4200 masl on the north aspect, while its upper limit is below 4000 masl on the south aspect (Panthi et al. 2007).

Our sampling was carried out along four vertical transects, two in each aspect. Because a single transect never spanned the elevational limits of our study area (3300–4000 masl), the entire range was represented by transects at two sites with similar physical environment and biotic composition. On the northern aspect, the two transects were laid at Humde village (3300–3800 masl, 28° 37' 39" N, 84° 05' 30" E) and near Gangapurna Glacier Lake (3800–4000 masl, 28° 39' 42" N, 83° 59' 44" E) (**Table 1**). South-aspect transects were laid near Ngawal village (3300–3800 masl, 28° 38' 23" N, 84° 05' 22" E) and above Braga village (3800–4000 masl, 28° 39' 54" N, 84° 03' 24" E – on the way to Ice Lake). The average slopes of the sampling sites on the northern and southern aspects were 22° and 17°, respectively. The forest on the northern aspect of the valley had been damaged severely by forest fire about 35 years prior to our study (Shrestha et

Table 2. Density (individuals/ha), basal area (BA, m²/ha) and Importance Value Index (IVI, %) of tree species (excluding seedling and sapling) at various elevation bands

Elevation (masl)	Northern Aspect				Southern Aspect			
	Tree species*	Density (per ha)	BA (m²/ha)	IVI (%)	Tree species*	Density (per ha)	BA (m²/ha)	IVI (%)
3300–3500	PW	1028	18.6	75.63	PW	570	11.8	61.64
	JI	200	0.15	11.86	JI	320	5.015	38.35
	BU	50	0.06	3	BU	0	NA	NA
	S	50	0.02	4.27	S	0	NA	NA
	AS	100	0.21	5.33	AS	0	NA	NA
	Total	**1428**	**19.04**	**100.09**	**Total**	**890**	**16.815**	**99.99**
3500–3800	PW	190	2.76	28.09	PW	59	0.42	34.01
	JI	100	0.037	19.44	JI	517	0.22	60.95
	BU	267	3.44	9.64	BU	0	NA	NA
	S	33	0.02	3.98	S	17	0.017	5.08
	AS	137	1.02	38.9	AS	0	NA	NA
	Total	**727**	**7.277**	**100.05**	**Total**	**593**	**0.674**	**100.04**
3800–4000	PW	240	2.26	39.16	PW	0	NA	NA
	JI	0	NA	NA	JI	375	NA	NA
	BU	225	1.46	41.82	BU	0	NA	NA
	S	0	NA	NA	S	0	NA	NA
	AS	150	0.36	19	AS	0	NA	NA
	Total	**615**	**4.08**	**100.08**	**Total**	**375**	**-**	**-**

* PW = *Pinus wallichiana*; JI = *Juniperus indica*; AS = *Abies spectabilis*; BU = *Betula utilis*; S = *Salix* species

al. 2007); we observed a large number of burnt logs. Because *P. wallichinana* is used as a construction material, there were many stumps, left behind after logs had been extracted.

Field sampling For the sake of analysis, we divided the elevational range into three bands: 3300–3500, 3500–3800 and 3800–4000 masl. These elevation bands are different in physical environment (both temperature and moisture) as well as in biotic composition (Mittermeier et al. 2004, Chaudhary 1998). For sampling, 35 square quadrats (10 m × 10 m) were randomly placed on each aspect such that five of them always occupied a 100 m elevation band. In each quadrat, we recorded the number and diameter at breast height (measured at 1.37 m above the ground surface) of individual trees (DBH ≥ 10 cm) of each species. Canopy cover was estimated visually. We divided each quadrat into four sub-quadrats (5 m × 5 m), and recorded the number of saplings (DBH <10 cm, height >30 cm) and seedlings (DBH <10 cm, height <30 cm, Rao et al. 1990) of each tree species in two diagonally located sub-quadrats, selected at random. Moisture and pH of soil were measured at the four corners and center of each quadrat with the use of a soil pH and moisture tester (Model DM 15, Takemura Electric Works Ltd., Japan); the values were averaged for data analysis.

Numerical methods and statistical analysis Density (individuals/ha), frequency (%), basal area (m²/ha), and the importance value index (IVI) (Holdridge et al. 1971) of trees were calculated from field data. We also determined the density of seedlings and saplings of tree species. To understand the regeneration status of *P. wallichiana,* trees in the three elevation bands were divided into 10 cm interval size classes based on DBH.

How do the physical and biotic differences between the north and south aspects affect seedling and sapling abundance? We compared the densities between the two aspects. Because the assumptions of parametric statistical tests (in this case, independent sample t-test) were violated (by non-normality in distribution of cases and heterogeneity of variances of groups being compared), we performed the non-parametric Wilcoxon–Mann–Whitney two-sample rank-sum test in SPSS 16.0 (SPSS Inc.).

Finally, we attempted to establish what determines density of seedling and sapling. We measured a range of environmental variables on the studied plots: soil pH, soil moisture, canopy cover, slope and altitude. Because these explanatory variables can correlate among themselves – which is always the case in field-based ecological studies – we tried to determine the significant predictors of density using multiple regression. However, the residuals in regression analysis were severely non-normal, and we observed both linear and non-linear relationships between explanatory and dependent variables. This indicated the violation of one of the assumptions of regression analysis and the likelihood

that power transformation would not be able to fix normality. Because it entails fewer assumptions, tree regression is a useful alternative to multiple regression analysis. We therefore performed tree regression analysis of our data in order to relate explanatory variables to the dependent variables in R 2.9.1 (The R Foundation for Scientific Computation).

One approach to building the best model (simplest model that best fits the data) is to begin with the simplest model and make it increasingly complex until the model keeps on improving in its fit to the data. This would entail growing the tree with more and more splits. As Breiman and colleagues have pointed out problems in this approach in their seminal work (Breiman et al. 1984), we first allowed the tree to grow to the maximum (full) size and then pruned it so that only important predictors remain in the simplified model (Rejwan 1999, De'ath 2000). The minimum cross-validated error rate was determined for tree models of various sizes. The smallest tree with an error rate within 1 SE of the minimum error rate was chosen as the best model (Breiman et al. 1984) after 1000 simulations.

All environmental variables (soil pH, soil moisture, canopy cover, slope and altitude) were accommodated in the model although cross-validation would eventually select only the important ones. On top of these, we used density of sapling as a potentially explanatory variable for explaining seedling abundance because the germination of seed and their growth to seedling is likely to be affected by nearby saplings which are bigger in size and therefore more effective competitors. For the same reason, we did not use seedling density as a potentially explanatory variable for explaining sapling abundance.

Results

Forest structure Total tree density and basal area decreased from low to high elevation on both aspects (**Table 2**). This pattern was generally followed by *P. wallichiana*. However, some species, e.g., *B. utilis* and *A. spectabilis*, had their lowest tree density and basal area values in the lowest elevation band. Because *P. wallichiana* is the most dominant species in these forests, its elevational pattern of density and basal area determined that of the forest as a whole. Forest density was greater on the northern aspect than on the southern. Above 3700 masl on the southern aspect no tree individuals of *P. wallichiana* were observed, and *J. indica* was represented only by bushes or scrub with minimal effect on basal area. The treeline occurred at a lower elevation on the southern aspect (3800 masl) than on the northern aspect (4100 masl).

Northern aspects were occupied by seedlings and saplings much more densely than southern aspects (**Figure 1**). Using the non-parametric Wilcoxon–Mann–Whitney two-sample rank-sum test, we confirmed that the north-south differences are highly significant.

Regeneration of Pinus wallichiana The density-diameter curve of tree populations of *P. wallichiana* in all elevation bands resembled an inverse J-shape on both aspects (**Figure 2**). We observed larger trees on north
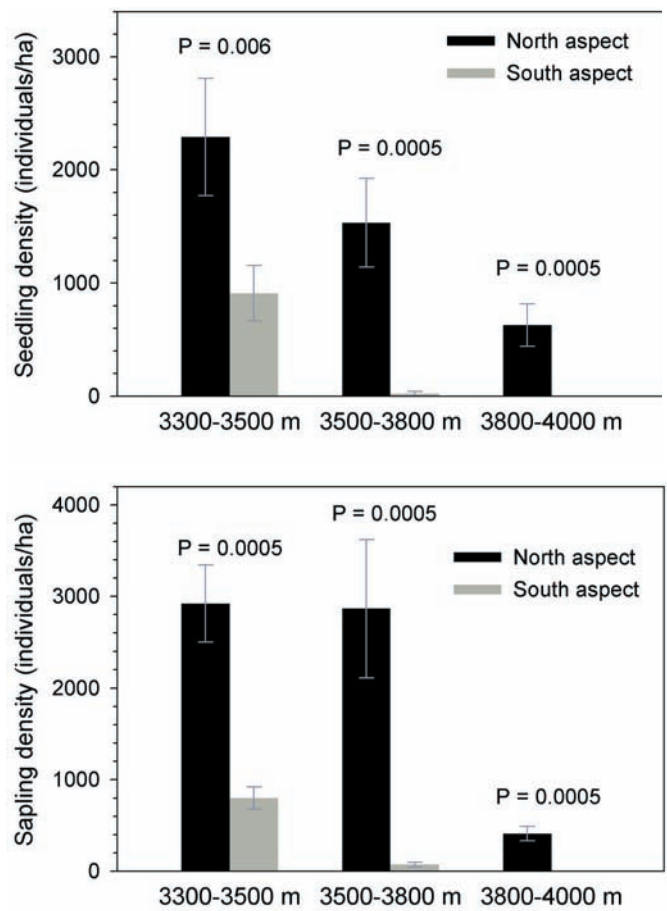

Figure 1. Seedling (top) and sapling (bottom) densities (mean ± 1 standard error). All the aspect-wise differences in the densities as determined by Wilcoxon–Mann–Whitney two-sample rank-sum test (two-tailed) are statistically highly significant.
Seedling density: Mann–Whitney $U = 14$, $n_1 = n_2 = 10$, $P = 0.006$ for 3300–3500m, Mann–Whitney $U = 15$, $n_1 = 15$, $n_2 = 14$, $P = 0.0005$ for 3500–3800 m, Mann–Whitney $U = 11$, $n_1 = 10$, $n_2 = 11$, $P = 0.0005$ for 3800–4000 m.
Sapling density: Mann–Whitney $U = 1.5$, $n_1 = n_2 = 10$, $P = 0.0005$ for 3300–3500 m, Mann–Whitney $U = 16.5$, $n_1 = 15$, $n_2 = 14$, $P = 0.0005$ for 3500–3800 m, Mann–Whitney $U = 0$, $n_1 = 10$, $n_2 = 11$, $P = 0.0005$ for 3800–4000 m.

aspect than on the south aspect (not shown in figure), and on valley floor and lower elevations than at higher elevations of both aspects (observation, **Figure 1**). Altogether 18% of plots on north and 40% of plots on south were devoid of mature trees.

Among the six variables, five environmental (soil pH, soil moisture, canopy cover, slope and altitude) and one biotic (density of saplings as a potential predictor for seedling density), the only significant predictor of both seedling and sapling abundance was altitude (**Figure 3**). On both aspects, seedling and sapling density decreased with altitude. Altitude explained approximately half of the total variance
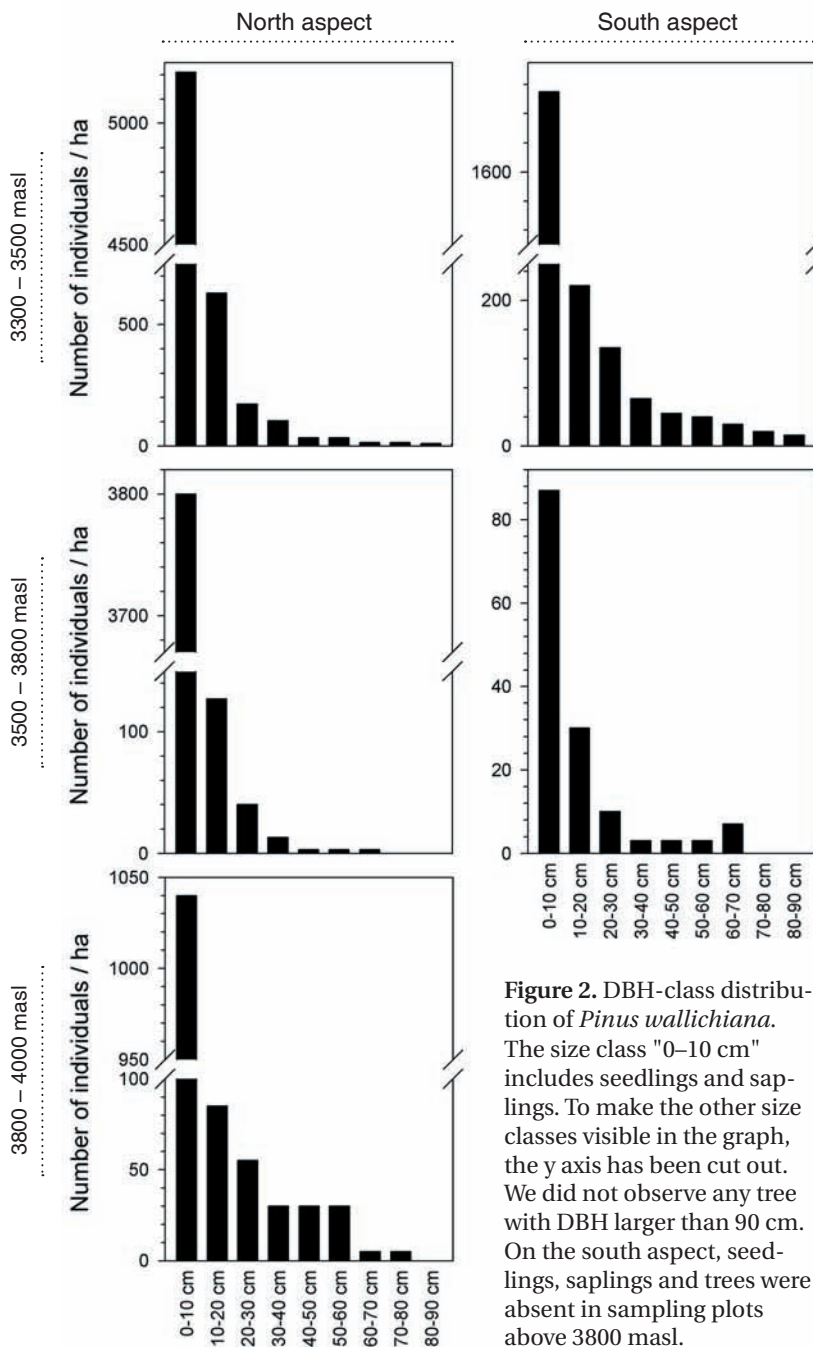
Figure 2. DBH-class distribution of *Pinus wallichiana.* The size class "0–10 cm" includes seedlings and saplings. To make the other size classes visible in the graph, the y axis has been cut out. We did not observe any tree with DBH larger than 90 cm. On the south aspect, seedlings, saplings and trees were absent in sampling plots above 3800 masl.

boundaries; for example, ring width of *Picea abies* and *P. cembra* growing near the alpine timberline in Switzerland positively correlates with summer temperature (Meyer 2000). Given the sharp decline in temperature with elevation – temperature lapse rate for the western Himalayas being 0.6–0.74°C/100 m for various months of the year (Jain et al. 2008) – and high sensitivity of physiological processes to temperature, it is not surprising that tree density, seedling density, sapling density and tree basal area decreased with elevation on both north and south aspects. High elevations are also characterized by shorter growing season, resulting in reduced annual growth (Tranquillini 1979, Vetaas 2000). This pattern of decrease in density and basal area with elevation, however, can vary with species because biotic interactions, importantly competition, also play a role in growth rate.

Northern aspects have denser forest than the southern aspects, with greater basal area and higher density of all size classes (seedling, sapling and mature plants) (**Figure 1, Table 2**). Five species of trees were found on northern aspects, but only three species on southern (**Table 2**). Higher species richness on northern aspects has been reported by previous studies (e.g., Panthi et al. 2007). The ecological significance of aspect is important because it influences diameter growth of tree, forest productivity, and species distribution (Hutchins et al. 1976, Verbyla and Fisher 1989). *B. utilis* and *A. spectabilis*, which are absent on the southern aspects, contributed substantially to the higher total tree density and, especially, total basal area found on the northern aspect.

The density-diameter curve of *P. wallichiana* populations resembled an inverse J-shape (**Figure 2**), indicating sustainable regeneration (Kimmins 1987, Shimano 2000). Shrestha et al. (2007) and Ghimire and Lekhak (2007) found inverse J-shape size-class distribution for *B. utilis* and *A. spectabilis,* respectively, in nearby forests on the northern aspect. Tree regression analyses show that altitude is the only significant predictor of seedling and sapling density on both aspects. Altitude explained half to three-quarters of the total variance in seedling and sapling density. Whereas we should not be surprised that altitude is the most important predictor of the seedling and sapling abundance, other variables (including canopy and aspect) that have been considered important

in abundance of seedlings and saplings in all cases except that it explained three-quarters of the total variance in sapling abundance on the southern aspect.

Discussion

Our transects were laid in the upper distributional ranges of the tree species. Towards the upper limit, environmental conditions become increasingly severe, resulting in the formation of a boundary of tree species called treeline. Various studies have repeatedly shown that some aspect of temperature – means or extremes – determine the position of treeline (Körner 1998, Jobbagy and Jackson 2000, Körner and Paulsen 2004). Studies have shown that annual increments in tree ring correlate with temperature at high elevation
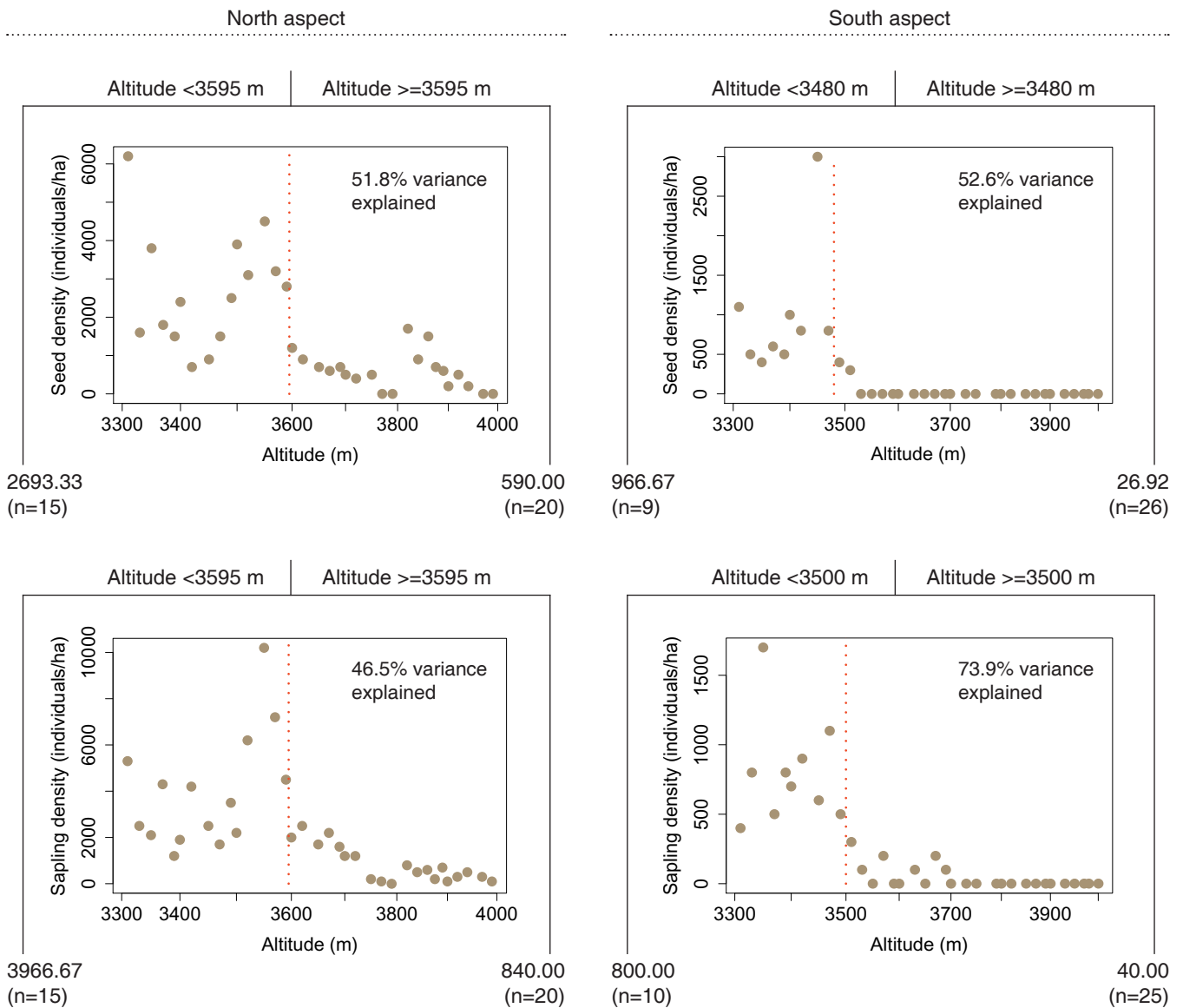
Figure 3. Regression trees relating abundance of seedlings and saplings to six potentially explanatory variables (soil pH, soil moisture, canopy cover, slope, altitude, and sapling density). In all cases the only significant explanatory variable was altitude, explaining approximately half of the total sum of squares except in one instance in which it explained 74% of the variance in sapling density. The tip of the tree branches list the mean of the response variable (density) assigned to the tree-branch and the sample size in parentheses.

in determining environmental conditions suitable for germination and growth of young plants do not turn out to be significant predictors.

Seedlings are generally light-demanding; they often require direct solar radiation (Tilman 1985). High canopy cover by large trees reduces the amount of direct sunlight that reaches floor; high canopy cover may also cause litter accumulation, which is not a favorable condition for seed germination and seedling establishment for *Pinus* (Neyisci 1993, Spanos et al. 2001, Bonnet et al. 2005). However, we failed to establish a relationship between canopy and density of seedlings and saplings. This finding may possibly reflect the actual absence of such a relationship in our study systems; we

might have observed a correlation between density and canopy (r = 0.44 for both seedling and sapling, Pearson Correlation, P = 0.0005) because both canopy and density covary with altitude, a more important predictor variable (Pearson Correlation *r* = –0.41 between altitude and canopy cover, –0.5 between altitude and seedling density, –0.43 between altitude and sapling density; P <0.0005 for all). In such a situation, variance explained by a less important variable in bivariate regression is captured by a more important variable when the variance in the dependent variable is explained simultaneously by multiple explanatory variables.

However, given the established relationship between canopy cover and density of seedlings and saplings (Neyisci

1993, Spanos et al. 2001, Bonnet et al. 2005) and our observation in the field that seedlings and saplings grew more densely in canopy gaps, the more likely explanations are the following. The non-parametric test (tree regression analysis) that we used may not have been powerful enough to detect the relationship between canopy cover and density of young plants. Another possibility is that the rule we adopted to determine the best model (tree) was very conservative yielding a sub-optimal tree that could not capture all the important predictor variables. Whereas Breiman et al. (1984) proposed both a minimum cross-validation rule and a 1 standard error (SE) rule, there is a lack of clear reason on the choice of the rule for determining best model. The minimum cross-validation rule assumes that the optimal tree is the one with the smallest cross-validation error; the 1-SE rule states that the optimal tree is the smallest tree within 1 standard error of the minimum cross-validation error. The negative relationship between cross-validation error and tree complexity (cross-validation error declines as tree grows until a minimum error is reached) makes it likely that 1 SE rule can yield a simpler model than minimum rule does, especially when multiple predictor variables exist. In such a situation, the 1 SE rule could yield sub-optimal trees. However, to avoid any potential false positives and to remain consistent with many studies in the practice of model selection, we applied the more conservative 1-SE rule, which might have been unable to capture some of the important predictor variables, such as canopy and moisture.

The quantity and duration of soil moisture depend on aspect because of the stark difference in the amount of sunlight received by north and south aspects (Parker 1991). As southern aspects receive more intense insolation than northern aspects, the northern aspects retain more soil moisture, which provides more suitable conditions for seed germination, seedling establishment and regeneration of *P. wallichiana*. The northern slopes in our study sites are moister – and have more canopy cover – than the southern slope; these two factors both have a positive influence on seedling germination. Some plots in moist gullies on the southern aspect also had higher seedling abundance than other dryer plots on the same aspect which were relatively dry. In contrast to the density pattern that we observed, Schickhoff (1996) found that *P. wallichiana* is more abundant on the relatively dry south-facing slopes than on the north-facing slopes in the Kaghan Valley of northern Pakistan.

Fire damage to forests has been reported to accelerate regeneration of *Pinus* species and increase seedling density; this is the case, for instance, with *P. brutia* on the Thasos Island of north Greece (Spanos et al. 2001) and *P. ponderosa* in the Black Hills of South Dakota, USA (Bonnet et al. 2005). Schweinfurth (1957) and Stainton (1972) have argued for the significant role of fire in the maintenance of *Pinus* forests in the Himalaya. Fire damage that occurred about 35 years ago on the north-facing slopes of our study site probably changed the course of forest regeneration. However, without detailed historical data, we are unable to relate that event to today's state of forest regeneration.

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