Article

Changes in Soil Chemistry and Foliar Metabolism of Himalayan Cedar (*Cedrus deodara*) and Himalayan Spruce (*Picea smithiana*) along an Elevational Gradient at Kufri, HP, India: The Potential Roles of Regional Pollution and Localized Grazing

Rakesh Minocha [1,*], Alexandra R. Contosta [2], Gregory B. Lawrence [3], Ravinder K. Kohli [4,†], Subhash C. Minocha [5] and Stephanie Long [1]

[1] USDA Forest Service, Northern Research Station, NE-4505, Durham, NH 03824, USA; stephanie.long2@usda.gov
[2] Earth Systems Research Center, University of New Hampshire, Durham, NH 03824, USA; Alix.Contosta@unh.edu
[3] U.S. Geological Survey, New York Water Science Center, Troy, NY 12180, USA; glawrenc@usgs.gov
[4] Botany Department, Panjab University, Chandigarh 160011, India; rkkohli45@yahoo.com
[5] Biological Sciences, University of New Hampshire, Durham, NH 03824, USA; Subhash.Minocha@unh.edu
* Correspondence: rakesh.minocha@usda.gov; Tel.: +1-603-868-7622
† Present address: Vice-Chancellor, Amity University, Mohali 140306, Punjab, India.

Abstract: We investigated changes in soil chemistry and foliar metabolism of Himalayan cedar [*Cedrus deodara* (Roxb. Ex Lamb.) G.Don] and Himalayan spruce [*Picea smithiana* (Wall.) Boiss] trees along a steep elevational gradient in the lower Himalayan Mountains at Kufri, Himachal Pradesh (HP), India. The foliar and soil samples were collected from four locations along a 300 m elevational gradient at ridge, high-, mid-, and low-elevation sites within the forested Shimla Water Catchment Wildlife Sanctuary that provides water for the city of Shimla, HP,. Observations at the time of sampling revealed that the high-elevation site was being heavily grazed. Soils collected at the four sites showed differences in soil chemistry along the gradient. Surface soils (top 10 cm) at the high-elevation site had the highest concentrations of carbon, nitrogen, calcium, magnesium, phosphorus, organic matter, and effective cation exchange capacity, possibly caused by grazing. Mineral soils were slightly acidic at all sites except the mid-elevation site, which was extremely acidic in the upper mineral soil. Similar to surface soil chemistry, foliar metabolism was also comparatively unique for high elevation. In Himalayan cedar foliage, higher concentrations of soluble proteins, polyamines, amino acids, and potassium were observed at the high-elevation site as compared to the ridge, mid and low elevations. No major differences were observed in the metabolic profiles of cedar between the ridge and low elevation ranges. Spruce foliage was sampled only from the ridge and low elevations and its metabolic profiles suggested healthier conditions at the low elevation. The results of the study demonstrate the impact of the interplay between local and regional drivers of forest health on cedar and spruce trees in a forested catchment that acts as a water source for downstream communities.

Keywords: amino acids; elevation; fertilizer; grazing; mountains; nitrogen; pollution; polyamines; stress

1. Introduction

Mountain systems cover approximately one-fifth of the Earth's continental areas. While most mountain systems feature human habitation, the Himalayas, Rockies, Andes, and the Alps all exhibit high biodiversity because of their huge altitudinal range. In these mountain ranges, climatic regimes change within short horizontal distances along an elevational gradient, thus creating diverse life-sustaining conditions with varying topography [1,2]. Mountains are also an important part of regional watersheds and the

hydrological cycle, and thus increases in environmental pollution related to urbanization, tourism, and industry will not only affect the montane environment but also downstream rivers [3,4]. The Himalayas constitute the youngest mountain system in the world. This mountain range plays a vital role in maintaining and controlling the monsoon system over the Asian continent [5]. For this reason, many climatologists, geologists, and biologists are attracted to the area for research.

Until recently, most studies in the Western Himalayan region (including Kufri Mountain) have included topics such as dendrochronology and climatology [6–8], medicinal plants for pharmaceutical value [9,10], and species composition and diversity [11–14]. Climate records from several high-elevation sites within the Western Himalayas indicate that the extent of temperature change in this region in the 20th century has been greater than that observed globally [5,8,15]. Several studies have shown the usefulness of Himalayan conifer genera (*Abies, Cedrus, Picea, and Pinus*) for their ability to reveal dendroclimatic events through their distinct annual growth and to survive over several centuries [8,16]. A strong dendroclimatic response makes these species useful for detailed studies on the effects of climate change and/or pollution on tree biology. However, to date there is a paucity of research focused on the physiological or metabolic effects of climate change or local environmental conditions, either alone or interacting with land-use history (e.g., grazing), on trees growing at high altitudes in the Western Himalayas, even for a predominant species such as Himalayan cedar [*Cedrus deodara* (Roxb. Ex Lamb.) G.Don].

A recent study by Ganguly and Thapa [17] used data from two monitoring sites, one urban site near the central bus stop and the other on a ridge representing the background environment, to investigate pollution levels in Shimla, HP, India. Located only 15 km from our study site (Kufri Mountain) at an elevational range of 2200–2300 m, Shimla is the largest city in HP and features a tourism-based economy. Ganguly and Thapa [17] reported that although the concentrations of nitrogen oxides (NO_x) and sulfur dioxide (SO_2) in Shimla city were below permissible limits [40 and 50 μg m^{-3}, respectively, according to the National Ambient Air Quality Standards (NAAQS)], the respirable suspended particulate matter (RSPM; criteria indicative of air quality) slightly exceeded the permissible limits of 60 μg m^{-3} (2004–2013) with a value of 69 μg m^{-3}, in 2009, the year sampling for this study was conducted. Continued monitoring until 2017 at these two sites showed that at the urban station, the average daily concentration of RSPM was 59 μg m^{-3} with a maximum daily average of 176 μg m^{-3}. The daily average at the background monitoring site was 45 μg m^{-3} with the maximum average recorded at 152 μg m^{-3}. Exceedance factors classified levels of RSPM at the urban location to be 'moderate to high' and the background monitoring site remained at the 'moderate' level [18]. These data indicate the fast rate at which pollutants are increasing over time in this region due to urbanization pressure in the nearby city.

Chen et al. [19] reported that levels of toxic chemicals in Himalayan cedar needles collected in 2002 from 17 sampling sites in urban areas around Dalian city in China were lower than or comparable to other international regions that were not impacted by evident point sources. This is one of the few studies available on Himalayan cedar. However, this research reports only on the absorption of persistent organic pollutants (POPs) and not the effects on the metabolism and health of this species. This may serve as a base for long-term spatial and temporal studies of specific POPs [e.g., polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/F)] and polychlorinated biphenyls (PCB) in cedar not only in China but in other places as well.

Studies conducted in the United States (US) at forested sites along an elevational gradient [20,21] revealed that the impacts of air and/or soil pollutants on the biology of select sensitive and responsive tree species were greater in magnitude and detectable earlier at higher elevations relative to lower elevations. Therefore, rigorous metabolic studies using these sensitive coniferous species in the Himalayan region are warranted for monitoring forest health in this important region.

Trees utilize complex biochemical processes to detect, respond to, and survive multiple concurrent environmental stresses to which they are exposed. Each stress factor affects cellular metabolism known to be sensitive to changes in local environmental conditions. Often the effects of multiple stressors are additive. Changes in environmental factors including acidic deposition, land-use history, N fertilization, atmospheric CO_2, ozone, soil Al mobility, nutrient deficiencies (Ca, N, P), drought, and soil warming and freeze–thaw are all known to impact C and N and metabolism, particularly metabolites such as polyamines and amino acids [21–28]. Most stress-linked metabolic pathways are interlinked via common signal transduction pathways that use Ca as a secondary messenger [24,29]. Additionally, these metabolic pathways share common precursors. Such tight interrelationships imply that changes in one metabolite will affect changes in several other metabolites [30]. In Arabidopsis, N supplementation led to genome-wide reprogramming of not only primary and secondary metabolism but even growth and development [31]. The production of defense compounds comes at a cost to plants in terms of taking energy away from growth processes [32].

Some indicator metabolites, such as the common polyamine putrescine (a small organic cation), amino acids, and chlorophyll in conjunction with other site characteristics (e.g., soil Ca, Al, and N) have successfully been used to suggest the overall health status of various conifer and hardwood tree species under environmental stress [20,22,33]. The detection of early metabolic changes in trees could lead to earlier mitigation programs and improved forest health. The objective of the present study was to evaluate the changes in soil chemistry and foliar metabolism of Himalayan cedar and Himalayan spruce trees at four sites along a steep elevational gradient at Kufri mountain, Himachal Pradesh, India. The goal was to understand the impact of multiple simultaneously acting environmental stressors on the health of foundational tree species in a forested catchment within the HP Himalayan mountain range that hosts high biodiversity and provides water to the communities downstream.

2. Materials and Methods

2.1. Site Description: Background on the Shimla Water Catchment Wildlife Sanctuary and the Sampling Site within This Sanctuary

This study occurred at the Shimla Water Catchment Wildlife Sanctuary, Himachal Pradesh in Northern India. (Figure 1). The water catchment regulates runoff into the Giri River, a tributary of the river Yamuna, one of the twelve major rivers in India. It is located between the geographical coordinates of 31.08° to 32.25° latitude and 77.2° to 77.25° longitude, at approximately 1915–2750 m above mean sea level. The slope of the elevational gradient of the study sites within the water catchment (Table 1) illustrates that the terrain is very steep and precipitous at places. The Shimla Water Catchment Wildlife Sanctuary derives its name from the hydrological function it has performed for more than 130 years since the establishment of British rule in India by supplying high-quality water to Shimla-the state capital of HP (https://www.shimlaonline.in/city-guide/water-catchment-sanctuary-shimla, accessed on 26 March 2021). The sanctuary is in the Lesser Himalayan mountain range, and its roughly rectangular shape covers an area of 1020 ha. The bedrock geology of the area of the study watershed is a complex sedimentary formation referred to as the Shimla Group that includes several carbonate-rich sequences [34]. The water catchment is buffered on all sides by dense forests managed by the Shimla Forest Division. The mean annual rainfall in the Shimla area is 140 cm with the highest rainfall occurring in July (34 cm) and lowest in November (7 cm). Rainfall during the southwest monsoon season, which is short-lived, accounts for approximately 71% of annual rainfall. The average annual maximum and minimum temperatures are 18.8 and 10.4 °C, respectively (http://www.imdpune.gov.in/caui/smartcities.html, accessed on 26 March 2021).
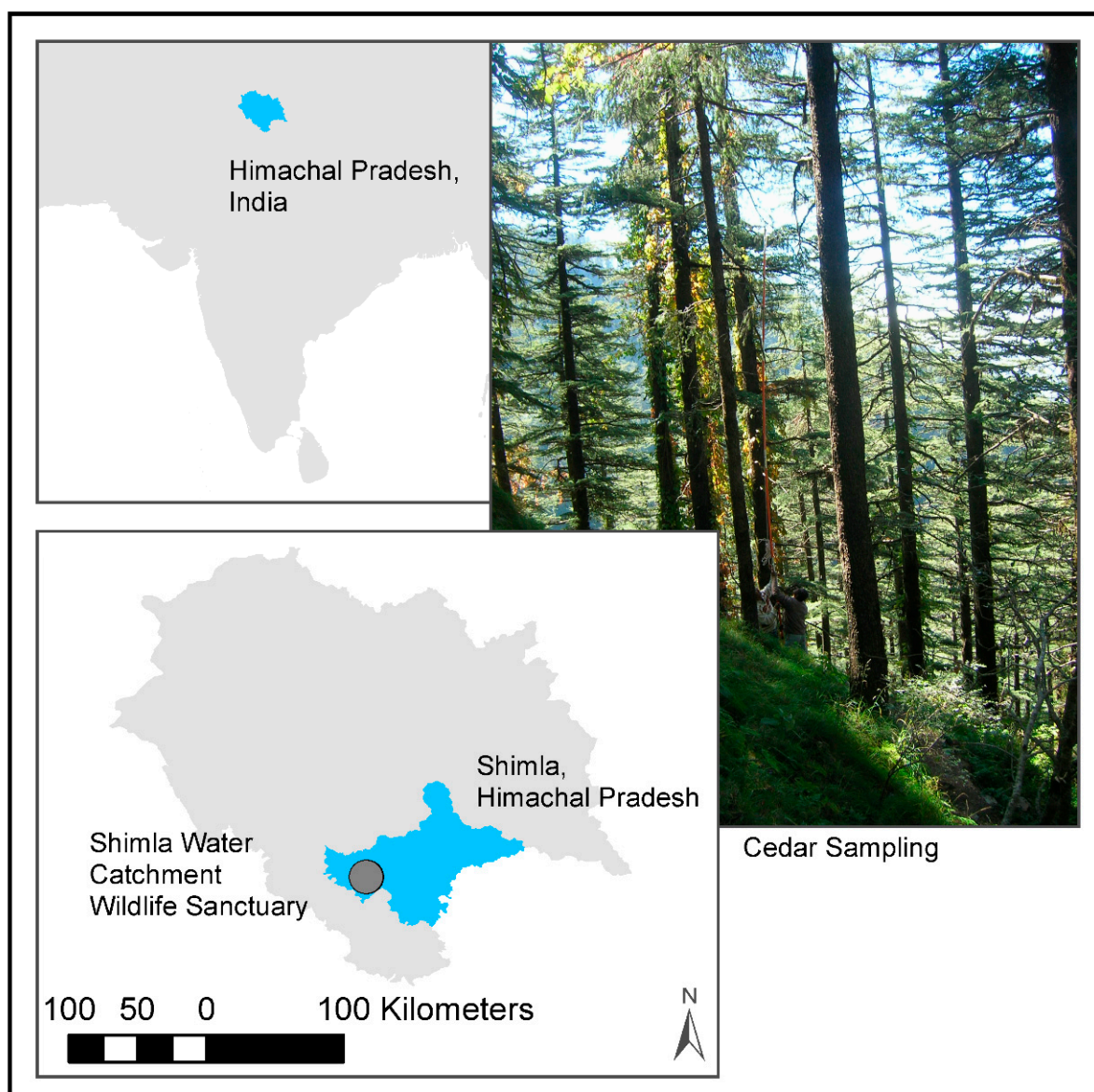
Figure 1. The geographical location of Shimla Water Catchment Wildlife Sanctuary, Shimla, Himachal Pradesh, India. Cedar sampling image was taken by coauthor Subhash C. Minocha. GIS mapping data were extracted from the GADM database (www.gadm.org, accessed on 26 March 2021), version 3.4, April 2018.

Table 1. GPS Coordinates in decimal degrees and elevation for sites sampled at Kufri, Himachal Pradesh, India.

Site	(Longitude)	(Latitude)	Point M (Distance from Ridge in m)	Elevation (m)	Slope (%) (Relative to Ridge)	Distance between Sites (m)	Slope (%) (between Sites)
Ridge	77.26365	31.09316	-	2649	_	_	_
High	77.26194	31.09194	207	2539	53.1	207	53.1
Mid	77.25983	31.09133	424	2425	52.8	217	52.5
Low	77.25876	31.09098	534	2371	52.1	110	49.1

The catchment area is rich in biodiversity and is home to many species of plants, animals, birds, and insects, including those of medicinal importance. Forests are two-storied, with Himalayan cedar being the dominant canopy species for which the Shimla Sanctuary is known. Additionally, known as "devdar" or "deodar", the Himalayan cedar is the state tree of Himachal Pradesh [35] and the national tree of Pakistan [36]. Its essential oils are antimicrobial, insecticidal, molluscicidal, germicidal, anti-tubercular agents and are widely used in the fragrance industry [9,37]. Due to its durability and antifungal properties, it is an important timber tree. Cedar is native to the Western Himalayas; typically found at elevations between 1500 and 3200 m. Although it prefers moist well-drained soils, cedar is a drought-resistant species. Of all the cedars, it is the most tolerant of heat and humidity [37]. This species can reach a height of 80 m, diameter at breast height (DBH) of 4 m, and live up to 600 years under favorable conditions. Its foliage is usually dark green, sometimes bluish green, sharp pointed, and varies in length from 2.3 to 3.7 cm [38,39].

Himalayan spruce [*Picea smithiana* (Wall.) Boiss] is another canopy tree species at this site. Additionally, known by the common names Indian spruce, Morinda, and West Himalayan spruce, it is native to India, Nepal, Pakistan, Afghanistan, and parts of Southern Tibet. Trees typically measure 1–2 m in DBH and grow to 40–55 m in height at elevations ranging from 2300 to 3600 m. They have long needles (3.3–5.5 cm) that are spread radially about the twig. Himalayan spruce is often found with cedar and West Himalayan fir [*Abies pindrow* (Royle ex D. Don)] in the Western Himalayan range (https://conifersociety.org/conifers/picea-smithiana/, accessed on 26 March 2021); all can tolerate temperatures as low as −12 °C. At the Shimla Water Catchment, spruce was only present at the ridge and low elevations, and mostly on the northern face of Kufri Mountain.

In addition to cedar and spruce, other canopy species at the site are acacia (*Acacia mollissima*), ban oak (*Quercus leucotrichophora)*, chir pine (*Pinus roxburghii*), Himalayan yew (*Taxus wallichiana*), kail (*Pinus wallichiana*), kainth (*Pyrus pashia*), khanor (*Aesculus indica*), kharsu oak (*Quercus semecarpifolia*), silver fir (*Abies spectabilis*), moru oak (*Quercus floribunda*), poplar (*Populus deltoides*), and rhododendron (*Rhododendron campanulatum, Rhododendron arboreum*). Shrubs include barberry (*Berberis aristate*), Desmodium (*Desmodium tiliaefolium*), Indigofera (*Indigofera pulchella*), and willow (*Salix fragilis*). Ground flora is comprised of various grass species along with a variety of ferns and vascular herbs.

Small villages popular with tourists lie on the periphery of the sanctuary, which leads to grazing sites for local horses entering the sanctuary. This practice creates biotic issues such as the potential introduction of diseases to other animals in the sanctuary, competition for food resources with other herbivores, and the effects of equine waste, especially ammonia. There have been recent efforts to control this problem by fencing certain areas. Grazing at these sites is not an authorized activity by the administration. Additionally, an access road with several shops and horse-back riding for tourists terminates close to the ridge site. Over the years, habitat degradation has resulted in a reduction in the supply of water to Shimla. The information provided here along with additional details regarding this sanctuary was obtained from the Shimla Water Catchment Wildlife Sanctuary Himachal Pradesh (2011–2012 to 2020–2021) received in April 2012 from the Himachal Pradesh Forest Department.

2.2. Soil Sample Collection and Analyses

Soil samples were collected at four elevations (Table 1) on the south-facing slope of the watershed from locations adjacent to where the cedar foliage sampling occurred. These locations spanned an elevation range of 2371 to 2649 to m. The watershed was extremely steep with slopes ranging from 49 to 53 percent at each of the sampling sites other than the ridge site, which had a relatively moderate slope of 23 percent. Profile descriptions and horizon designations are based on the United States Department of Agriculture National Resources Conservation Service Field Book [40]. Soils at each sampling location were comprised of a thin surface layer of litter and organic matter (Oe horizon) 2 to 3 cm thick overlying mineral soil with an A horizon of variable thickness. At the mid- and low-

elevation sites, canopy openings allowed sufficient light for the growth of intermittent patches of grass. At the low-, mid-, and high-elevation sites, upper and lower B horizons exhibited distinct differences in texture with abrupt boundaries. The deeper layer had a higher amount of coarse fraction material (gravel and stones), but clay was an important component of the texture throughout the excavated profile, so the boundaries were not considered lithologic discontinuities. At the ridge site, texture varied vertically over a subtle gradient within the B horizon. The low-elevation site had an abundance of macropores in the B2 horizon not seen in the profiles of the other sites. The B2 horizon of the mid-elevation site had a greater abundance of stones and was more compacted than the B2 horizon at the other sites. Hereafter, upper and lower layers of the B horizon are referred to as B1 and B2. At each of these four sites, one soil pit was also excavated for a profile description and sampling for the chemistry of the mineral soils. Samples of A, B1, and B2 horizons were collected at vertical midpoints of each horizon. Further information on soils and areas near soil sampling are provided in Table 2.

Table 2. Pit descriptions and site characteristics observed near soil pits at each elevation at Kufri, Himachal Pradesh, India.

Site-Soil Horizon	Depth of Horizon (cm)	Notes	Elevation (m)	Aspect	Canopy	Overstory (in Order of Abundance)	Understory
Ridge-A	0–4	Small A horizon. More friable than B horizons	2649	South	Not closed (but not as open as Low and Mid), moist conditions	*Cedrus deodara, Pinus wallichiana, Quercus dentata*	More diverse; sedge, *Cedrus* saplings, soil compaction from heavy foot traffic
Ridge-B1	4–27	Very fine textured, heavy clay.					
Ridge-B2	27–56	No large stones					
Ridge-BW	56–>80						
High-A	2–30	Roots common to the bottom of the pit, though few in B2	2539	South	Open (but not as much as Low and Mid), dry conditions	Dominated by *Cedrus deodara*	Mostly sedge-like grass, few *Cedrus* saplings, no seedlings of any tree species, signs of horses grazing
High-B1	30–55	Some small stones					
High-B2	55–>73	Very stony, compacted. No macropores					
Mid-A	3–26	Fine roots to 5 cm, but not overly dense. Roots common to bottom of the pit	2425	South	Open, dry conditions	Dominated by *Cedrus deodara*, (some *Quercus dentata* and Rhododendron, though not numerous)	Mostly sedge-like grass, few *Cedrus* saplings, no seedlings of any tree species
Mid-B1	26–50	Some small stones					
Mid-B2	50–>70	Very stony, compacted. May not have macropores					
Low-A	2–20	Thick A layer. Roots common to bottom of the pit	2371	South	Open, dry conditions	*Cedrus deodara, Quercus dentata, Picea smithiana*	Mostly sedge-like grass, few *Cedrus* saplings, no seedlings of any tree species
Low-B1	20–33	Clay, some small stones					
Low-B2	33–>57	Very stony with major macropores which roots tap into					

Soil cores (8–10 cm) were collected from the surface at each of the four sites, near the excavated soil pit and also the foliage sampling. The cores collected the 2 to 3 cm-thick Oe horizon plus several cm of the uppermost A horizon. Five replicate cores were collected at each of the four sampling locations along the elevational gradient.

Air-dried soils (20–40 g) were analyzed by the Soil Testing Service Laboratory at the University of Maine, Orono, ME, USA per the North American Proficiency Testing (NAPT) Program (http://anlab.umesci.maine.edu, accessed on 26 March 2021). Soil pH was determined in distilled water at ratios of either 1:5 for organic horizons or 1:1 for mineral soils [41]. The percentage of soil organic matter was determined by loss-on-ignition using a muffle furnace at 550 °C for 5 h [42]. Total N (TN) and total C (TC) were measured by combustion analysis at 1350 °C with a CN analyzer.

Exchangeable acidity was measured in soils extracted at a ratio of 1 g (organic) or 2.5 g (mineral) in 50 mL 1 M KCl and end-point titration using NaOH [43]. Exchangeable P, Al, and base cations were extracted in NH_4Cl [42] and the extracts were analyzed using

inductively coupled plasma optical emission spectrometry (ICP-OES). Effective cation-exchange capacity (ECEC) was calculated as the sum of the exchangeable base cations (Ca, Mg, K, and Na) plus exchangeable acidity [41]. Base saturation was calculated as the sum of exchangeable base cations divided by the ECEC.

2.3. Foliar Samples Collection and Analyses

Samples of Himalayan cedar and spruce were collected on 22 October 2009, from Kufri beat (a 174 hectare area that was a part of Seog block within the sanctuary). Foliar samples of Himalayan cedar were collected from the southern face of the mountain from four elevations described in Table 1. Spruce foliar samples were collected from the northern face of the mountain, approximately 50 m from the ridge, and low-elevation sites where the cedar trees were sampled. Samples of both species were obtained on the same day as the soil samples closer to where soil core and pit samples were taken.

Samples of both species were consistently taken from sunlit branches in the mid-canopy using a tall pole pruner. Visually healthy current-year needles were collected from 10 cedar trees from each of the four locations, and 10 spruce trees from the low and ridge locations, for a total of 60 foliar samples. Approximately 500 mg (FW) sample of foliage from a single branch per tree was chopped using scissors. The clippings were mixed, and two sub-samples per tree were taken for biochemical analyses. The first sub-sample, ~200 mg, was placed in a pre-weighed 2 mL microfuge tube to which one 1 mL of 5% perchloric acid (PCA) was added. The remainder of the sample was placed in a separate tube. On the day of collection, all samples were transported on ice and then stored at $-20\,^{\circ}C$ until further analysis. The frozen samples were later shipped to the USDA Forest Service laboratory in Durham, NH, USA, where they were frozen and thawed 3 times, weighed, vortexed, and centrifuged at $14{,}000\times g$ for 8 min according to the procedure described in Minocha et al. (1994). The supernatant was used for analyses of PCA-extractable (soluble) polyamines, amino acids, and inorganic ions. The second sub-set of samples was used for analyses of soluble proteins and total chlorophyll. Samples from each tree were analyzed separately for the chosen variables.

Soluble inorganic ions: Supernatants of frozen-thawed samples in PCA were diluted ($100\times$) with distilled deionized water for the estimation of soluble inorganic ions (defined as the fraction of total ions within cells that is extractable in 5% PCA) including Ca, K, P, Mg, Mn, Al, Fe, and Zn. The analysis was conducted using a simultaneous axial Inductively Coupled Plasma Optical Emission Spectrophotometer (Vista CCD, Varian, Palo Alto, CA, USA) and Vista Pro software (version 4.0) per EPA SW-846 compendium, method 6010 (https://www.epa.gov/hw-sw846, accessed on 26 March 2021).

Polyamines and amino acids: Supernatants of frozen-thawed samples in PCA were derivatized with a fluorescent dansyl chloride tag for the simultaneous quantitation of polyamines and amino acids according to Minocha and Long (2004) with minor modifications as described here. Samples were incubated at 60 °C for 30 min, cooled for 3 min and microfuged at $14{,}000\times g$ for 30 s. The reaction was terminated by adding 45 μL of glacial acetic acid. Sample tubes were kept open for 3 min under a flow hood to allow CO_2 to escape. The acetone in which dansyl chloride was dissolved was evaporated using a SpeedVac Evaporator (Savant, Farmingdale, NY, USA) for 8 min. Finally, 1.735 mL of filtered HPLC-grade methanol was added to each tube bringing the final volume to 2 mL. Analytes were separated using a PerkinElmer (Waltham, MA, USA) series 200 HPLC system and fluorescence detector. Data were processed using PerkinElmer TotalChrom software (version 6.2.1). With this protocol, the peaks of arginine and threonine could not be completely resolved. For their quantitation, the peak areas and concentrations of these two amino acids were added together to formulate a combined calibration curve; at equal concentrations, Arg occupied 2.5 fold of the combined peak area.

Chlorophyll analysis: For chlorophyll analysis, 1 mL of 95% ethanol was added to 10 mg of thawed needles. Samples were then incubated in the dark at 65 °C for 16 h and centrifuged at $13{,}000\,g$ for 5 min [44]. The supernatant was scanned for absorbances ranging

from 350 to 710 nm using a spectrophotometer (Hitachi Ltd., Tokyo, Japan; bandwidth 2 nm, wavelength accuracy of ±0.3 nm, wavelength setting reproducibility of ±0.1 nm; with Hitachi UV Solutions software version 2.0). Equations from Lichtenthaler (1987) were used to calculate total chlorophyll and chlorophyll a and b concentrations.

Soluble proteins analysis: For soluble proteins, 0.25 mL of extraction buffer [100 mM Tris-HCl, 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM EDTA, and 10% (*v/v*) glycerol, pH 8.0] was added to 50 mg of thawed needles [45]. Samples were then frozen and thawed three times. The supernatant (after centrifugation at 13,000 *g* for 5 min) was quantified for total soluble protein concentration as per Bradford [46] using Bio-Rad protein assay dye reagent (Bio-Rad Laboratories, Hercules, CA, USA). Absorbance was recorded at 595 nm with a Hitachi U2010 spectrophotometer (Hitachi Ltd., Tokyo, Japan) and analyzed with Hitachi UV Solutions software version 2.0.

2.4. Statistical Analyses

All statistical analyses were conducted in R version 3.1.2. A mixed-effects modeling approach [47–49] with the *nlme* package [50] was used to examine differences in nutrients, polyamines and amino acids, chlorophyll, and soluble proteins as a function of landscape position. Elevation was the hypothesized fixed effect. Possible random effects were replicate trees nested within elevations, which were evaluated using the likelihood ratio test [49]. Unequal variance within each elevation was also assessed using the *varIdent* function and was compared to models without the weighting function using the Bayesian Information Criterion (BIC). Final model diagnostics evaluated model residuals for normality and homoscedasticity. If final model-level *p*-values were <0.05, pairwise comparisons between means at different elevations were conducted using the *glht* function in the multcomp package with Bonferroni corrections for multiple comparisons [51]. Using vegan package [52] in R, non-metric multidimensional scaling (NMDS) analyses were then conducted to both examine elevational patterns in foliar variables and to compare patterns between soil chemistry and foliar chemistry and foliar metabolites for cedar (Table S1) and spruce (Table S2). Plots of beta diversity for cedar and spruce based on Bray–Curtis dissimilarities of foliar variables were generated after 20 random starts of a two-dimensional solution. Data were log transformed prior to the ordination. *Adonis* (MANOVA using Bray–Curtis distance matrices) was used to determine the significance of landscape position in explaining difference in foliar variables for cedar (Table S3) and spruce (Table S4). The *envfit* function in vegan was used to examine correlations between individual foliar nutrients, foliar metabolites, and soil chemical variables and the NMDS ordination. Only significant foliar or soil variables (*p* < 0.05) were overlaid as vectors onto the NMDS ordination using the function *envfit* (1000 permutations) in vegan.

3. Results

3.1. Soil Cores (Top 10 cm of Soil)

The mean concentration of TN was significantly higher in samples from the high-elevation site than in samples from the other three elevations (Table 3). At the ridge site, TN was 45% lower than at the high-elevation site and ~32% lower than at mid- and low-elevation sites. The TC concentration was significantly lower at the ridge sites compared to other elevations whereas low and mid-elevation TC was not different from either high or ridge elevation. Loss on ignition (LOI) and Ca concentrations were significantly lower in samples from the ridge than those at high and mid-elevation sites but were not different to those at low-elevation sites (Table 3).

Table 3. Differences in chemical properties of the top 10 cm of soil (including 0–3 cm of O horizon) from soil cores collected along an elevational gradient at Kufri, Himachal Pradesh, India. Data are the mean $\pm$ SE (n = 5). Superscripted letters indicate significant differences between elevations ($p \leq 0.05$).

| Soil Chemistry	Ridge (2649 m)	High (2539 m)	Mid (2425 m)	Low (2371 m)
TN (%)	0.29 $\pm$ 0.03 [a]	0.53 $\pm$ 0.07 [b]	0.43 $\pm$ 0.02 [c]	0.42 $\pm$ 0.03 [c]
TC (%)	5.75 $\pm$ 1.03 [a]	8.64 $\pm$ 1.07 [b]	7.61 $\pm$ 0.31 [a,b]	7.49 $\pm$ 0.43 [a,b]
Ca (mg kg^{-1})	3291 $\pm$ 480 [a]	5056 $\pm$ 419 [b]	5105 $\pm$ 514 [b]	3775 $\pm$ 478 [a,b]
K (mg kg^{-1})	412 $\pm$ 69 [a,b,c]	290 $\pm$ 30 [a,c]	540 $\pm$ 95 [b]	288 $\pm$ 28 [c]
Mg (mg kg^{-1})	314 $\pm$ 28 [a]	446 $\pm$ 20 [b]	457 $\pm$ 54 [b]	347 $\pm$ 40 [a]
P (mg kg^{-1})	2.0 $\pm$ 0.2 [a]	4.3 $\pm$ 0.8 [b]	3.6 $\pm$ 0.3 [b]	3.5 $\pm$ 0.3 [b]
Al (mg kg^{-1})	6.8 $\pm$ 3.4	4.2 $\pm$ 0.8	5.5 $\pm$ 1.9	3.6 $\pm$ 0.8
Fe (mg kg^{-1})	1.3 $\pm$ 0.6	1.1 $\pm$ 0.3	0.9 $\pm$ 0.3	1.4 $\pm$ 0.5
Mn (mg kg^{-1})	4.2 $\pm$ 0.6	5.0 $\pm$ 1.8	3.6 $\pm$ 1.0	7.7 $\pm$ 2.0
Na (mg kg^{-1})	16.1 $\pm$ 9.1	7.7 $\pm$ 0.9	12.2 $\pm$ 3.6	7.5 $\pm$ 0.8
Zn (mg kg^{-1})	0.29 $\pm$ 0.09	na	0.27 $\pm$ 0.03	0.57 $\pm$ 0.14
pH	6.2 $\pm$ 0.1	6.3 $\pm$ 0.1	6.8 $\pm$ 0.2	6.3 $\pm$ 0.1
Acidity (cmolc kg^{-1})	0.24 $\pm$ 0.05	0.19 $\pm$ 0.00	0.19 $\pm$ 0.00	0.19 $\pm$ 0.00
ECEC (cmolc kg^{-1})	20.4 $\pm$ 2.8 [a]	29.8 $\pm$ 2.3 [b]	30.8 $\pm$ 3.2 [b]	22.6 $\pm$ 2.6 [a]
LOI (%)	12.5 $\pm$ 1.6 [a]	18.1 $\pm$ 1.7 [b]	17.0 $\pm$ 1.0 [b]	15.8 $\pm$ 0.8 [a,b]

LOI = loss on ignition; measurement of organic at 55 °C. TN = total nitrogen. TC = total carbon. Acidity = exchangeable acidity. ECEC = effective cation exchange capacity; the sum of concentrations of Ca, K, Mg, Na, and acidity expressed as cmolc kg^{-1}.

The concentration of P was significantly lower at the ridge relative to the other elevations, which were not different from each other. Effective cation exchange capacity and Mg concentration were similar at high and mid-elevations but higher than the values at the ridge and low-elevation site. Potassium at the ridge was not different from the other three elevations but was highest at mid-elevation and was significantly different from the high- and low-elevation sites. Other chemical properties, including pH and acidity, and concentrations of Al, Fe, Mn, Na, and Zn were not different among the four elevations.

NMDS analyses conducted on Bray–Curtis dissimilarities calculated from natural log-transformed data (stress = 0.094) revealed that soil characteristics at high, mid, and low elevations were spatially closer to each other as compared to the ridge (Figure 2). MANOVA analysis also showed that soil chemistry significantly varied among elevations (*adonis* test, p = 0.086). Out of 13 soil variables tested, four (TC, Ca, ECEC, Fe) were significantly correlated with the dissimilarity matrix of soil chemistry at $p < 0.05$, and another three (TN, LOI, and P) were significantly correlated at $p < 0.1$.

3.2. Soil Pits

Soil chemistry from samples collected at the ridge, high elevation, and low-elevation sites exhibited similar values when compared by horizon, and similar changes with depth from the A horizon downward to the B2 horizon (Table 4). Concentrations of TN, TC, ECEC, and LOI decreased with depth from the A to the B2 horizon at all sites. Concentrations of Ca, Mg, P, and Mn also decreased with depth at three of four sites, and Mn was below the detection limit at the high-elevation site in the B1 and B2 horizons (Table 4). Concentrations of K, Na, Fe, and Zn exhibited variable relationships with depth among the four sites. Values of pH, acidity, and Al showed no changes with depth at any of the sites except the mid-elevation site, where acidity and Al both decreased with depth and pH increased consistently with depth. Aluminum and acidity, however, were minimal at all sites other than the mid-elevation site.
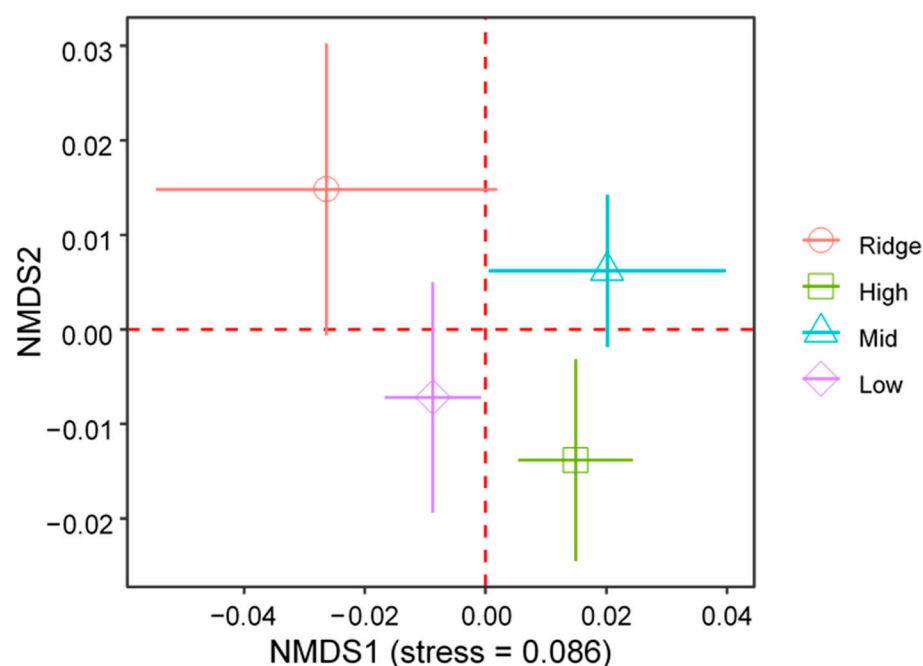
Figure 2. Non-metric multidimensional scaling (NMDS) plots of soil chemical constituents grouped by elevation. The further away the elevations are on the plot the more dissimilar they from each other.

The mid-elevation site differed from the other sites for most measurements in the A and B1 horizons and differed from the other sites in how concentrations varied with depth (Table 4). Whereas pH and acidity at the ridge, high-, and low-elevation sites reflected strong acid buffering throughout the profile, the mid-elevation site exhibited substantial acidification in the A horizon, a moderate degree of acidification in the B1 horizon, and a small degree of acidification in the B2 horizon. The pH of the A horizon at mid-elevation was approximately 2 pH units lower than that of the other sites, which equates to an H^+ ion concentration approximately 100 times greater. Thus, exchangeable acidity in the A horizon at the mid-elevation site was also nearly 100 times greater than in the A horizon at the other sites, and only the mid-elevation site had more than a nominal value of acidity in the B1 horizon. The concentration of Ca in the mid-elevation A horizon was approximately 25% that of the A horizons at other sites, it was lower in the B1 and B2 horizons than at the other sites as well. The acidic characteristics of the mid-elevation site were also reflected in the base saturation of the A horizon, which was only 33%, whereas the other sites were near the maximum value of 100% in all horizons. Base saturation of the B1 and B2 horizons at the mid-elevation site was also less than 100%.

Other large concentration differences between the mid-elevation site and the other sites included the concentration of Al, which was more than 100 times greater in the A horizon of the mid-elevation site than the other sites, and substantially higher in the B1 and B2 horizon as well. Like Al, Mn was higher at the mid-elevation site than the other sites in all three horizons. Concentrations of Fe, Zn, and P were substantially higher in the A horizon of the mid-elevation site than the other sites, but not substantially different from the other sites in the B1 and B2 horizons, although Zn was below detection limits at the high elevation and ridge site.

Table 4. Chemical properties of A, B1, and B2 horizon samples that were collected from soil pits at four sites along an elevational gradient; samples were collected at vertical midpoints of each horizon at Kufri, Himachal Pradesh, India.

Soil Chemistry	pH	LOI (%)	TN (%)	TC (%)	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	P (mg kg^{-1})	Al (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Na (mg kg^{-1})	Zn (mg kg^{-1})	Acidity (cmolc kg^{-1})	ECEC (cmolc kg^{-1})	Base Saturation
Ridge-A	6.2	9.7	0.24	3.87	2446	281	246	1.5	2.0	0.9	0.90	3.8	<0.2	0.19	15.2	98.6
Ridge-B1	6.0	8.0	0.18	2.89	2146	165	181	1.1	9.1	1.3	0.66	6.1	<0.2	0.19	12.9	98.3
Ridge-B2	6.2	3.7	0.06	0.58	1453	81	189	<1.0	14.7	3.1	<0.2	5.5	<0.2	0.39	9.4	97.9
High-A	6.0	10.7	0.26	3.82	2786	146	293	1.9	7.9	1.2	1.68	13.6	<0.2	0.19	17.0	98.7
High-B1	6.3	6.4	0.14	1.65	1901	105	207	1.5	1.2	1.7	<0.2	9.1	<0.2	0.19	11.7	98.2
High-B2	6.3	5.1	0.09	1.11	1409	131	184	1.3	3.9	0.8	<0.2	9.6	<0.2	<0.2	8.9	99.8
Mid-A	4.2	12.2	0.30	4.76	663	167	262	6.3	984.1	16.0	33.68	35.0	1.76	12.22	18.3	33.1
Mid-B1	5.3	6.7	0.13	1.81	1223	174	188	1.7	147.2	1.4	4.16	15.5	0.56	1.75	9.9	82.3
Mid-B2	5.9	6.1	0.12	1.61	1153	171	160	1.4	38.5	1.4	1.82	71.5	0.62	0.58	8.4	92.9
Low-A	6.2	11.0	0.26	4.09	2931	212	257	1.8	3.6	0.4	0.98	6.6	<0.2	<0.2	17.3	99.8
Low-B1	6.3	7.4	0.15	2.20	2057	164	217	1.9	4.3	0.2	0.38	7.3	0.60	<0.2	12.5	99.8
Low-B2	6.4	5.6	0.09	1.13	1458	61	184	1.5	7.1	<0.2	0.96	9.6	0.22	0.19	9.2	97.7

LOI = loss on ignition; measurement of organic matter at 55 °C. TN = total nitrogen. TC = total carbon. Acidity = exchangeable acidity. ECEC = effective cation exchange capacity; the sum of concentrations of Ca, K, Mg, Na, and acidity expressed as cmolc kg^{-1}.

3.3. Himalayan Cedar

There were no differences in the foliar concentrations of soluble Ca or P for any of the elevations (Figure 3a,f). The foliar concentration of soluble Mg was highest at low elevation relative to the ridge and high elevations (Figure 3b). At high elevation, while foliar soluble Mn and Al were lower, K was significantly higher relative to the ridge, mid, and low elevations (Figure 3c,e,d). Concentrations of these three ions were similar among the other three elevations.
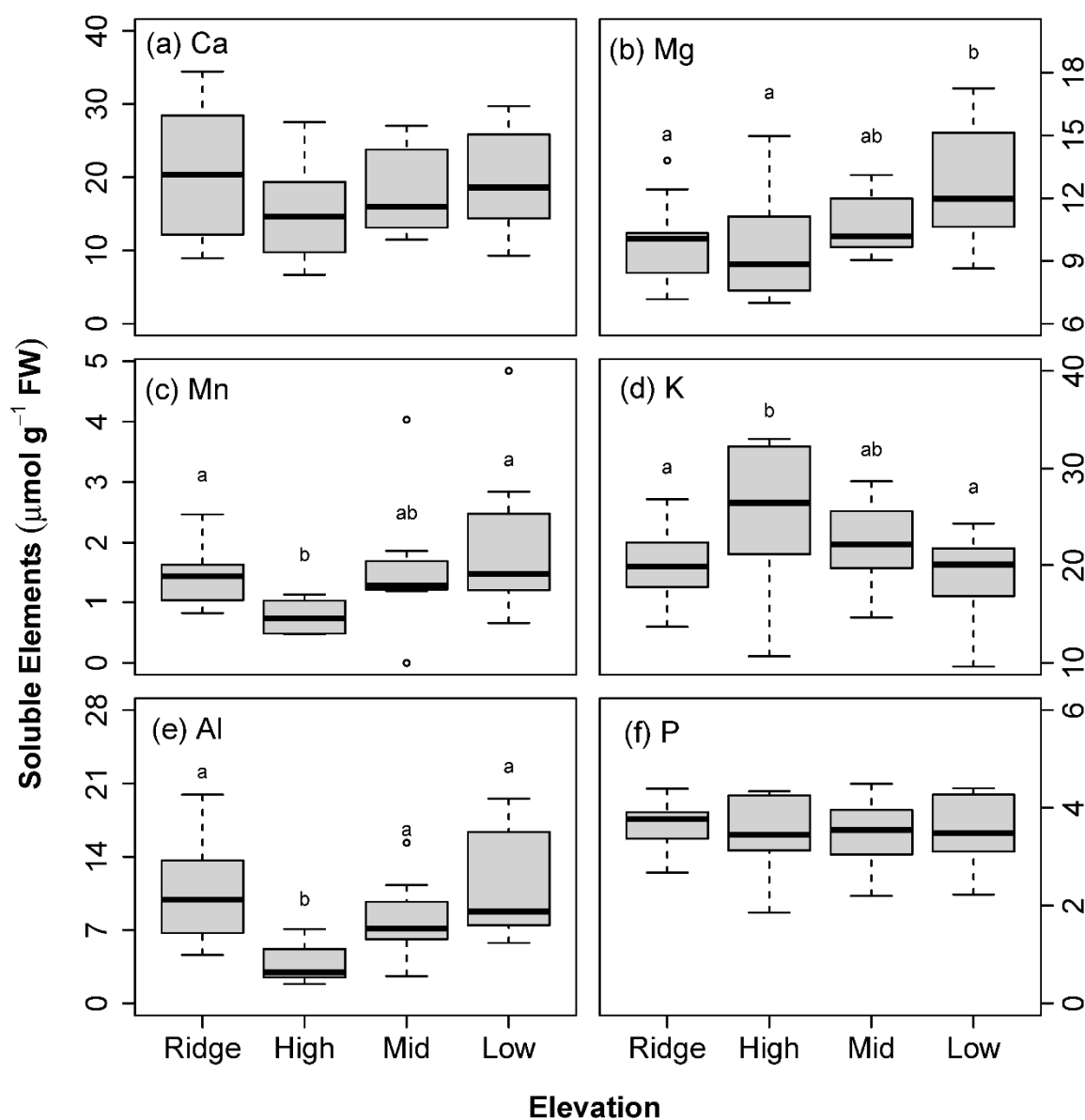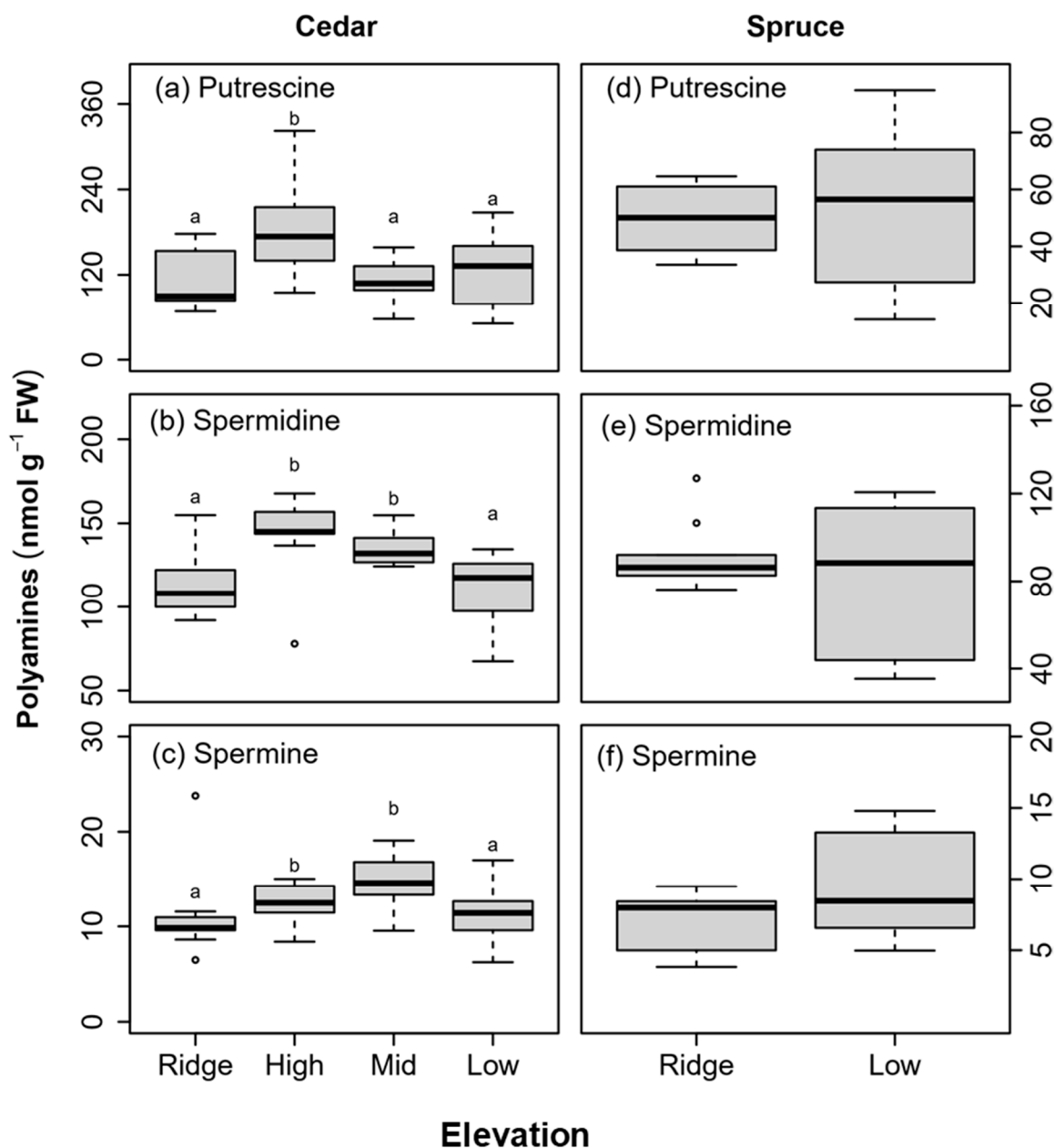

Figure 3. Foliar soluble (dilute acid extractable) element concentrations in Himalayan cedar along an elevational gradient of 2649 to 2371 m at Kufri Mountain in the foothills of the Northwestern Himalayas in 2009. Data are presented as box and whisker plots (n = 10). Letters represent significant differences between sites ($p \leq 0.05$).

Among the organic metabolites, all three common polyamines, putrescine, spermidine, and spermine were significantly higher in high elevation foliage (Figure 4a–c). In comparison with ridge and low elevation, concentrations of foliar spermidine and spermine were also higher at mid-elevations (Figure 4b–c). Amino acids, arginine + threonine, proline, γ-aminobutyric acid (GABA), ornithine, glycine, serine, lysine, leucine, isoleucine, and tryptophan were significantly higher at high elevation (Figure 5b–e,g,h,j–m). In comparison with ridge and low elevation, foliar ornithine and lysine were also higher at mid-elevation

(Figure 5e,j). The foliar concentrations of other common amino acids were either too low to detect or were not different among the elevations (Figure 5). There were no significant differences in foliar concentrations of total chlorophyll, chlorophyll a and chlorophyll b among elevations. However, the ratio of chlorophyll a:b was significantly higher at high elevation relative to the other three elevations (Figure 6a–d). Soluble protein was significantly higher in foliage from high elevation versus that from mid and low elevations, but not different from the ridge (Figure 7a).

Figure 4. Foliar free polyamines concentrations in Himalayan cedar and Himalayan spruce along an elevational gradient of 2649 to 2371 m at Kufri Mountain in the foothills of the Northwestern Himalayas in 2009. Data are presented as box and whisker plots (*n* = 10). Letters represent significant differences between sites ($p \leq 0.05$).

NMDS analyses conducted on Bray–Curtis dissimilarities calculated from natural log-transformed data (stress = 0.086) revealed that cedar trees growing at high and mid-elevations were spatially closer and thus more similar to each other as compared to the ridge and low elevations, both of which were also spatially closer to one another (Figure 8a). The MANOVA analysis performed with the *adonis* function indicated a significant difference ($p < 0.001$) in foliar chemistry and foliar metabolites among elevations. All 10 of the soil variables tested using the *envfit* function were significant ($p < 0.05$) in explaining the dissimilarities between the elevations. The soil C, N, P, and LOI vectors pointed more towards the high-elevation site, indicating that variation in foliar chemistry and foliar metabolites among elevations was largely stemming from the different soil chemistry at the high-elevation site.
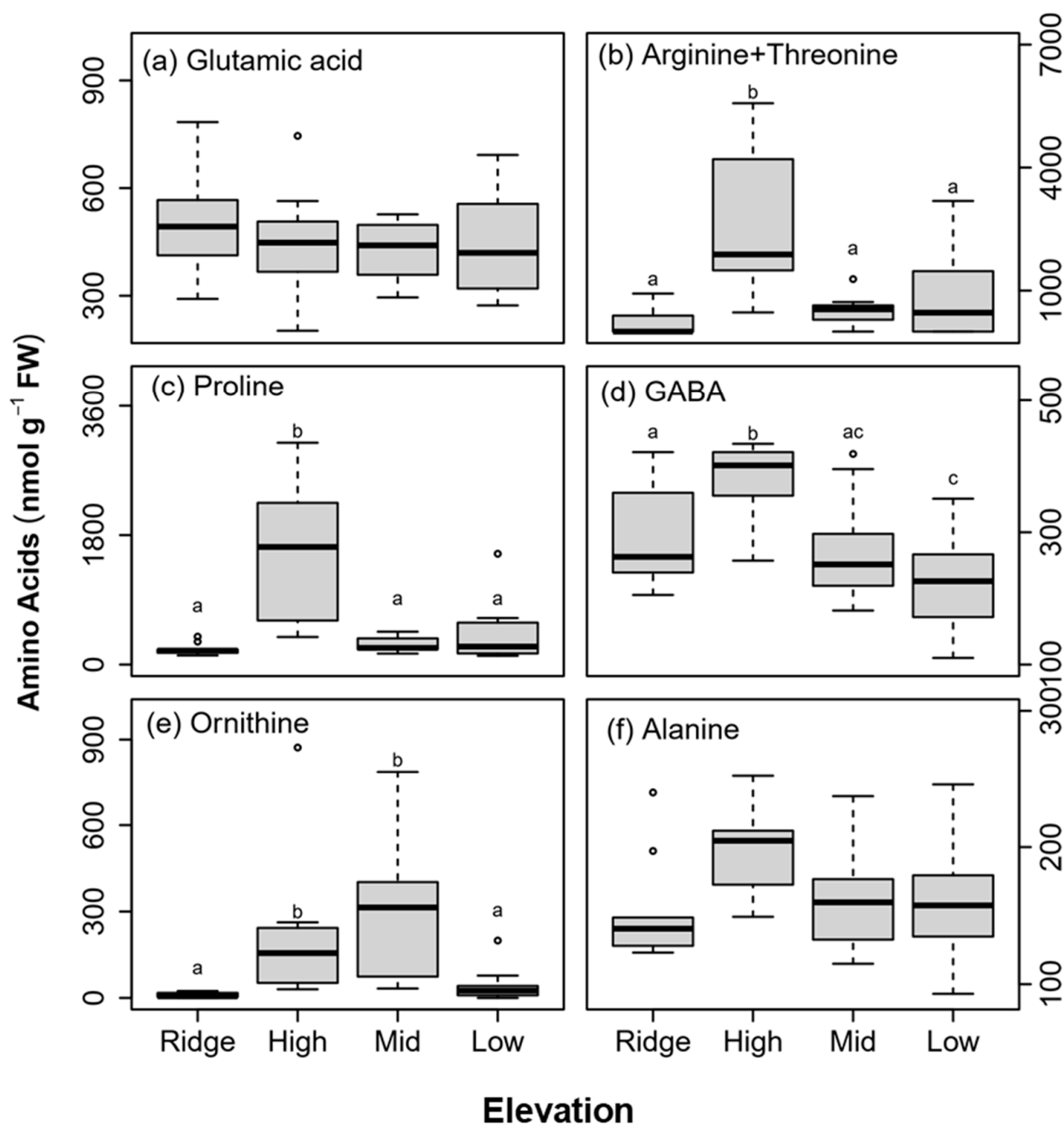
Figure 5. *Cont.*
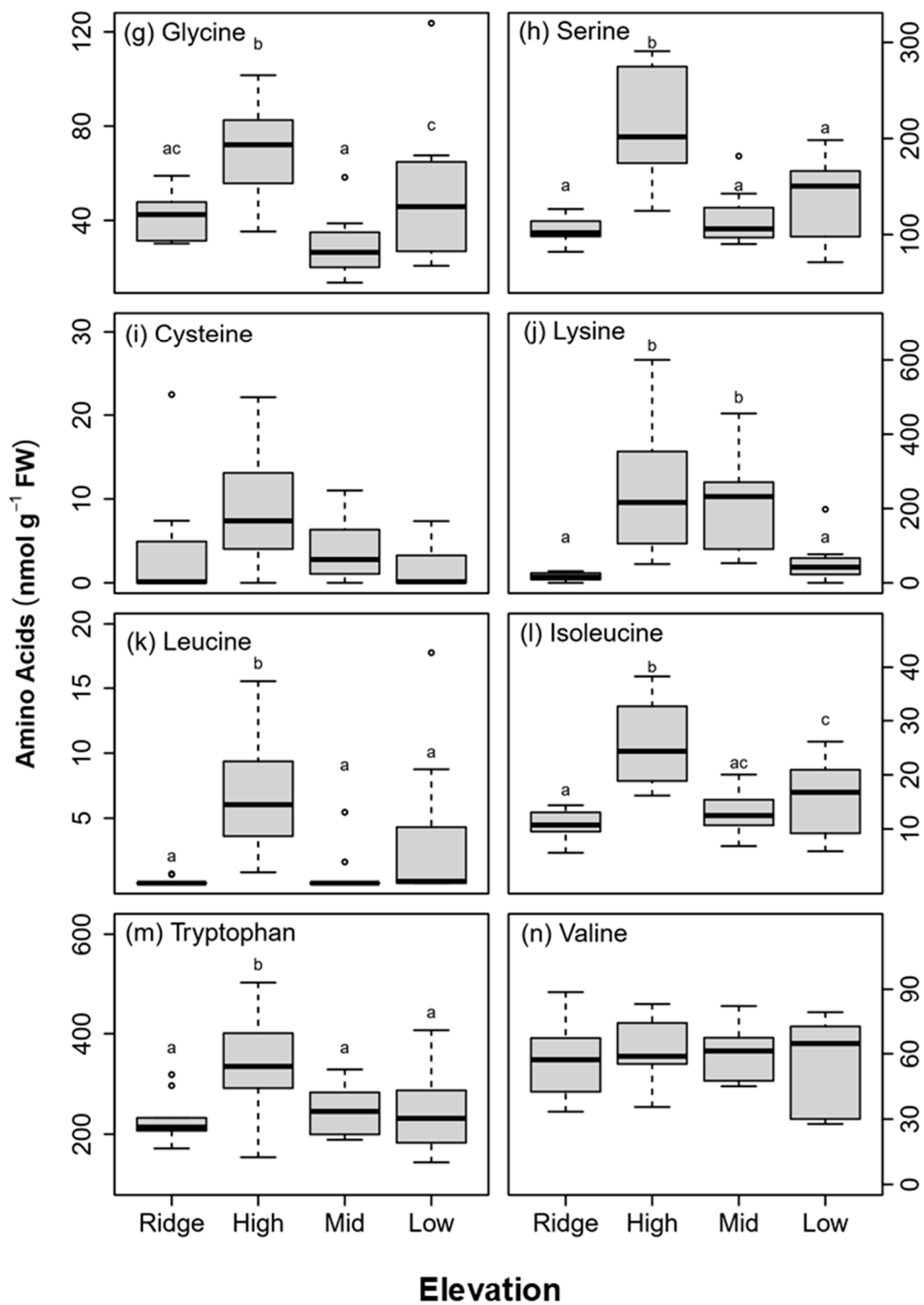
Figure 5. Foliar free amino acid concentrations in Himalayan cedar along an elevational gradient of 2649 to 2371 m at Kufri Mountain in the foothills of the Northwestern Himalayas in 2009. Data are presented as box and whisker plots (*n* = 10). Letters represent significant differences between sites ($p \leq 0.05$).
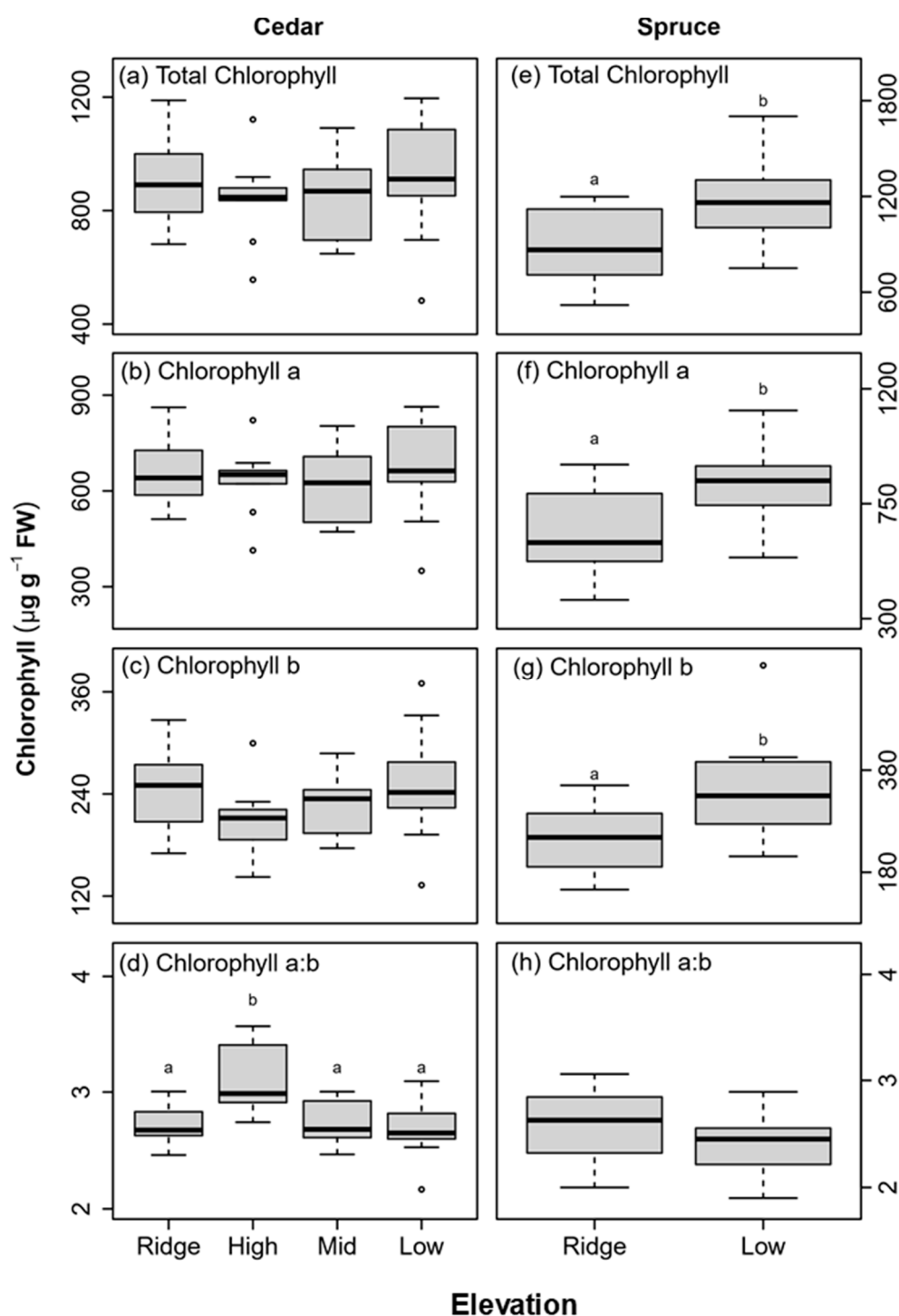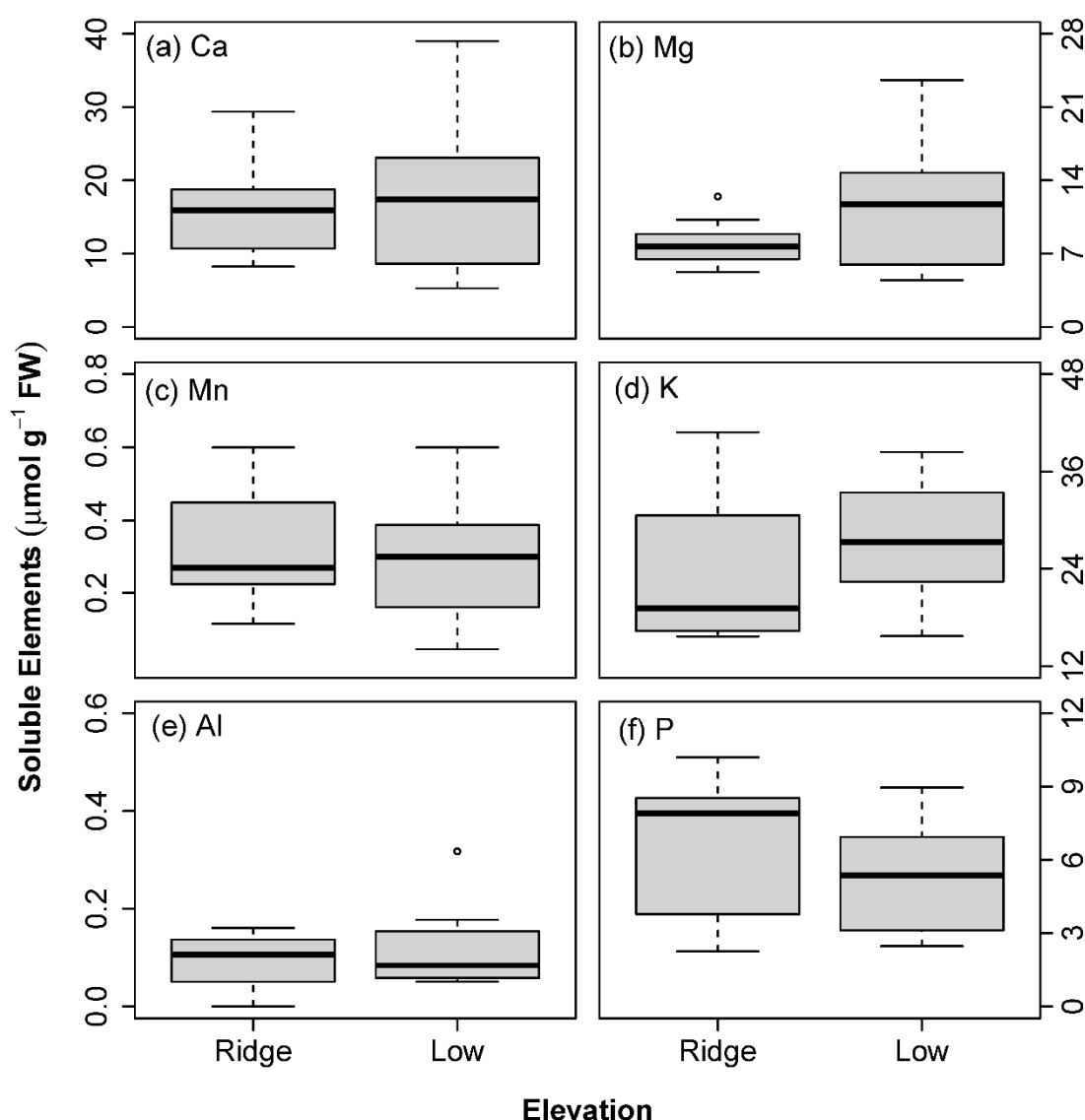
Figure 6. Foliar chlorophyll concentrations in Himalayan cedar and Himalayan spruce along an elevational gradient of 2649 to 2371 m at Kufri Mountain in the foothills of the Northwestern Himalayas in October of 2009. Data are presented as box and whisker plots ($n = 10$). Letters represent significant differences between sites ($p \leq 0.05$).
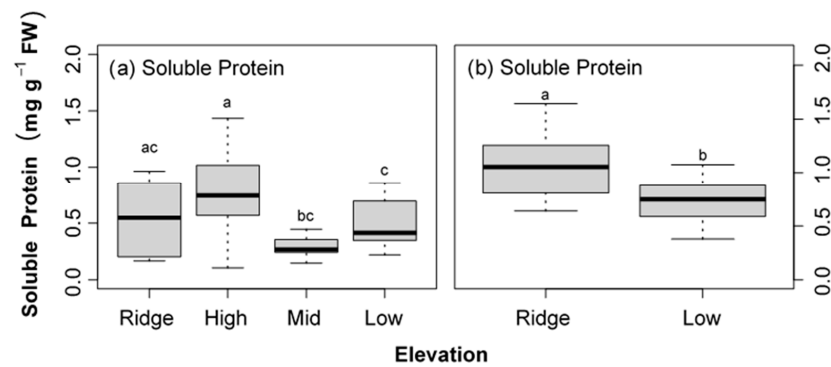
Figure 7. Foliar soluble protein concentrations in Himalayan cedar and Himalayan spruce along an elevational gradient of 2649 to 2371 m at Kufri Mountain in the foothills of the Northwestern Himalayas in 2009. Data are presented as box and whisker plots (n = 10). Letters represent significant differences between sites ($p \leq 0.05$).
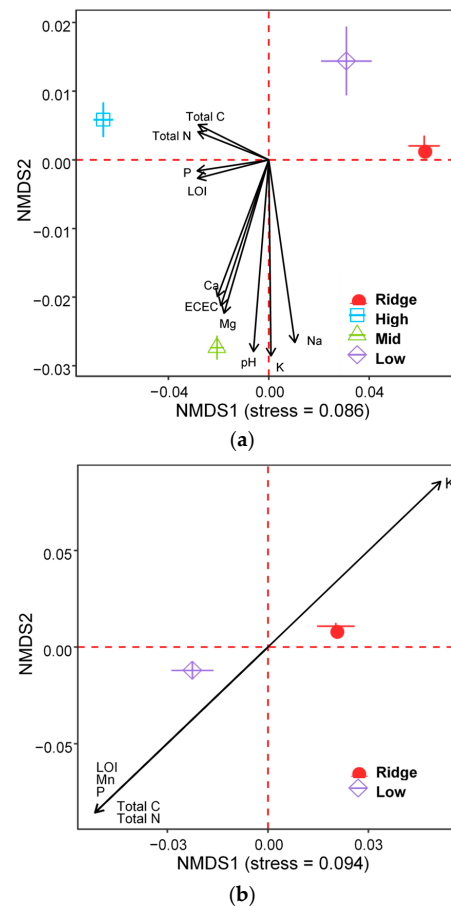
Figure 8. Non-metric multidimensional scaling (NMDS) plots of foliar chemistry grouped by elevation for (**a**) cedar and (**b**) spruce. Arrows fit on top of the ordination represent correlations between soil and foliar chemistry. Only significant soil factors ($p \leq 0.05$) were overlaid as vectors onto the NMDS using the *envfit* function (999 permutations) in vegan. The direction of arrows indicates the direction of the relationship while the length indicates the strength of the relationship. LOI = loss on ignition; measurement of organic at 55 °C. ECEC = effective cation exchange capacity; the sum of concentrations of Ca, K, Mg, Na, and acidity expressed as cmolc kg^{-1}.

3.4. Himalayan Spruce

Unlike cedar, neither foliar nutrients nor foliar polyamines varied as a function of elevation in Himalayan spruce. There were no significant differences in soluble elements (Figure 9) or polyamines (Figure 4d–f) observed between the foliage of trees growing at the ridge and low elevation plots. However, foliar glutamic acid, GABA, glycine, serine, leucine, isoleucine, tryptophan, and valine were all significantly higher at the low elevation relative to the ridge (Figure 10a, 10d, 10g–h, 10k–n). Other amino acids were in concentrations too low to detect or were similar among elevations (Figure 10). The concentration of chlorophyll (total, a and b) was significantly higher at low elevation relative to the ridge (Figure 6e–g). The ratio of chlorophyll a:b did not change between the two elevations (Figure 6h). Soluble proteins were significantly lower in foliage from low elevation as compared to the ridge (Figure 7b).

Figure 9. Foliar soluble (dilute acid extractable) element concentrations in Himalayan spruce along an elevational gradient of 2649 to 2371 m at Kufri Mountain in the foothills of Northwestern Himalayas in 2009. Data are presented as box and whisker plots (*n* = 10). Letters represent significant differences between treatments (*p* $\leq$ 0.05).

NMDS analyses conducted on Bray–Curtis dissimilarities calculated from natural log-transformed data (stress = 0.094) revealed that spruce growing on the ridge and low elevations were spatially distant (Figure 8b) and significantly different (Manova test, $p < 0.008$). All five of the soil variables tested using the *envfit* function were significantly correlated ($p < 0.05$) with the dissimilarity matrix. The soil C, N, P, Mn, and LOI vectors pointed more towards the low-elevation site, indicating that variation in foliar chemistry and foliar metabolites among the two elevations was largely stemming from the different soil chemistry between the two sites.
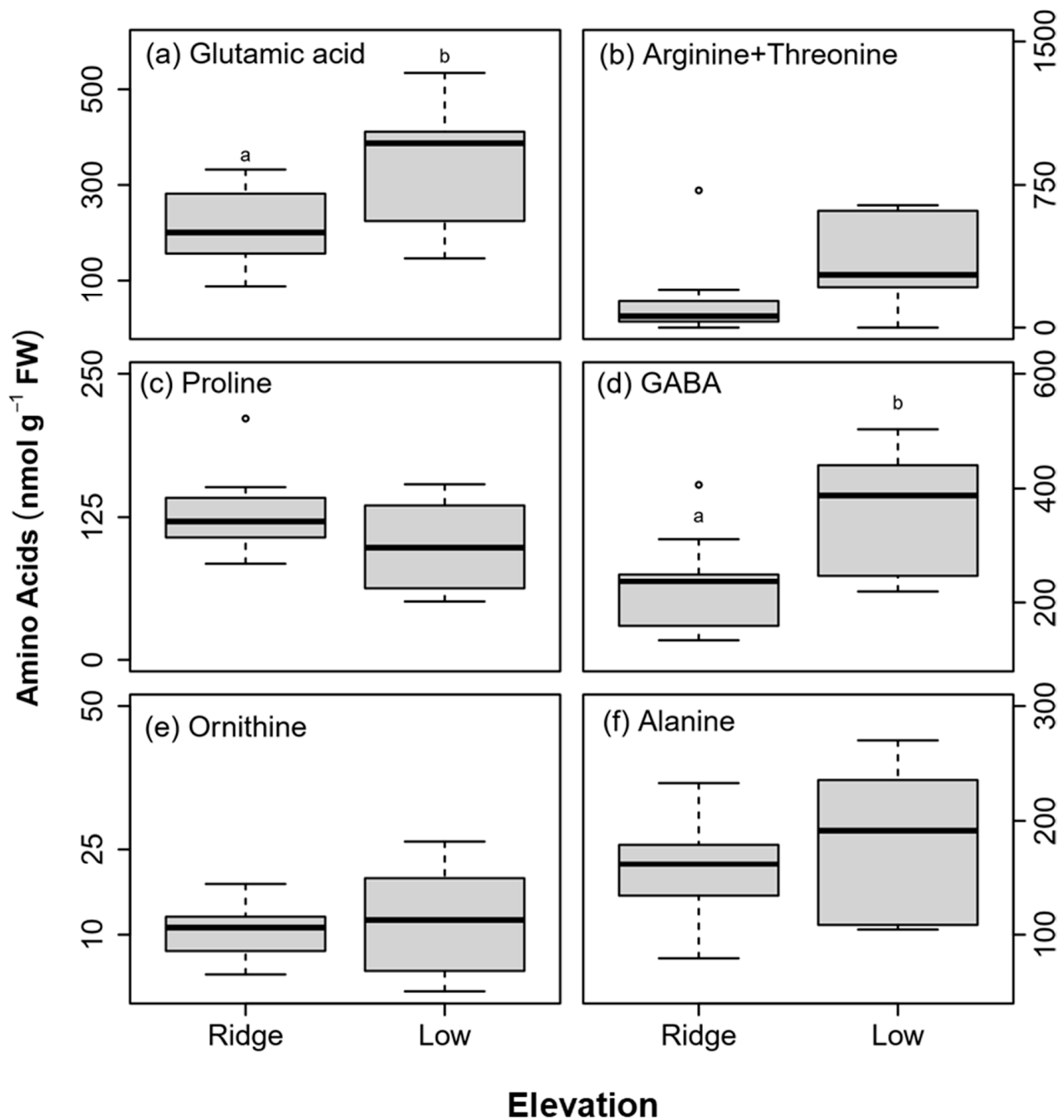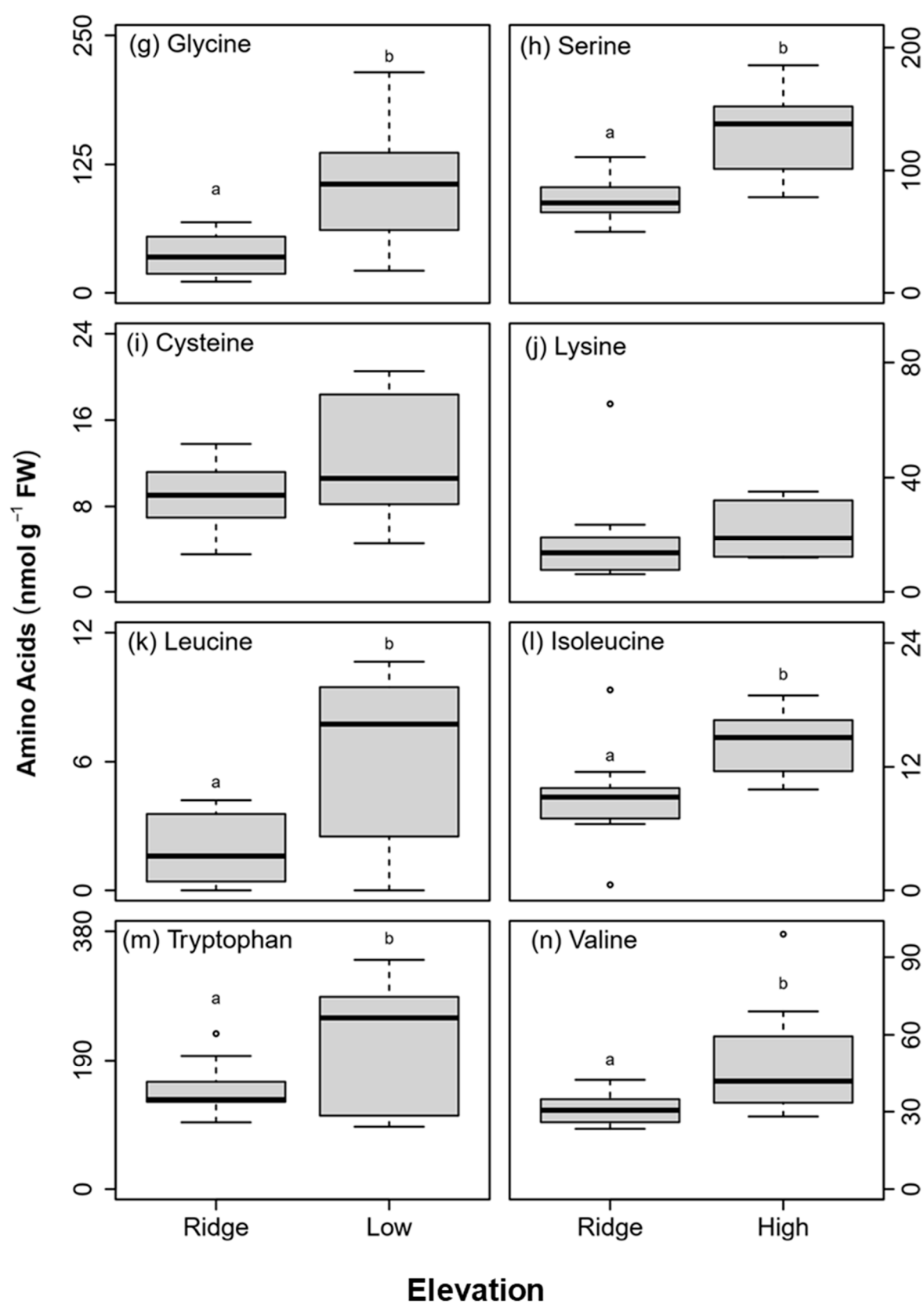

Figure 10. *Cont.*

Figure 10. Foliar free amino acid concentrations in Himalayan spruce along an elevational gradient of 2649 to 2371 m at Kufri Mountain in the foothills of the Northwestern Himalayas in 2009. Data are presented as box and whisker plots (n = 10). Letters represent significant differences between sites ($p \leq 0.05$).

4. Discussion

Mountain environments typically have shorter growing seasons and are generally subject to harsh environmental conditions such as higher UV radiation, lower temperatures, higher levels of precipitation, and lower nutrient availability resulting from limited soil development. These factors make montane ecosystems more sensitive to environmental disturbances than low elevations [3,4]. In addition to natural factors, mountains tend to receive a greater deposition of air pollutants than low elevations, increasing the risk of harm to these sensitive ecosystems [53]. The evaluation of changes in soil chemistry and foliar metabolism of trees along an elevational gradient has allowed the assessment of the impact of multiple simultaneously acting environmental stressors on the health of foundational tree species in this forested catchment at Kufri Mountain. Both site history and the data from the present study revealed that there was potentially an additional source of N input besides regional pollution via animal grazing at high elevation (just one elevation below the ridge). Therefore, it is possible that grazing contributed to the observed changes in the metabolism of trees growing not only at high elevation but downslope at mid and low elevations as well. Similarly, the presence of an access road with several shops and horseback rides for tourists near the ridge site might have impacted tree metabolism at the ridge differently compared to the other sites. Though grazing within this sanctuary is not an authorized activity by the regional forest administration, there was ample evidence along with the personal observations (made midway through our sampling) of horse-grazing at high elevation. Therefore, the discussion of results from the current study and their interpretation are presented in light of the combined effects of land-use history and the elevational gradient.

The effects of regional pollution (including acid deposition and/or urbanization containing S and N, and ozone) on the metabolism of mature forest trees growing in experimental forests and/or along a natural elevational gradient has been observed previously in the Northeastern U.S. [20,21,25,33,54–57] and around the globe [58–61]. In addition, regional pollution is known to have species-specific effects on the metabolism of conifer and hardwood trees [21,23,25,54]. The present study is the first one of its kind from this area that observed the effects of N inputs from regional pollution (known to vary along an elevational gradient) and potentially from grazing (human land-use patterns) localized only at high elevations on soil chemistry, and the metabolism of Himalayan cedar and Himalayan spruce.

4.1. Soil

The effects of pollution often include soil acidification if the geologic substrate does not provide sufficient acid-buffering capacity. Steep slopes, high levels of precipitation, and pollutant deposition increase the susceptibility of soils to become acidified. Soil acidification in montane environments generally increases with increasing elevation [4,62] as well as with increasing depth in the soil profile [63,64]. However, the variations of soil chemistry measured at the four elevations at Kufri did not fit these patterns. The variability in pH and base saturation, two measurements that reflect acidification status, was minimal among elevations and soil horizons, with the noteworthy exception of the mid-elevation site. The lack of variation at the low, high, and ridge sites occurred because the soils at these sites were able to nearly fully neutralize all sources of strong acids and had achieved a stable balance between geologic, biologic, and atmospheric controls of weak acidity generated by inorganic C that originates from underlying carbonate sediments [34].

Consistent soil pH and base saturation throughout much of the watershed reflects a highly acid-buffered system that overrides the effects of elevation range, steep slopes, and high precipitation; factors that all work to create spatial patterns in soil acid-base chemistry related to hillslope position and soil depth. The high acid-buffering capacity is the result of the complex sedimentary geology of the region around Kufri that includes multiple carbonate-rich formations that generated remarkably similar acid-buffering in soils at three of the four sampling sites [34]. However, there was a significant amount of

acidification in the A horizon at the mid-elevation site. Low-buffering soil parent material that was present only at the mid-elevation may have led to soil acidification under the high levels of pollutant deposition that presumably fell throughout the watershed [17,18,65]. The compacted B2 horizon at this elevation relative to the B2 horizon of the low-elevation site also suggests that the upper part of the mid-elevation soil profile was subject to greater soil water flux and therefore greater leaching than the low-elevation site. High subsurface water flux through the upper mineral soil at the mid-elevation site, may have accelerated leaching of the A and B1 horizons, atypical of the other elevation sites. The influence of subsurface flow paths on patterns of soil variations has been documented in hillslope watersheds in North America [66,67], although we are not aware of similar studies being conducted in the steep, carbonate watersheds of the lesser Himalaya. However, there were no apparent differences in subsurface hydrologic conditions between the mid- and low-elevation sites to explain the difference in acidification levels in late October during the time of sampling, which is a seasonally dry period in this area.

Increases in TN, TC, Mg, and P in the organic soil horizon at high elevation, and in some cases at mid and low elevation, were possibly the impact of liquid and solid wastes from the animal-grazing activity nearby [68,69]. Unlike the nutrient-depleted soils in the Northeastern U.S. and many of the European forests, where the addition of N as fertilizer or acidic inputs cause an increase in soil Mn and Al accompanied by a decrease in Ca [57], in the present study there was no change in soil Al or Mn with a significant increase in N. This was also a reflection of the high acid-buffering capacity of the soils at Kufri, which provides sufficient Ca to maintain the soil pH at levels that are only slightly acidic. These soils had N (%) concentrations that were at least 20-fold lower and C (%) 7–10-fold lower than the soils in Northeastern U.S. forests [e.g., Harvard Forest, MA, and Hubbard Brook Experimental Forest, NH, both a part of The Long-Term Ecological Research (LTER) Network in the United States]. For some tree species, the soils at this site may be limited in both N and C as shown by lower soil C concentrations. In general, except for the mid-elevation site, several elements decreased with depth through the soil. The highest % N, % C, Al, Mn, and P accompanied with the lowest pH and Ca in the A horizon at mid-elevation suggested that the soil at this site was most affected by the possible N leaching from the animal activity that occurred above at high elevation.

4.2. Himalayan Cedar

Overall conditions for cedar were seemingly better at high elevation with the input of N from animal activity as shown by an increase in growth-related variables (putrescine, spermidine, total chlorophyll). The absence of any indication of stress from excess N, e.g., an increase in putrescine with little or no change in spermidine along with an increase in N-pathway related amino acids (glutamine, arginine, Pro, GABA, Ala, and Orn) at this elevation also support this conclusion.

In low pH soils of forests around the world, high concentrations of soluble Al often cause leaching, and subsequent deficiency of Ca [70]. If mineral weathering replaces the leached base cations at a slower rate than their loss, then trees can encounter nutrient deficiencies [71,72]. Under these conditions, especially when Ca is deficient, trees become more vulnerable to other stresses, as has been observed in studies with sugar maple (*Acer saccharum*) and red spruce (*Picea rubens*) [23,57,73–75]. That was not the case at this site where the changes in soil chemistry at high elevation might have led to a significant decrease in foliar soluble Al, Mn and an increase in K without any effect on Ca, Mg, and P. Comparatively, soils at Kufri have a higher pH and high concentrations of Ca, conditions that often do not negatively impact foliar metabolism because of higher soil buffering capacity. This is also evident in the present study from the observations that the increase in putrescine at high elevation was not accompanied by a decrease in Ca, which is often observed in cases where soils have higher pH and are Ca deficient [20,21,57]. Instead, the simultaneous increase both in putrescine and spermidine indicated better growth conditions for this species with the increase in N, indicating potential N limitation

for cedar growing at this site. Polyamines are required for growth and development via involvement in a multitude of physiological functions in plants and have also been considered a reliable indicator of stress responses of plants [22]. Similar observations were made with sugar maple trees after chronic exposure to N fertilization at Bear Brook Watershed in Maine, U.S. [25]. Most amino acids (10 out of 14) that were quantified were in the highest concentrations at high elevation indicating the assimilation of additional N into amino acids. In addition to their physiological roles, increased polyamines and amino acids can also be used as reusable N storage metabolites in plants when excess N is available [25,54,76]. At high elevation relative to mid and low elevations, some of this N was also used in the production of higher concentrations of soluble proteins. These proteins contain up to 50% RuBisCO needed in photosynthesis [77]. An increase in the ratios of chlorophyll a:b at high elevation indicated the higher intensity of light at this elevation relative to lower elevations. An increase in light intensity has been shown to degrade chlorophyll b, which results in the production of more chlorophyll a thus leading to an increase in the ratio of chlorophyll a:b [78]. Cedar trees at high elevation could be experiencing changes similar to those observed in Arabidopsis [31] where a genome-wide reprogramming of primary and secondary metabolism in response to N led to increased protein synthesis and improved growth and development.

The increase in spermidine and spermine along with the precursor of polyamines (i.e., ornithine-an uncommon non-proteinogenic amino acid) at mid-elevation indicated possible downstream effects of the grazing activity at high elevation. Furthermore, high and mid were the elevations that had significantly higher organic soil ECEC and LOI. Since the soils at this site may have been C limited, it is conceivable that the significant increase simultaneously in both C and N at the high elevation from animal inputs may have led to an increase in a large suite of metabolic changes. No direct growth data were available to us for this site but based on all the metabolic differences we observed; we hypothesize that these differences indicate better growth conditions for cedar.

4.3. Himalayan Spruce

Contrary to cedar, spruce was present only at the ridge and low elevations on a slightly north-facing aspect of the mountain, but not far from where cedar trees were sampled. At higher altitudes, a combination of natural environmental factors limits the distribution of certain species. Additionally, higher elevations are especially prone to exposure to higher levels of air pollutants, which can disproportionately elevate stress in many marginally functional ecosystems, which can be detected by the use of metabolic markers [53]. As mentioned earlier, tourism is the main attraction at this site, which is not far from the ridge sampling site where we found some predictable land-use changes as described in Boavida-Portugal et al. [79]. In contrast, the low-elevation site was in a pristine area located down a very steep decline from the ridge. Relatively higher chlorophyll concentrations and lower soluble proteins at low elevation indicate a significantly lower level of stress at low versus ridge elevations. An increase in several amino acids without any changes in other stress indicators, such as high levels of putrescine without any change in spermidine, also indicated that N was being invested into productivity at the lower elevation. A lack of difference in nutrient chemistry or polyamines between the two elevations could be the result of inputs from tourist activities into the soil at the ridge. Changes in nutrient inputs with inhabitation of land have previously been observed under conditions of urbanization [33]. Again, in lieu of actual growth measurements, our metabolic data suggest that the spruce growing at low elevation is less stressed and more productive than those at the ridge top.

5. Conclusions

The soils at Kufri Mountain, including those at the ridge site, were higher in pH and Ca than soils of the Northeastern U.S. that have been strongly impacted by acidic deposition, with the notable exception of the mid-elevation site. Consequently, soils generally had a higher buffering capacity to withstand environmental acidic deposition. However, waste from horse grazing at high elevation possibly led to significantly higher inputs of N and C in soils at this site. These changes in soil chemistry, in turn, changed the primary and secondary metabolism of cedar trees. The metabolic data suggest that there were possible C and N limitations, and the inputs from grazing resulted in better growth conditions for the cedar trees at high elevation. For cedar, there were more similarities in the metabolism of trees growing at high and mid-elevation sites possibly due to the subsurface flow of animal wastes generated at high elevation that to some extent affected the soils downslope. Soil chemistry and foliar metabolites in cedar were comparatively more similar at the ridge and low-elevation sites. Despite the apparent alleviation of nutrient limitations at the high and mid-elevation sites, N concentrations may someday reach above a system threshold, at which point there could be severe negative impacts of grazing-induced N-fertilization on cedar health. Metabolic data imply that, for spruce, growth conditions were more favorable at low elevation relative to the ridge. The thresholds and tolerance limits for spruce are different to those for cedar and, in general, spruce is a genus known to be sensitive to pollution.

References

1. Körner, C.; Jetz, W.; Paulsen, J.; Payne, D.; Rudmann-Maurer, K.; Spehn, E.M. A global inventory of mountains for bio-geographical applications. *Alp. Bot.* **2017**, *127*, 1–15. [CrossRef]
2. Beniston, M.; Haeberli, W. Sensitivity of mountain regions to climatic change. In *Climate of the 21st Century: Changes and Risks*; Lozan, J., Grassl, H.L., Hupfer, P., Eds.; GEO Publications: Hamburg, Germany, 2001.
3. Johnson, C.E.; Driscoll, C.T.; Siccama, T.G.; Likens, G.E. Element Fluxes and Landscape Position in a Northern Hardwood Forest Watershed Ecosystem. *Ecosystems* **2000**, *3*, 159–184. [CrossRef]
4. Bailey, S.W.; Horsley, S.B.; Long, R.P.; Hallett, R.A. Influence of Edaphic Factors on Sugar Maple Nutrition and Health on the Allegheny Plateau. *Soil Sci. Soc. Am. J.* **2004**, *68*, 243–252. [CrossRef]
5. Borgaonkar, H.P.; Sabin, T.P.; Krishnan, R. Deciphering climate variability over western Himalaya using instrumental and tree-ring records. In *Himalayan Weather and Climate and Their Impact on the Environment*; Dimri, A.P., Bookhagen, B., Stoffel, M., Yasunari, T., Eds.; Springer Nature Switzerland AG: Cham, Switzerland, 2020; pp. 205–238.
6. Wu, J.; Xu, Y.; Gao, X.-J. Projected changes in mean and extreme climates over Hindu Kush Himalayan region by 21 CMIP5 models. *Adv. Clim. Chang.* **2017**, *8*, 176–184. [CrossRef]
7. Sohar, K.; Altman, J.; Lehečková, E.; Doležal, J. Growth–climate relationships of Himalayan conifers along elevational and latitudinal gradients. *Int. J. Clim.* **2017**, *37*, 2593–2605. [CrossRef]
8. Borgaonkar, H.P.; Ram, S.; Sikder, A.B. Assessment of tree-ring analysis of high-elevation *Cedrus deodara* D. Don from Western Himalaya (India) in relation to climate and glacier fluctuations. *Dendrochronologia* **2009**, *27*, 59–69. [CrossRef]
9. Kumar, S.; Kumar, A.; Kumar, R. Himalayan (Himachal region) cedar wood (*Cedrus deodara: Pinaceae*) essential oil, its processing, ingredients and uses: A review. *J. Pharmacogn. Phytochem.* **2019**, *8*, 2228–2238.
10. Vidyarthi, S.; Samant, S.S.; Sharma, P. Traditional and indigenous uses of medicinal plants by local residents in Himachal Pradesh, North Western Himalaya, India. *Int. J. Biodivers. Sci. Ecosys. Serv. Manag.* **2013**, *9*, 185–200. [CrossRef]
11. Gairola, S.; Sharma, C.M.; Suyal, S.; Ghildiyal, S.K. Composition and diversity of five major forest types in moist temperate climate of the Western Himalayas. *For. Stud. China* **2011**, *13*, 139. [CrossRef]
12. Kumar, A.; Singh, K.; Lal, B.; Chawla, A.; Uniyal, S.; Kaushal, R.; Singh, R.; Ahuja, P. Regeneration pattern of tree species in forested landscapes of Himachal Pradesh, Western Himalayan region, India. *Proc. Natl. Acad. Sci. India B* **2014**, *84*, 695–707. [CrossRef]
13. Rawat, B.S. Variability in cone and seed characteristics and seed testing in various provenances of Himalayan spruce (*Picea smithiana*). *J. For. Res.* **2011**, *22*, 603–610. [CrossRef]
14. Dimri, S.; Baluni, P.; Mohan, S.C. Growing stock of various pure conifer forest types of Central (Garhwal) Himalaya, India. *Int. J. Curr. Res. Rev.* **2014**, *6*, 45–49.
15. Beniston, M.; Diaz, H.F.; Bradley, R.S. *Climatic Change at High Elevation Sites: An Overview*; Springer: Dordrecht, The Netherlands, 1997; Volume 36, pp. 233–251.
16. Khan, S.; Moinuddin, A.; Syed, S. Climatic signal in tree-ring chronologies of *Cedrus deodara* from Chitral Hindukush Range of Pakistan. *Geochronometria* **2013**, *40*, 195. [CrossRef]
17. Ganguly, R.; Thapa, S. An assessment of ambient air quality in Shimla city. *Curr. Sci.* **2016**, *111*, 509–516. [CrossRef]
18. Ganguly, R.; Sharma, D.; Kumar, P. Trend analysis of observational PM10 concentrations in Shimla city, India. *Sustain. Cities Soc.* **2019**, *51*, 101719. [CrossRef]
19. Chen, J.; Zhao, H.; Gao, L.; Henkelmann, B.; Schramm, K.-W. Atmospheric PCDD/F and PCB levels implicated by pine (*Cedrus deodara*) needles at Dalian, China. *Environ. Pollut.* **2006**, *144*, 510–515. [CrossRef]
20. Minocha, R.; Shortle, W.C.; Lawrence, G.B.; David, M.B.; Minocha, S.C. Relationships among foliar chemistry, foliar polyamines, and soil chemistry in red spruce trees growing across the northeastern United States. *Plant Soil* **1997**, *191*, 109–122. [CrossRef]
21. Minocha, R.; Long, S.; Thangavel, P.; Minocha, S.C.; Eagar, C.; Driscoll, C.T. Elevation dependent sensitivity of northern hardwoods to Ca addition at Hubbard Brook Experimental Forest, NH, USA. *For. Ecol. Manag.* **2010**, *260*, 2115–2125. [CrossRef]
22. Minocha, R.; Majumdar, R.; Minocha, S.C. Polyamines and abiotic stress in plants: A complex relationship. *Front. Plant Sci.* **2014**, *5*, 175. [CrossRef]
23. Schaberg, P.G.; Minocha, R.; Long, S.; Halman, J.M.; Hawley, G.J.; Eagar, C. Calcium fertilization at the Hubbard Brook Experimental Forest increases the capacity for stress tolerance and carbon capture in red spruce (*Picea rubens*) trees during the cold season. *Trees* **2011**, *25*, 1053–1061. [CrossRef]
24. Wuddineh, W.; Minocha, R.; Minocha, S.C. Polyamines in the context of metabolic networks. In *Polyamines: Methods and Protocols*; Alcázar, R., Tiburcio, A.F., Eds.; Springer: New York, NY, USA, 2018; pp. 1–23.
25. Minocha, R.; Long, S.; Turlapati, S.A.; Fernandez, I. Dynamic species-specific metabolic changes in the trees exposed to chronic N+S additions at the Bear Brook Watershed in Maine, USA. *Ann. For. Sci.* **2019**, *76*, 25. [CrossRef]
26. Mekonnen, D.W.; Flugge, U.I.; Ludewig, F. Gamma-aminobutyric acid depletion affects stomata closure and drought tolerance of Arabidopsis thaliana. *Plant Sci.* **2016**, *245*, 25–34. [CrossRef] [PubMed]
27. Parankusam, S.; Adimulam, S.S.; Bhatnagar-Mathur, P.; Sharma, K.K. Nitric Oxide (NO) in Plant Heat Stress Tolerance: Current Knowledge and Perspectives. *Front. Plant Sci.* **2017**, *8*, 1582. [CrossRef]
28. Guan, C.; Cui, X.; Liu, H.-Y.; Li, X.; Li, M.-Q.; Zhang, Y.-W. Proline biosynthesis enzyme genes confer salt tolerance to Switchgrass (*Panicum virgatum* L.) in cooperation with polyamines metabolism. *Front. Plant Sci.* **2020**, *11*, 46. [CrossRef] [PubMed]

29. Page, A.F.; Cseke, L.J.; Minocha, R.; Turlapati, S.A.; Podila, G.K.; Ulanov, A.; Li, Z.; Minocha, S.C. Genetic manipulation of putrescine biosynthesis reprograms the cellular transcriptome and the metabolome. *BMC Plant Biol.* **2016**, *16*, 113. [CrossRef] [PubMed]

30. Mohapatra, S.; Minocha, R.; Long, S.; Minocha, S.C. Transgenic manipulation of a single polyamine in poplar cells affects the accumulation of all amino acids. *Amino Acids* **2010**, *38*, 1117–1129. [CrossRef] [PubMed]

31. Scheible, W.-R.; Morcuende, R.; Czechowski, T.; Fritz, C.; Osuna, D.; Palacios-Rojas, N.; Schindelasch, D.; Thimm, O.; Udvardi, M.K.; Stitt, M. Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. *Plant Physiol.* **2004**, *136*, 2483–2499. [CrossRef]

32. Perchlik, M.; Tegeder, M. Leaf amino acid supply affects photosynthetic and plant nitrogen use efficiency under nitrogen stress. *Plant Physiol.* **2018**, *178*, 174–188. [CrossRef]

33. McDermot, C.R.; Minocha, R.; D'Amico, V.; Long, S.; Trammell, T. Red maple (*Acer rubrum* L.) trees demonstrate acclimation to urban conditions in deciduous forests embedded in cities. *PLoS ONE* **2020**, *15*, e0236313. [CrossRef]

34. Kumar, R.; Brookfield, M.E. Sedimentary environments of the Simla group (Upper Precambrian), lesser Himalaya, and their palaeotectonic significance. *Sediment. Geol.* **1987**, *52*, 27–43. [CrossRef]

35. Kumar, A.; Lal, B.; Subramani, R.; Chawla, A.; Kaushal, R. Landscape mapping and tree diversity assessment of Pangi valley: A remote tribal area of Himachal Pradesh in Western Himalaya, India. *Int. J. Conserv. Sci.* **2013**, *4*, 503–508.

36. Mesaik, A.; Nazim, K.; Siddiqui, M.; Wahab, M.; Khan, N.; Khan, N.; Hussain, S. Community description of Deodar forests from Himalayan range of Pakistan. *Pak. J. Bot.* **2010**, *42*, 3091–3102.

37. Sharma, A.; Prashar, B.; Arorat, P. *Cedrus deodara*: A medicinal herb. *Int. J. Curr. Res. Rev.* **2018**, *10*, 65758–65762.

38. Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R.; Simons, A. *Agroforestree Database: A Tree Reference and Selection Guide, Version 4.0*; World Agroforestry Centre ICRAF: Nairobi, Kenia, 2009; Available online: http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp (accessed on 26 March 2021).

39. Slathia, P.S.; Bhagat, G.R.; Singh, S.; Kher, S.K.; Paul, N. Traditional knowledge on utility of *Cedrus deodara* (Roxb.) Loud. in Doda district of Jammu province. *Indian J. Tradit. Knowl.* **2007**, *6*, 518–520.

40. Schoeneberger, P.J.; Wysocki, D.; Benham, E.C.; Broderson, W.D. (Eds.) *Field Book for Describing and Sampling Soils, Version 2.0*; National Soil Survey Center: Lincoln, NE, USA, 2002.

41. Fernandez, I.J. Preliminary protocols for sampling and analysis of ash and sludge amended forest soils. *Maine Agric. Exp. Stn. Bull.* **1998**, *818*, 1–24.

42. Robarge, W.P.; Fernandez, I.J. *Quality Assurance Methods Manual for Laboratory Analytical Techniques*; US Environmental Protection Agency, US Department of Agriculture, Forest Service: Washington, DC, USA, 1987.

43. Zimmerman, C.F.; Keefe, C.W.; Bashe, J. *Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis, USEPA Method 440.0*; EPA/600/R-15/009; U.S. Environmental Protection Agency: Washington, DC, USA, 1997.

44. Minocha, R.; Martinez, G.; Lyons, B.; Long, S. Development of a standardized methodology for the quantification of total chlorophyll and carotenoids from foliage of hardwood and conifer tree species. *Can. J. For. Res.* **2009**, *39*, 849–861. [CrossRef]

45. Jones, C.G.; Daniel Hare, J.; Compton, S.J. Measuring plant protein with the Bradford assay. *J. Chem. Ecol.* **1989**, *15*, 979–992. [CrossRef] [PubMed]

46. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]

47. Littell, R.C.; Henry, P.R.; Ammerman, C.B. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* **1998**, *76*, 1216–1231. [CrossRef]

48. Pinhiero, J.; Bates, D. *Mixed Effects Models in S and S-Plus*; Springer: New York, NY, USA, 2000.

49. Zuur, A.F.; Iena, E.N.; Walker, N.; Saveliev, A.A.; Smith, G.M. *Mixed Effects Models and Extensions in Ecology With R*; Springer: New York, NY, USA, 2009.

50. Pinheiro, J.; Bates, D.; DebRoy, S.; Sarkar, D. R Core Team. nlme: Linear and nonlinear mixed effects models. R package version 3.1-128. *Bioinformatics* **2016**, *26*, 1–98.

51. Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric models. *Biom. J.* **2008**, *50*, 346–363. [CrossRef] [PubMed]

52. Oksanen, J.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; Henry, M.; Stevens, H.; et al. *Vegan: Community Eeology Package. R Package Version*; R Foundation for Statistical Computing: Vienna, Austria, 2010; pp. 2–3.

53. Grill, D.; Pfanz, H.; Lomsky, B.; Bytnerowicz, A.; Grulke, N.E.; Tausz, M. Physiological responses of trees to air pollutants at high elevation sites. In *Plant Responses to Air Pollution and Global Change*; Omasa, K.N.I., De Kok, L.J., Eds.; Springer: Tokyo, Japan, 2005; pp. 37–44.

54. Minocha, R.; Turlapati, S.A.; Long, S.; McDowell, W.H.; Minocha, S.C. Long-term trends of changes in pine and oak foliar nitrogen metabolism in response to chronic nitrogen amendments at the Harvard Forest, MA. *Tree Physiol.* **2015**, *35*, 894–909. [CrossRef]

55. Verrico, B.M.; Weiland, J.; Perkins, T.D.; Beckage, B.; Keller, S.R. Long-term monitoring reveals forest tree community change driven by atmospheric sulphate pollution and contemporary climate change. *Divers. Distrib.* **2020**, *26*, 270–283. [CrossRef]

56. Riikonen, J.; Kivimäenpää, M.; Ossipov, V.; Saunier, A.; Marquardt, P. Metabolite composition of paper birch buds after eleven growing seasons of exposure to elevated CO_2 and O_3. *Forests* **2020**, *11*, 330. [CrossRef]

57. Wargo, P.M.; Minocha, R.; Wong, B.L.; Long, R.P.; Horsley, S.B.; Hall, T.J. Measuring changes in stress and vitality indicators in limed sugar maple on the Allegheny Plateau in north-central Pennsylvania. *Can. J. For. Res.* **2002**, *32*, 629–641. [CrossRef]
58. Matyssek, R.; Wieser, G.; Nunn, A.J.; Löw, M.; Then, C.; Herbinger, K.; Blumenröther, M.; Jehnes, S.; Reiter, I.M.; Heerdt, C.; et al. How sensitive are forest trees to ozone? New research on an old issue. In *Plant Responses to Air Pollution and Global Change*; Omasa, K.N.I., De Kok, L.J., Eds.; Springer: Tokyo, Japan, 2005; pp. 29–35.
59. Nihlgard, B. The ammonium hypothesis: An additional explanation to the forest dieback in Europe. *Ambio* **1985**, *14*, 2–8.
60. Ericsson, A.; Walheim, M.; Norden, L.G.; Näsholm, T. Concentrations of mineral nutrients and arginine in needles of *Picea abies* trees from different areas in southern Sweden in relation to nitrogen deposition and humus form. *Ecol. Bull.* **1995**, *44*, 147–157.
61. Näsholm, T.; Nordin, A.; Edfast, A.B.; Hogberg, P. Identification of coniferous forests with incipient nitrogen saturation through arginine and nitrogen-15 abundance in trees. *J. Environ. Qual* **1997**, *26*, 302–309. [CrossRef]
62. Lawrence, G.B.; Fuller, R.D.; Driscoll, C.T. Spatial relationships of aluminum chemistry in the streams of the Hubbard Brook Experimental Forest, New Hampshire. *Biogeochemistry* **1986**, *2*, 115–135. [CrossRef]
63. Lawrence, G.B.; Sullivan, T.J.; Burns, D.A.; Bailey, S.W.; Cosby, B.J.; Dovciak, M.; Ewing, H.A.; McDonnell, T.C.; Minocha, R.; Quant, J.; et al. Acidic deposition along the Appalachian Trail corridor and its effects on acid-sensitive terrestrial and aquatic resources: Results of the Appalachian Trail MEGA-transect atmospheric deposition effects study. In *Natural Resource Report NPS/NRSS/ARD/NRR—2015/996*; National Park Service: Fort Collins, CO, USA, 2015.
64. Fraser, O.L.; Bailey, S.W.; Ducey, M.J. Decadal change in soil chemistry of northern hardwood forests on the White Mountain National Forest, New Hampshire, USA. *Soil Sci. Soc. Am. J.* **2019**, *83*, S96–S104. [CrossRef]
65. Ganguly, R.; Kumar, P. The air quality assessment of northern hilly city in India. *Int. J. Environ. Prot.* **2018**, *38*, 983–997.
66. Sabzevari, T.; Noroozpour, S. Effects of hillslope geometry on surface and subsurface flows. *Hydrogeol. J.* **2014**, *22*, 1593–1604. [CrossRef]
67. Weiler, M.; McDonnell, J.J.; Tromp-van Meerveld, I.; Uchida, T. Subsurface Stormflow. In *Encyclopedia of Hydrological Sciences*; McDonnell, J.J., Anderson, M.G., Eds.; John Wiley & Sons Ltd: Hoboken, NJ, USA, 2006.
68. Chastain, J.P.; Moore, K.P. Plant nutrient and carbon content of equine manure as influenced by stall management in South Carolina. In Proceedings of the American Society of Agricultural and Biological Engineering and Canadian Society for Bioengineering Joint Meeting, Montreal, QC, Canada, 13–16 July 2014; ASABE: St. Joseph, MI, USA, 2014; pp. 1–12.
69. Sugino, T.; Sumarini, N.; Suwandi, R.; Rosliani, D.; Setyorini, W.; Hartatik, R.; Saraswati. *Horse Manure Can Replace Half the Amount of Conventional Chemical Fertilizer Application Without Any Yield Loss in West Java Highland*; Japan International Research Center for Agricultural Sciences (JIRCAS): Tsukuba, Japan, 2015; *Research Highlight*. Available online: https://www.jircas.go.jp/en/publication/proceedings/2015 (accessed on 26 March 2021).
70. Shortle, W.C.; Smith, K.T. Aluminum-induced calcium deficiency syndrome in declining red spruce. *Science* **1988**, *240*, 1017–1018. [CrossRef]
71. Johnson, D.W.; Fernandez, I.J. Soil-mediated effects of atmospheric deposition on eastern U.S. spruce-fir forests. In *Ecology and Decline of Red Spruce in the Eastern United States*; Eagar, C., Adams, M.B., Eds.; Springer: New York, NY, USA, 1992; pp. 235–270.
72. Driscoll, C.T.; Driscoll, K.M.; Mitchell, M.J.; Raynal, D.J. Effects of acidic deposition on forest and aquatic ecosystems in New York State. *Environ. Pollut.* **2003**, *123*, 327–336. [CrossRef]
73. Long, R.P.; Horsley, S.B.; Lilja, P.R. Impact of forest liming on growth and crown vigor of sugar maple and associated hardwoods. *Can. J. For. Res.* **1997**, *27*, 1560–1573. [CrossRef]
74. Schaberg, P.G.; DeHayes, D.H.; Hawley, G.J. Anthropogenic calcium depletion: A unique threat to forest ecosystem health? *Ecosys. Health* **2001**, *7*, 214–228. [CrossRef]
75. Hallett, R.A.; Bailey, S.W.; Horsley, S.B.; Long, R.P. Influence of nutrition and stress on sugar maple at a regional scale. *Can. J. For. Res.* **2006**, *36*, 2235–2246. [CrossRef]
76. Chen, H.; Zheng, Y.; Zhan, J.; He, C.; Wang, Q. Comparative metabolic profiling of the lipid-producing green microalga Chlorella reveals that nitrogen and carbon metabolic pathways contribute to lipid metabolism. *Biotechnol. Biofuels* **2017**, *10*, 153. [CrossRef]
77. Ellis, R.J. The most abundant protein in the world. *Trends Biochem. Sci.* **1979**, *4*, 241–244. [CrossRef]
78. Horie, Y.; Ito, H.; Kusaba, M.; Tanaka, R.; Tanaka, A. Participation of chlorophyll b reductase in the initial step of the degradation of light-harvesting chlorophyll a/b-protein complexes in *Arabidopsis*. *J. Biol. Chem* **2009**, *284*, 7449–17456. [CrossRef]
79. Boavida-Portugal, I.; Rocha, J.; Ferreira, C.C. Exploring the impacts of future tourism development on land use/cover changes. *Appl. Geogr.* **2016**, *77*, 82–91. [CrossRef]