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Original article

Biomass Allocation of *Xanthium strumarium* L.: A key factor for invasive success at high elevation

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ABSTRACT

This study aims to investigate the importance of biomass allocation in the invasive success of *Xanthium strumarium*, an alien invasive species across an elevation gradient in Pakistan. This plant was selected as a model due to its lowland invasion capacity and going up the elevation gradient. Sites were sampled in three different elevation groups, $\leq$ 200m, $\leq$ 600m, and $\leq$ 1000m while $\geq$ 1400m above sea level, to analyze *X. strumarium* biomass allocation, related environmental variables, and community diversity. Root/Below ground, stems, leaves, fruits, total dry biomass and, its ratios were found to vary with elevation ($P < 0.05$). The total dry biomass and density of the species had a significant negative effect on community diversity, where the crown cover was the main contributing factor to total biomass accumulation. Environmental factors such as elevation, sand, clay, nitrogen, organic matter, phosphorus, and pH were most strongly correlated in CCA-ordination, which indicates that several topographic, edaphic, and soil variables influence the spatial pattern of biomass allocation in this invasive species. It was concluded that *X. strumarium* invasion continues at a higher elevations, adapting to new environmental conditions, particularly reproductive dry biomass.

Keywords: dry biomass, community diversity, environmental variables, performance efficiency

Introduction

Recently, concerns about the possible invasion hazards posed by alien species and the ensuing effect on biodiversity have been increased dramatically (Liu *et al*. 2020; Shackleton *et al*. 2020). Several studies focused on the dynamics of invasive species' to understand the invasion process and its mechanism. The invasion depends on the plant species' ability to modify itself for improved fitness in a new habitat, which has been poorly understood and proven compared to non-invasive plant species (Clements & Ditommaso 2011). Invasive plants are considered triggers for causing ecosystem biodiversity disruption with changes in floristic composition and habitat fragmentation that is often suppressive to native plant species (Dutta *et al*. 2021). Invasive plants are considered intensively and extensively for manageable control because of their long-term damage to the ecological

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and economical system (Gallardo *et al*. 2016). Identifying the traits that assist in invasion success, classification, and the various life forms may be considered when assessing economic losses caused by invading species (Pyšek *et al*. 2009).

Generally three major ecological mechanisms function as successive filters affecting invasion outcomes (Richardson & Pyšek 2012). The species must spread (the first filter) into the appropriate habitat (the second filter) and manage to develop biotic interactions in the recipient community (the third filter). Dispersal, *e.g.*, propagules pressure (Simberloff 2009), abiotic environment, *e.g.*, climatic matching (Thuiller *et al*. 2005), and biotic interactions, *e.g.*, enemy release (Mitchell *et al*. 2006), are all important drivers of biological invasion, according to previous studies (Higgins *et al*. 2000; Roura-Pascual *et al*. 2010). It is unclear if competitive interspecific interactions, in particular, play a role in successful invasion and how they may interact with environmental gradients (Simberloff 2006).

Biomass structure, environmental degradation, and changes in species diversity are the major causes that enable invasive plant species to establish in a new habitat despite control strategies (Dwire *et al*. 2004). Plant biomass investment in reproduction and growth provide requirements for regeneration (Bazzaz & Reekie 1985). Increased biomass productivity of invasive plant species is an essential feature that can enhance the establishment and promulgation of invasive species populations in new habitats across elevation gradients (Ordonez *et al*. 2010). It is generally believed that invasive plant species grow faster and healthier than native plants and can outcompete and diminish the native flora and, eventually, biodiversity (Hsu & Kao 2014). Biomass can provide a key to the growth of different plant parts and their role in plant propagation and reproduction (Ordonez *et al*. 2010). Biomass production results from normal plant growth and development that provides information about the success of invasion and, later on, disturbance of the plant community, which usually results in loss of biodiversity and crop production that may impact a country's economy and export (Coleman *et al*. 1994).

Most plant invasion research has been conducted in lowland areas from 0 to 500 m (*a.s.l.*) (Najberek *et al*. 2020), and high-altitude areas have been assumed to have fewer anthropogenic activities, and harsh environments and are therefore more capable of resisting subtropical invaders (Pauchard *et al*. 2009). In this context, elevation is a significant indicator of microclimate changes affecting the invasion of plants (Wilson *et al*. 1992). In addition, fewer resources, shorter growing seasons, reduced microbial activity, and a lower human population further make the habitats resistant to invaders (Pauchard *et al*. 2009). However, recent globalization and climate change have turned the tide towards invasive species, providing them opportunities to spread and establish in high-altitude regions (Shrestha *et al*. 2019). Consequently, elevation-based alterations may significantly influence the functional characteristics of plant species (Trunschke & Stocklin 2017). The selection of stress-resistant genotypes, progressive exposure to cold resistance and intrinsic hybrids with cold-tolerant species are among the main drivers of alien plant invasion across elevation gradients (Pauchard *et al*. 2009). High biomass production of invasive species in their native habitat may allow them to invade adjacent areas (Van Kleunen *et al*. 2011). Adaptations to the functionality of an alien species support the survival and proliferation in a range of climate and edaphic regimes (Watermann *et al*. 2020). Although plasticity and adaptive characteristics differences are common across invasive plants, they have not yet been studied thoroughly in subtropical invasive species (Colautti *et al*. 2017). For the current study, *Xanthium strumarium* (Asteraceae) has been selected as a model plant since it is one of the most common subtropical invaders globally (Shaheen *et al*. 2019) and has invaded high elevation areas quickly.

Xanthium strumarium is an annual invasive weed plant that originated in North America. In recent years it has spread to most of Europe and Asia, affecting biodiversity and crop production growing along roadsides and grasslands (Xiong *et al*. 2013). This invasive species has recently invaded and spread in the Khyber Pakhtunkhwa province of Pakistan with the movement of Afghan refugees along the roadside and waste fields (Hashim & Marwat 2002). The spiny nature of its fruit allows them to spread far and long-distance quickly and provides an opportunity for the plant species to invade many areas along the elevation gradient where it affects the native plant communities (Marwat *et al*. 2010). Biomass allocation plays an essential role in the effective invasion and adaptability of a species in changing environmental conditions and species diversity, particularly across the elevation gradient because invasive species expand successfully across the mountainous regions (Pauli & Halloy 2019). Examining adaptations to the elevation gradient in invasive species is essential to assess their potential spread. Therefore, this research was designed to examine the changes in biomass allocation and its ratios reproductive and vegetative of *X. strumarium* in Khyber Pakhtunkhwa province of Pakistan across an elevation gradient. The primary aim of the research was to determine a) how the biomass of an invasive species changes in the mountain environment across elevation gradients; b) how the environmental variables and species diversity change with biomass allocation across the elevation gradient. The study was based on the hypothesis that *X. strumarium* progressively colonized the altitudinal zones with variation in biomass across the altitude and allometric biomass ratios allocating strategies for successful invasion and continuously adapting to the new environment across the spatial variation in environmental factors and changes in species diversity.

Materials and Methods

Study site

The study was conducted in three distinct zones of Khyber Pakhtunkhwa, Pakistan, based on the differences in edaphic and species diversity characteristics across elevation gradients. The areas including in the 1st zone (Group I) were situated at an elevation of ≤ 200m above sea level (*a.s.l*), mostly including plain areas of the Peshawar division. The 2nd zone (Group II) at the elevation of ≤ 600 m asl mostly includes transition areas of two-division, *i.e.*, Malakand and Hazara, partly plain and partly hilly. At the same time, the 3rd zone (Group III) lies at an elevation between ≤ 1000 ≥ 1400 m asl and includes hilly areas of the two divisions as mentioned earlier (Fig. 1).

The Environmental Protection Agency (EPA) has classified the province into four agro-ecological zones (Tab. S1). The region is typified by sloping valleys and lowlands surrounded by high mountains, which have a major impact on the south to north and northwest climate. High elevation zones have severe winters and mild summer, with a marked increase in temperature towards the south (Ali *et al*. 2018). The warmest month is June, with mean maximum and minimum temperatures of 35.0±1.4 °C and 19.1±1.5 °C, respectively. The coldest month is January, with mean high and low temperatures of 13.7±1.4 °C and 0.7±0.9 °C, respectively (Rahman & Khan 2013). Precipitation in the province ranges from 384 mm to 639 mm annually, and relative humidity ranges from 54.8±2.2% to 77.3±3.1% (Ali *et al*. 2018). The climate of a region is important when analyzing vegetation structure since it affects economic, social, hydrologic, and agricultural activities (Deo & Sahin 2015).

Ecological and biomass data collection

Field sampling was conducted by selecting fifteen *X. strumarium* dominated sites along the elevation gradient with distinct zonation in spatial variation. Three elevation gradients, *i.e.*, 500 m, 1000 m, and 1500 m *a.s.l*, were identified as suitable for altimetric and allometric variation and community diversity level changes. A total of 150 quadrates in 15 sites were established for collecting phytosociological and biomass data. Geographic coordinates were recorded using GPS, while the elevation was recorded using an altimeter. Floristic data were recorded using the Quadrat method to calculate phytosociological attributes following Pragada & Venkaiah (2012). The size of the quadrate ranges from 3-5 m^2, while edge effects were reduced by taking a buffer zone of 10 m^2 between the quadrates following Call & Nilsen (2003). The plant species found in communities of *X. strumarium* were identified using Flora of Pakistan (Nasir & Ali 1972), and voucher specimens were deposited in the herbarium of the Department of Botany, University of Malakand Khyber Pakhtunkhwa, Pakistan. Biomass of *Xanthium strumarium* was estimated using a destructive method by collecting forty-five plants with fifteen plants from each elevation zone and three plants from each site. The plants collected were washed, divided into different parts, *i.e.*, root (up to a depth of 20-150 cm), stem, leaves, fruits, and flowers, and dried at 70 °C for 48 h and then weighed to calculate the dry biomass (DBM).

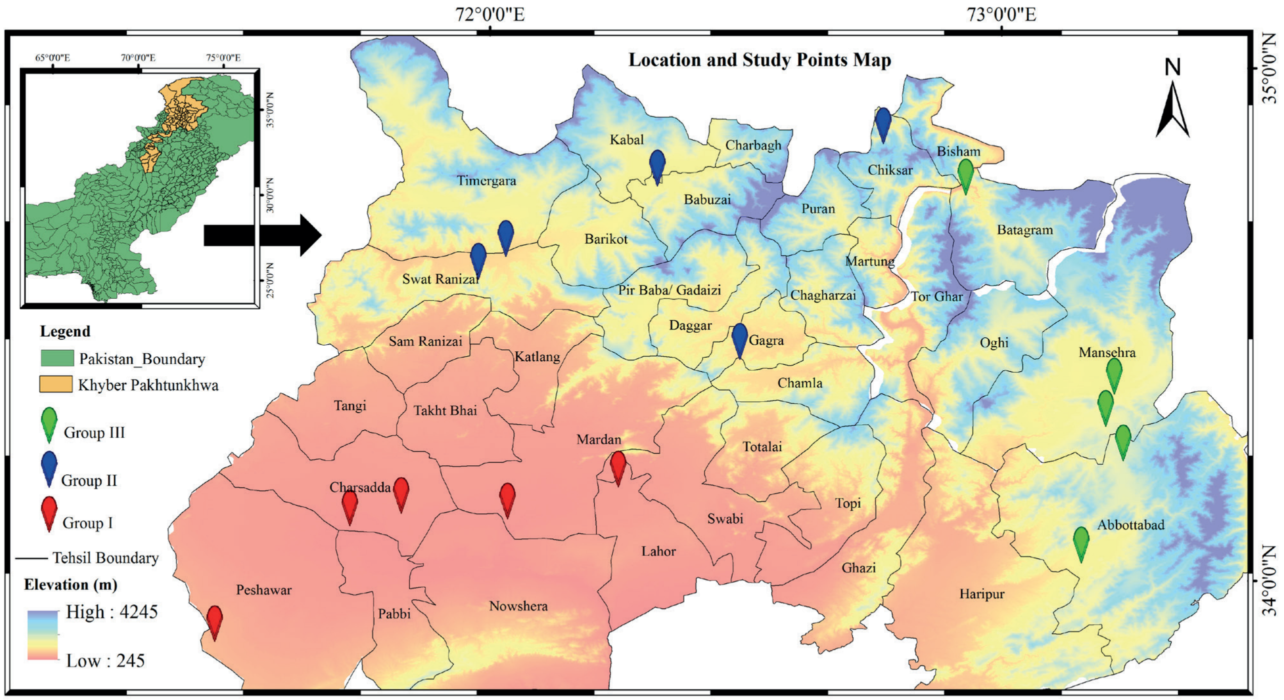

Figure 1. Sampling locations of *Xanthium strumarium* for biomass and corresponding environmental variables in north Pakistan.

The plants were divided into categories, *i.e.*, below-ground biomass (R/BDBM), stem dry biomass (SDBM), Leaves dry biomass (LDBM), Inflorescence dry biomass (IDBM), Fruits dry biomass (FDBM), above-ground biomass (ADBM), Vegetative dry biomass (VDBM), reproductive dry biomass (RDBM) and total dry biomass (TDBM). Standard procedure of Wang *et al.* (2008) was adopted for biomass estimation. In addition, important morphological characteristics were measured using a tape measure, *i.e.*, crown cover (area of the ground occupied by plant) and plant height (Ground or collar to the apex of the stem).

Soil collection and analysis

A total of 15 soil samples at a depth of 0-30 cm, each in three replicates, were collected randomly in each site and divided into three groups based on altitudinal zonation for floristic data and brought to Agriculture Research Institute, Swat (ARI). Soil texture and important nutrients, *i.e.*, NPK, were measured following Yusof *et al.* (2016). Electrical conductivity and pH were determined by following the procedures adopted by Aini *et al.* (2014). Organic matter and lime percentage were determined by following Salehi *et al.* (2011) and Nelson & Sommer (1982). Available water (AW), saturation point (SP), total dissolved solids (TDS), bulk density (BD), and field capacity (FC) were calculated using Saxton & Rawls (2006) online calculator.

Statistical analysis

Ward's agglomerative cluster analysis, joined by Euclidean Pythagorean distance measure, was used to group the sampling sites along the elevation gradient by following Grandin (2006). Descriptive statistics and one-way ANOVA of biomass components (response variable), environmental and edaphic characteristics (predictor variables) were performed. Moreover, Tukey's post hoc tests were used to examine pairwise variation in samples from various elevations. A linear regression models were used to assess the allometric biomass variation between different plant organs and relate allometric variation in biomass. We used linear regression models to establish the relationship between elevation and total dry biomass, crown cover (CC), and plant height (H). Linear regression models were used to investigate *X. strumarium* total dry biomass and density effects on the Shannon-Wiener diversity index. The floristic data were subjected to different diversity indexes, *i.e.*, species richness, Shannon diversity index, Simpson diversity index, and species evenness, to understand the effects of *X. strumarium* biomass and density on species diversity. Canonical Correspondence Analysis (CCA) was used to examine the relation of biomass with environmental variables and identify which factors have an influential effect on biomass distribution (Zuur *et al.* 2007). The CCA was performed using the following options: 1) Environmental and edaphic parameters were taken as response variables 2) Using Hill's method (Hill 1979) 3) taking stand scores linear combination of variables 4) Listed correlation coefficient for second matrix 5) Null hypothesis was tested by opting no relationship between matrices. The analysis was performed in SPSS version 22. PC-ord version 6.0 and Canoco version 4.5 (Xiao *et al.* 2013) was used for cluster and CCA analysis. Statistical significance of the variables was tested at $p < 0.05$.

Results

The two-way cluster depicted in Figure S1 includes all the biomass variables and the ratios with the response variable as elevation in the second matrix. The results demonstrated three distinctly related groups with elevation as the prominent gradient for biomass classification. The three cluster groups are formed at 87.5 % information retrieved from the cluster indicating elevation gradient as the predominant factor in plant total biomass distribution and its different biomass components and ratios.

Biomass allocation along the elevation gradient

The distribution of *X. strumarium* dry biomass decreased with increasing elevation (Fig. 2). The site of Group I, which was at low elevation (348±129 m), shows high biomass of all the parameters, *i.e.*, root/below ground dry biomass, stem dry biomass, leaf dry biomass, inflorescence dry biomass, and fruit dry biomass, compared to Group II and Group III, which were at the middle and upper elevation, *i.e.*, 572±27 m and 1312±79 m respectively. The highest total dry biomass was 144.3±17.93 g for Group I, followed by 95±5.9 g in Group II and 71.78±5.8 g in Group III. The highest biomass was accumulated by stem dry biomass, *i.e.*, 59.4±7.5 g in group I, followed by Group II (31.9±2.6 g) and Group III (24.1±3.3 g). The same pattern was followed by root/below ground dry biomass, leaf dry biomass, inflorescence dry biomass, and fruit dry biomass. Below ground dry biomass/above ground dry biomass ratio was higher in Group II, *i.e.*, 0.16±0.02 g, followed the Group I (0.15±0.009 g) and lowest for Group III plants, *i.e.*, 0.11±0.003 g. The non-significant cases were for inflorescence dry biomass, leaf dry biomass/inflorescence dry biomass; leaf dry biomass/fruit dry biomass, inflorescence dry biomass/fruit dry biomass and vegetative dry biomass/reproductive dry biomass (Fig. 2).

Allometric biomass variation

Broad intra-specific allometric biomass relationship is presented in Table 1, which shows a significant linear relationship among the different dry biomass parameters. The highest regression was revealed by below ground dry biomass/total dry biomass and below ground dry biomass/vegetative dry biomass ($P<0.0001$). In the regression model, a significant increase was observed for vegetative dry biomass to reproductive dry biomass having a=0.24 and bx=10.94, revealing the plant's ability to allocate higher quantities for vegetative dry biomass to increase the plant size and therefore maintain physiological functioning and

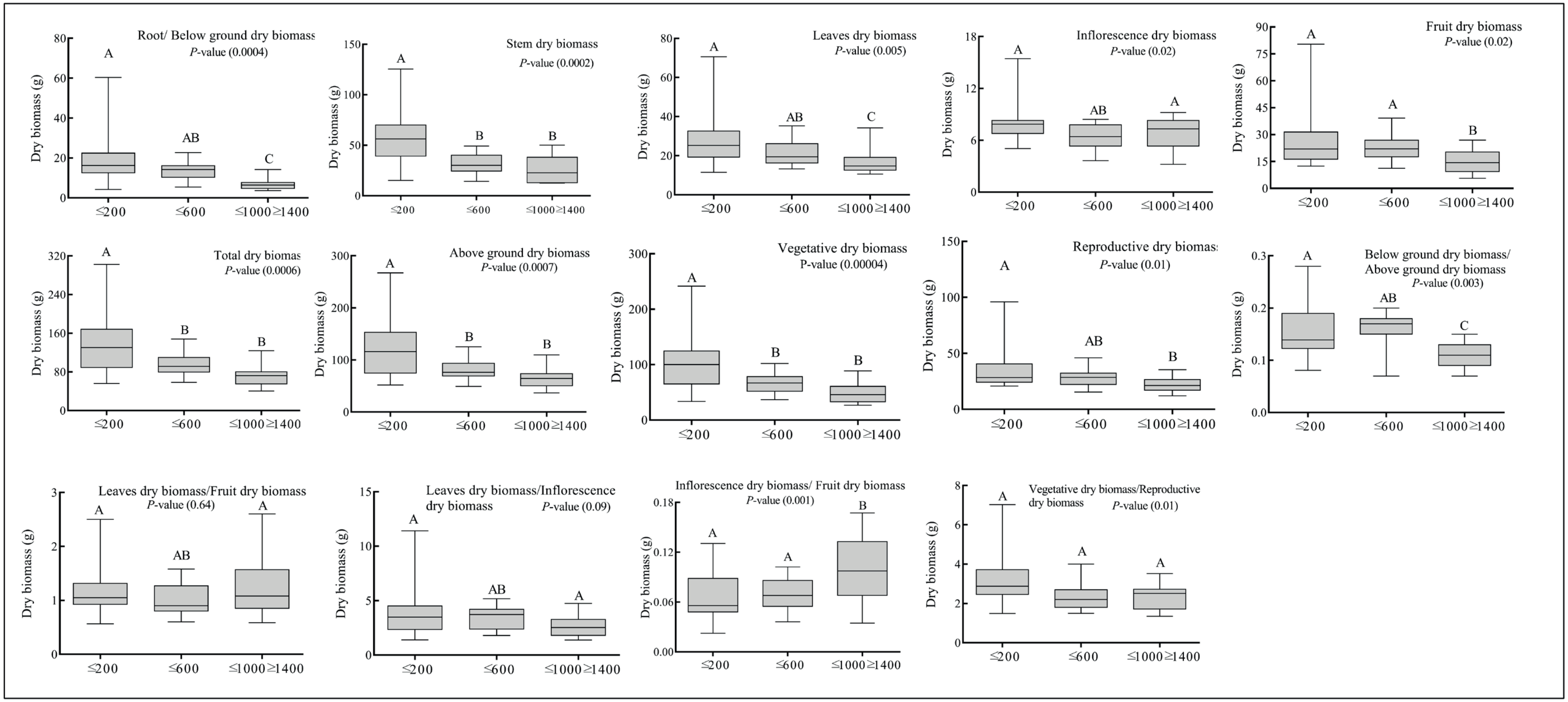

Figure 2. Variation in biomass (g) of *Xanthium strumarium* with altitude. Different box-whisker plots not sharing a common letter are significantly different ($P < 0.05$).

Table 1. Regression analysis for intra-specific biomass allocation of different plant parts and simulation between Elevation, TDBM-crown cover and plant height for *X. strumarium* along the elevation gradient.

X/Y	Adj. R^2	$\hat{y} = a+bx$	*F*-value	*p*-value
BDBM/SDBM	0.81	$\hat{y} = 2.37X + 6.92$	62.98	0.0001[a]
SDBM/LDBM	0.79	$\hat{y} = 0.44X + 4.43$	55.08	0.0001[a]
LDBM/IDBM	0.64	$\hat{y} = 0.06X + 5.50$	25.28	0.0001[a]
IDBM/FDBM	0.208	$\hat{y} = 3.38X - 1.61$	4.66	0.05[a]
BDBM/TDBM	0.9	$\hat{y} = 5.68X + 26.45$	127.95	0.0001[a]
VDBM/RDBM	0.66	$\hat{y} = 0.24X + 10.94$	28.28	0.001[a]
BDBM/VDBM	0.92	$\hat{y} = 4.57X + 12.40$	162.45	0.0001[a]
BDBM/RDBM	0.59	$\hat{y} = 1.105X + 14.0$	21.81	0.0001[a]
BDBM/ADBM	0.86	$\hat{y} = 4.68X + 26.45$	86.88	0.0001[a]
E/TDBM	0.3	$\hat{y} = -0.054X + 145.92$	5.75	0.03
E/CC	0.57	$\hat{y} = -0.021X + 119.84$	20.28	0.001
E/H	0.14	$\hat{y} = 0.0081X + 64.79$	2.28	0.15

Note: Adjusted R^2 (Adj. R^2), Elevation (E), Crown cover (CC), Height (H), Total dry biomass (TDBM).

survival. All regression models show high significance ($p < 0.0001$) except for inflorescence dry biomass/fruit dry biomass wich was significant at $p < 0.025$.

Relationship between biomass and plant morphology along the elevation gradient

The crown cover showed the best results in establishing the relation of total dry biomass, crown cover, and plant height along the elevation gradient, as evident from the regression parameters (Tab. 1). The height of the plant showed less significant relation with elevation than the crown cover, indicating crown cover is the main contributor to change in biomass allocation of individual plant species. The slope and intercept value for the data yielded positive values, indicating the direct relation of elevation with total dry biomass, crown cover, and height.

Dry biomass and diversity indices

Xanthium strumarium total dry biomass was found to significantly affect the diversity of communities sampled in the region. The increase of total dry biomass directly reduced the Shannon-Wiener diversity index and species richness. The density of the plants species remained the same, indicating these plants ability to propagate along with altitude, while the decrease in biomass was because of a reduction in plant cover owing to the more extreme environment (Tab. 2). Higher elevation sites were found to have higher species richness presumably due to less grazing intensity.

The total dry biomass of *X. strumarium* had a negative influence on the Shannon-Wiener diversity index (Fig. 3A; $r^2 = -0.50$; $P < 0.05$) but the density of *X. strumarium* did not (Fig. 3B; $r^2 = 0.09$; $P > 0.05$).

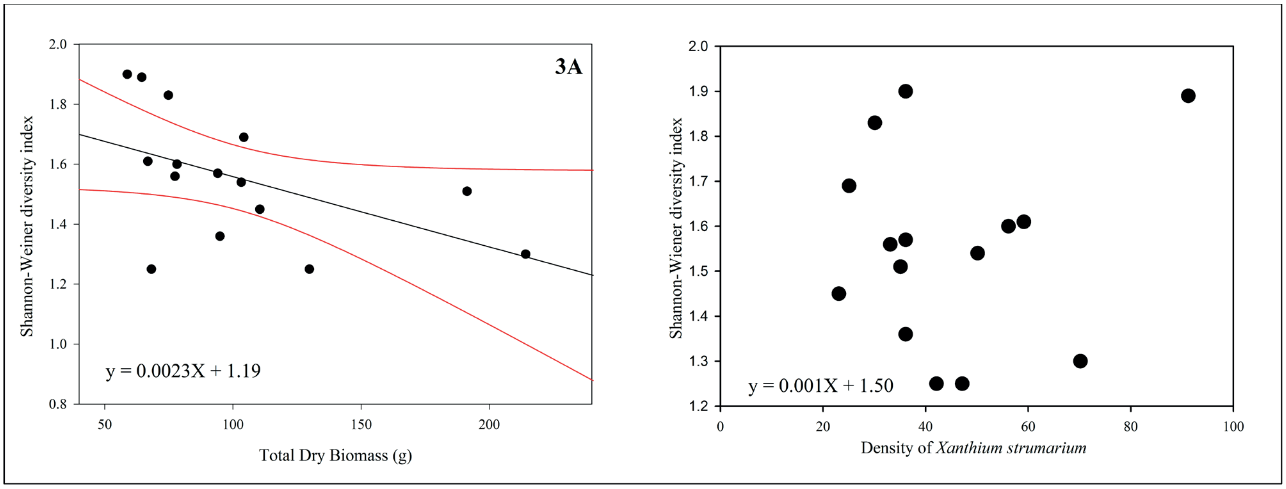

Figure 3. The relationship of Shannon-Wiener diversity index with *Xanthium strumarium* total dry biomass (3A) and density (3B).

Table 2. Relationships between TDBM, diversity and density of *X. strumarium* in different elevation stratum.

St. No	Elevation	TDBM	S	H’	1/D	J	N	D(X)	%age
1	≤200	213.7±32.21	6	1.30	2.54	0.73	121	70	57.85
2		190.91±55.90	7	1.51	3.25	0.78	69	35	50.72
3		110.23±21.60	7	1.45	3.30	0.74	41	23	56.10
4		129.50±8.31	6	1.25	2.71	0.70	82	47	57.32
5		104±22.23	7	1.69	4.30	0.87	63	25	39.68
6	≤600	103±11.70	8	1.54	2.97	0.74	92	50	54.35
7		78±12.51	9	1.60	2.45	0.73	114	56	49.12
8		77.19±11.21	7	1.56	3.41	0.80	68	33	48.53
9		93.81±4.07	7	1.57	3.37	0.80	73	36	49.32
10		94.71±14.72	6	1.36	2.89	0.76	67	36	53.73
11	≤ 1000 ≥ 1400	66.64±7.09	10	1.61	3.26	0.70	114	59	51.75
12		58.63±17.46	11	1.90	4.18	0.79	81	36	44.44
13		74.64±11.10	10	1.83	4.54	0.80	75	30	40.00
14		64.28±9.65	15	1.89	3.30	0.70	179	91	50.84
15		68.04±6.23	11	1.25	2.04	0.52	61	42	68.85

Note: Species richness (S), Shannon-Weiner diversity index (H’), Simpson diversity index (1/D), Evenness index (J), Density in stand (N), Density of *Xanthium strumarium* (D (X)), %age (Percentage density of *X. strumarium*).

Environmental and soil parameters effects on X. strumarium dry biomass

In environmental and soil variables (Tab. S2), the highest coefficient of variance was shown by nitrogen concentration followed by aspect angle and elevation in the studied parameters. Organic matter and organic carbon were also found to have a significant coefficient of variance of more than 50 %.

Elevation was found to be the most prominent factor that varied in all the plant communities (*F*-value=73 and $P < 0.001$), followed by organic matter and organic carbon (*F*-value=7.4 and $p < 0.01$). Nitrogen concentration also varied significantly in three cluster and elevation groups (*F*-value=3.84 and $P < 0.05$), while electrical conductivity and total dissolved solid also significantly contributed and varied with elevation gradient (*F*-value= 4.19 and $P < 0.05$).

Relation of TDBM with Environmental Variables

The effect of different environmental variables was assessed on biomass allocation and allometric variations the CCA axes summary revealed a total of 85.9 % of cumulative variances, in which 50.6 % was contributed by axis 1. In addition, axes 2 and 3 also contribute a significant correlation coefficient (Tab. 3).

Table 3. Eigen value and variance explained (%) explained by CCA-ordination and Pearson correlations between environmental variables and the three Canonical axes.

Axis No	Axis 1	Axis 2	Axis 3
Eigenvalue	0.007	0.003	0.002
% of variance explained	50.6	22.4	12.9
Cumulative % explained	50.6	73	85.9
Pearson Correlation, Spp-Envt*	0.96	0.963	0.995
Kendall (Rank) Corr., Spp-Envt	0.848	0.886	0.943
Environmental variables			
X_{Elev}	-0.604	-0.069	0.327
X_{AA}	-0.126	-0.384	-0.783
X_{Clay}	-0.231	0.183	0.312
X_{Silt}	0.038	0.345	-0.429
X_{Sand}	0.108	-0.536	0.308
X_{pH}	0.224	0.352	0.071
X_{OM}	-0.403	-0.245	0.528
X_{Lime}	0.339	-0.12	-0.435
X_{N}	-0.046	-0.593	0.014
X_{P}	-0.312	-0.129	0.395
X_{K}	0.137	-0.23	0.47
X_{EC}	-0.349	-0.145	0.394
X_{BD}	0.2	-0.4	-0.254

Variable (X), Elevation (Elev), Aspect angle (A.A), Clay percentage (Clay), Silt percentage (Silt), Sand percentage (Sand), Protenz hydrogen (pH), Organic matter (OM), Lime percentage (Lime), Nitrogen percentage (N), Phosphorus (mg/kg) (P), Potassium (mg/kg) (K), Electrical conductivity (μs/cm) (EC), Bulk density (g/cm^3) (BD).

The CCA bi-plot (Fig. 4) shows the key environmental factors affecting biomass allocation and allometric variation. The biplot indicated that soil texture (Silt, Clay, and Sand percentage), Nutrients (Nitrogen, Organic matter, Lime percentage, and Potassium in mg/Kg), Environmental variables (aspect angle), and soil factor (Bulk density in g/cm^3) significantly affected biomass along the elevation gradient.

There was a negative correlation between elevation and axis 1 (r=-0.60, $P < 0.01$) and aspect angle and axis 3 (r=-0.73, $P < 0.01$) (Tab. 3). In soil nutrients, organic matter percentage has a significant positive correlation on axes 3 (r=0.52, $P < 0.05$). In contrast, nitrogen percentage showed a significant negative correlation on axes 2 (r=-0.59, $P < 0.05$) and was considered the factor that varied significantly along elevation. Lime percentage was found negatively, and potassium concentration positively correlated on axes 3 ($P < 0.05$).

Discussion

Development, growth, and reproduction consequently resulted in the biomass allocation to different plant parts. Total plant biomass and ratios among different plant structures, can be critical indicators of invasive plant behavior in the pace of environmental factors (Badeck *et al.* 2004). *X. strumarium* total dry biomass, plant parts dry biomass, and different dry biomass ratios, *i.e.* leaf dry biomass/inflorescence dry biomass, leaf dry biomass/fruit dry biomass, and vegetative dry biomass/root dry biomass, decreases along the elevational gradient. In contrast, inflorescence and fruit biomass ratios increase and are supposed to be an efficient reproductive performance strategy in newly introduced habitats by allocating higher biomass to reproductive traits for efficient propagation. Similarly, Rathee *et al.* (2021) reported that *Parthenium hysterophorus* follows the same biomass allocation trends while invading along the elevation gradient. Elevational gradient origin was also reported by Watermann *et al.* (2020), supporting the view that elevation gradient affects vegetative and reproductive biomass allocation and above/below ground biomass ratios as an overall trend in the studied species.

The reduction of biomass in a single and multiple exotic species across the elevation is well studied (Monty *et al.* 2009; Haider *et al.* 2018). Biomass reductions may be linked with changes in temperature, precipitation, and length of the growing season, which changes with elevation gradient (Jia *et al.* 2010). In contrast to the work of these researchers, our study was conducted as open field sampling where the plant species were affected by a variety of conditions in a more appropriate way than the control experiments. There is a decrease in reproductive biomass if the species originate from a higher elevation (Hautier *et al.*

2009; Halbritter *et al*. 2018). The findings of this study are contrary to this, where the studied plant originates from plains and invades the higher elevation with an increase in reproductive dry biomass compared to vegetative dry biomass. Bustamante *et al*. (2018) suggested that individuals might be under reproductive stress; however, increased reproductive investment, as indicated by the results in the present study, can be considered an appropriate strategy to meet limiting environmental factors. Although we cannot directly attribute these effects to a particular factor, different environmental conditions linked to higher elevations could lead to a (relative and absolute) increased investment into reproduction and thus entail fitness advantages for the populations. In particular, increased investment into reproduction (inflorescence biomass in *X. strumarium*) at higher elevations might be a suitable strategy to counteract the risk of inbreeding in the absence of adequate pollinators (Fabbro & Körner 2004; Arroyo *et al*. 2013). In addition, genotypic divergence may also be caused by non-adaptive evolutionary processes, implying genetic drift, mutation, and recombination can also be considered for variations in biomass (Lachmuth *et al*. 2011).

One of the important factors behind successful invasion may be that invasive plants have a maximum rate of photosynthesis for which the importance of stem (for exposure) and leaves (for photosynthesis) are important and vital organs (Durand & Goldstein 2001). In the present study, the invasive plant produced biomass ratios of stem>leaves>fruits>root> inflorescence. The biomass production in this sequence indicates that leaves have considerable biomass, enabling the plant to grow and propagate vegetatively. The result is that the production of high fruits biomass enables the plant to continue its generation and life cycle for the next year. Similar results have also been reported by Hsu & Kao (2014) by studying invasive *Bidens pilosa* var. *Radiate* with minor differences in biomass accumulation which may be attributed to species and environmental factors. The higher biomass production regarding vegetative, reproductive, and over-dry, fresh biomass included *X. strumarium* as one of the topmost invasive weed plants of different crops in Khyber Pakhtunkhwa Pakistan (Muhammad *et al*. 2011).

Allometric relationships benefit the plant life history with propagation playing a key role in plant survival. Optimal allocation theory states if a plant maximizes seed and fruit production, it will invest all its resources in the growth of stem, leaves, and root indicating 100 % investment in the vegetative phase and 0 % allocation for reproductive

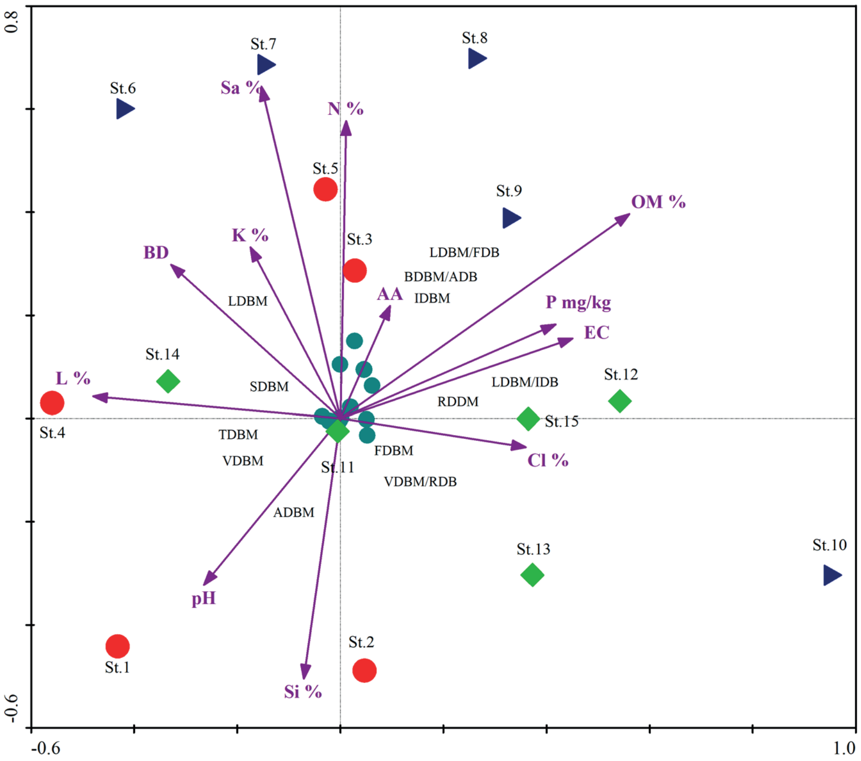

Figure 4. CCA-biplot show the effect of environmental variables on the biomass production of *Xanthium strumarium* and its different ratios. Note: Root ground dry biomass (RGDB), Stem dry biomass (SDBM), Total dry biomass (TDBM), Vegetative dry biomass (VDBM), Leaf dry biomass (LDBM), Leaf dry biomass/Inflorescence dry biomass (LDBM/IDBM), Above ground dry biomass (ADBM), Fruit dry biomass (FDBM), Reproductive dry biomass (RDBM), Below ground dry biomass/Above ground dry biomass (BDBM/ADBM), Inflorescence dry biomass (IDBM), Leaf dry biomass/Fruit dry biomass (LDBM/FDBM), Vegetative dry Biomass/Reproductive dry biomass (VDBM/RDBM), Organic matter (OM), Silt percentage (Si), clay percentage (Cl), Sand percentage (Sa), Nitrogen (N), Potassium (K), Phosphorus (P), Electrical conductivity (EC), Bulk density (BD).

growth and then at a certain stage of life it will allocate 0 % resources in vegetative growth and 100 % investment in reproductive structures (Cohen 1968; Ellner 1987). The vegetative to reproductive biomass ratio of *X. strumarium* reveals that this plant allocates sufficient biomass to the vegetative and reproductive phase and can better propagate in existing environmental conditions by allocating and investing biomass properly in different stages of its life cycle. Similar studies were also conducted by Wang *et al.* (2008) and Hai-Gen *et al.* (2004) to investigate the spread of invasive species in China and revealed that the invasive species had caused biotic homogenization.

Results of this study indicated that species diversity indices decrease with an increase in biomass production of *X. strumarium*. The plant species have caused homogenization of native plant communities at lower elevation by decreasing species richness, evenness, diversity, and number of species with the increase of *X. strumarium* density and biomass shown in Table 2 and Figure 3A, B. An increase in species diversity with environmental gradients and elevation has been reported by many authors such as Boscutti *et al*. (2018), Anderson *et al*. (2004), Casper & Jackson (1997), Gough *et al*. (1994), and Grace *et al*. (1999). This decrease of species diversity at low elevation and its gradual changes across elevation provide a clue that the invasive success across the altitude will affect diversity, resulting in the disturbance of the ecosystem at a higher elevation enabling the invasive species to possibly invade the stable forest ecosystems. The current study is limited in comparison of the diversity pattern between un-invaded and invaded sites; however, in the same region, Ullah *et al*. (2021) have reported high species richness (92 species) in the un-invaded area, *i.e.* native communities of *Vitex negundo* compared to the sites invaded by *X. strumarium*. Likewise, Qureshi *et al*. (2019) compared the diversity indices of *X. strumarium* invaded and uninvaded sites and has revealed that the invaded site has lower species diversity.

Environmental variables and nutrient concentration have also played a vital role in species diversity (Wijewardene *et al.* 2021). At lower elevation, organic matter and nitrogen contents increase, favoring the growth of plant species and reproduction, indicating environmental variables which also contribute significantly to species diversity. These results were in continuation with the works of Schlesinger *et al.* (1996) and Nordin *et al*. (2001). CCA ordination reveals the importance of elevation, bulk density, saturation point, available water, nitrogen, organic matter, and sand percentage play a crucial role in biomass production of the plant species.

Conclusion and Recommendation

Higher elevation sites were previously considered to be resistant to invasive plant species, but the recent increase in anthropogenic disturbance and climate changes make such habitats prone to tropical species. *X. strumarium* is one such tropical invader that successfully invades across the elevation because of its efficient biomass production. The changes in biomass allocation can allow the species to expand its distribution, as reported in *Parthenium hysterophorus* invasive success by Rathee *et al*. (2021). The present research found that reproductive biomass in general and inflorescence biomass, in particular, remained unchanged along the elevation gradient, revealing the reproductive fitness of the species at higher altitudes. Therefore, strategies for eradicating the species are needed to protect the native communities. Our findings add knowledge of how invasive species allocate biomass to different structures to improve their performance along altitudinal gradients, potentially enhancing their invasion capacity.

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References

Aini IN, Ezrin MH, Aimrun W. 2014. Relationship between soil apparent electrical conductivity and pH value of Jawa series in oil palm plantation. Agriculture and Agricultural Science Procedia 2: 199-206.

Ali A, Khan TA, Ahmad A. 2018. Analysis of Climate Data of Khyber Pakhtunkhwa, Pakistan. International Research Journal of Engineering and Technology 5: 4266-4283.

Anderson TM, McNaughton SJ, Ritchie ME. 2004. Scale-dependent relationships between the spatial distribution of a limiting resource and plant species diversity in an African grassland ecosystem. Oecologia 139: 277-287

Arroyo MTK, Pacheco DA, Aguilera P. 2013. Floral allocation at different altitudes in highly autogamous alpine *Chaetanthera euphrasioides* (Asteraceae) in the central Chilean Andes. Alpine Botany 123: 7–12.

Badeck FW, Bondeau A, Bottcher K, *et al*. 2004. Responses of spring phenology to climate change. New Phytologist 162: 295-309.

Bazzaz FA, Reekie EG. 1985. The meaning and measurement of reproductive effort in plants. Studies on Plant Demography. London, Academic Press.

Boscutti F, Casolo V, Beraldo P, Braidot E, Zancani M, Rixen C. 2018. Shrub growth and plant diversity along an elevation gradient: Evidence of indirect effects of climate on alpine ecosystems. PLOS ONE 13: 1-12. https://doi.org/10.1371/journal.pone.0196653

Bustamante RO, Duran AP, Peña-Gómez FT, Véliz D. 2018. Genetic and phenotypic variation, dispersal limitation and reproductive success in the invasive herb *Eschscholzia californica* along an elevation gradient in central Chile. Plant Ecology and Diversity 10: 419-429.

Call LJ, Nilsen ET. 2003. Analysis of spatial patterns and spatial association between the invasive tree-of-heaven (*Ailanthus altissima*) and the native black locust (*Robinia pseudoacacia*). The American Midland Naturalist 150: 1-14.

Casper BB, Jackson RB. 1997. Plant competition underground. Annual Review of Ecology and Systematics 28: 545-570.

Clements DR, Ditommaso A. 2011. Climate change and weed adaptation: can evolution of invasive plants lead to greater range expansion than forecasted? Weed Research 51: 227-240.

Cohen D. 1968. A general model of optimal reproduction in a randomly varying environment. Journal of Ecology 56: 219-228.

Colautti RI, Ågren J, Anderson JT. 2017. Phenological shifts of native and invasive species under climate change: insights from the Boechera–lythrum model. Philosophical Transactions of the Royal Society B: Biological Sciences 372: 1-10. https://doi.org/10.1098/rstb.2016.0032.

Coleman JS, McConnaughay KDM, Ackerly DD. 1994. Interpreting phenotypic variation in plants. Trends in Ecology & Evolution 9: 187-191.

Deo RC, Şahin M. 2015. Application of the Artificial Neural Network model for prediction of monthly Standardized Precipitation and Evapotranspiration Index using hydro-meteorological parameters and climate indices in eastern Australia. Atmospheric Research 161: 65-81.

Durand LZ, Goldstein G. 2001. Photosynthesis, photo inhibition, and nitrogen use efficiency in native and invasive tree ferns in Hawaii. Oecologia 126: 345-354.

Dutta W, Basuthakur P, Ray P. 2021. Gardening the menace! Environmental and Sustainability Indicators 12: 1-5.

Dwire KA, Kauffman JB, Brookshire ENJ. 2004. Plant biomass and species composition along an environmental gradient in montane riparian meadows. Oecologia 139: 309-317.

Ellner S. 1987. Alternate plant life-history strategies and coexistence in randomly varying environments. Vegetatio 69: 199-208.

Fabbro T, Körner C. 2004. Altitudinal differences in flower traits and reproductive allocation. Flora 199: 70–81.

Gallardo B, Zieritz A, Adriaens T, *et al*. 2016. Trans-national horizon scanning for invasive non-native species: a case study in Western Europe. Biological Invasions 18: 17-30.

Gough L, Grace JB, Taylor KL. 1994. The relationship between species richness and communities: The importance of environmental variables. Oikos 70: 271-279.

Grace JB. 1999. The factors controlling species density in herbaceous plant communities: An assessment. Perspectives in Plant Ecology, Evolution and Systematics 2: 1-28.

Grandin U. 2006. PC-ORD version 5: A user-friendly toolbox for ecologists. Journal of Vegetation Science 17: 843-844.

Haider S, Milbau A, Naylor BJ, *et al*. 2018. Mountain roads and non-native species modify elevational patterns of plant diversity. Global Ecology and Biogeography 27: 667-678.

Hai-Gen XU, Sheng Q, Zheng-Min HAN, *et al*. 2004. The distribution and introduction pathway of alien invasive species in China. Biodiversity Science 12: 626-638.

Halbritter AH, Fior S, Keller I, *et al*. 2018. Trait differentiation and adaptation of plants along elevation gradients. Journal of Evolutionary Biology 21: 784-800.

Hashim S, Marwat KB. 2002. Invasive weeds a threat to the biodiversity: a case study from Abbotabad District, N-W Pakistan. Pakistan Journal of Weed Science Research 8: 1-12.

Hautier Y, Randin CF, Stöcklin J, Guisan A. 2009. Changes in reproductive investment with altitude in an alpine plant. Journal of Plant Ecology 2: 125-134.

Higgins SI, Bond WJ, Trollope WS. 2000. Fire, resprouting and variability: a recipe for grass–tree coexistence in savanna. Journal of Ecology 88: 213-229.

Hill MO. 1979. DECORANA - A Fortran program for detrended correspondence analysis and reciprocal aver- aging. Ithaca, Cornell University.

Hsu HM, Kao WY. 2014. Vegetative and Reproductive Growth of an Invasive Weed *Biden spilosa* L. var. radiata and its Non-invasive Congener *Bidens bipinnata* in Taiwan. Taiwania 59: 119-126.

Jia X, Pan XY, Sosa A, Li B, Chen JK. 2010. Differentiation in growth and biomass allocation among three native *Alternanthera philoxeroides* varieties from Argentina. Plant Species Biology 25: 85-92.

Lachmuth S, Durka W, Schurr FM. 2011. Differentiation of reproductive and competitive ability in the invaded range of Senecio inaequidens: the role of genetic Allee effects, adaptive and non-adaptive evolution. New Phytologist 192: 529–541.

Liu X, Blackburn TM, Song T, Wang X, Huang C, Li Y. 2020. Animal invaders threaten protected areas worldwide. Natural Communications 11: 1-9.

Marwat KB, Hashim S, Ali H. 2010. Weed management: a case study from north-west Pakistan. Pakistan Journal of Botany 42: 341-353.

Mitchell CE, Agrawal AA, Bever JD, *et al*. 2006. Biotic interactions and plant invasions. Ecology Letters 9: 726-740.

Monty A, Lebeau J, Meerts P, Mahy G. 2009. An explicit test for the contribution of environmental maternal effects to rapid clinal differentiation in an invasive plant. Journal of Evolutionary Biology 22: 917-926.

Muhammad Z, Wazir SM, Farooq A, Ullah S, Hussain Z. 2011. Distribution and checklist of weeds in maize crop of frontier region Bannu, Khyber Patunkhwa, Pakistan. Pakistan Journal of Weed Science Research 17: 373-379.

Najberek K, Okarma H, Chmura D, Kr´ol W, Walusiak E, Solarz W. 2020. Enemy pressure exerted on alien and native plants may differ between montane and lowland regions. Arthropod-Plant Interactions 14: 275–287.

Nasir E, Ali SI. 1972. Flora of West Pakistan. Karachi, Fakhri Press.

Nelson DW, Sommer LE. 1982. Total Carbon, Organic Carbon and Organic Matter. In: Page AL, Miller MH, Kenny DR. (eds.). Methods of Soil Analysis, Part 2. 2nd Edn. American Society of Agronomy. pp 539–77.

Nordin A, HÖgberg P, Näsholm T. 2001. Soil N form availability and plant N uptake along a boreal forest productivity gradient. Oecologia 129: 125-132.

Ordonez A, Wright IJ, Olff H. 2010. Functional differences between native and alien species: a global-scale comparison. Functional Ecology 24: 1353-1361.

Pauchard A, Kueffer C, Dietz H, *et al*. 2009. Ain't no mountain high enough: plant invasions reaching new elevations. Frontiers of Ecology and Environment 7: 479-486.

Pauli H, Halloy SR. 2019. High Mountain Ecosystems Under Climate Change. Oxford Research Encyclopedia of Climate Science. https://doi.org/10.1093/acrefore/9780190228620.013.764

Pragada PM, Venkaiah M. 2012. Phytosociological attributes of weed flora in major crops of north coastal Andhra Pradesh, India. Pakistan Journal of Weed Science Research 18: 107-126.

Pyšek P, Křivánek M, Jarošík V. 2009. Planting intensity, residence time, and species traits determine invasion success of alien woody species. Ecology 90: 2734-2744.

Qureshi H, Anwar T, Arshad M, Osunkoya OO, Adkins SW. 2019 Impacts of *Xanthium strumarium* L. invasion on vascular plant diversity in Pothwar Region (Pakistan). Annali di Botanica 9: 73-82.

Rahman A, Khan AN. 2013. Analysis of 2010-flood causes, nature and magnitude in the Khyber Pakhtunkhwa, Pakistan. Natural Hazards 66: 887–904.

Rathee S, Ahmad M, Sharma P, *et al*. 2021. Biomass allocation and phenotypic plasticity are key elements of successful invasion of *Parthenium hysterophorus* at high elevation. Environmental and Experimental Botany 184: 104392.

Richardson DM, Pyšek P. 2012. Naturalization of introduced plants: ecological drivers of biogeographical patterns. New Phytologist 196: 383-396.

Roura-Pascual N, Krug RM, Richardson DM, Hui C. 2010. Spatially-explicit sensitivity analysis for conservation management: exploring the influence of decisions in invasive alien plant management. Diversity and Distributions 16: 426-438.

Salehi MH, Beni OH, Harchegani HB, Borujeni IE, Motaghian HR. 2011. Refining soil organic matter determination by loss-on-ignition. Pedosphere 21: 473-482.

Saxton KE, Rawls WJ. 2006. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. Soil science society of America Journal. 70: 1569-1578.

Schlesinger WH, Raikes JA, Hartley AE, Cross AF. 1996. On the spatial pattern of soil nutrients in desert ecosystems: ecological archives E077-002. Ecology 77: 364-374.

Shackleton RT, Foxcroft LC, Pyšek P, Wood LE, Richardson DM. 2020. Assessing biological invasions in protected areas after 30 years: revisiting nature reserves targeted by the 1980s SCOPE programme. Biological Conservation 243: 108424.

Shaheen H, Batool A, Gillani SF, Dar MEI, Habib T, Aziz S. 2019. Diversity and distribution of invasive plant species in suburban vegetation of Kashmir Himalayas. Polish Journal of Environmental Studies 28: 2823-2833.

Shrestha BB, Shrestha UB, Sharma KP, Thapa-Parajuli RB, Devkota A, Siwakoti M. 2019. Community perception and prioritization of invasive alien plants in Chitwan-Annapurna Landscape, Nepal. Journal of Environmental Management 229: 38-47.

Simberloff D. 2006. Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? Ecology Letters 9: 912-919.

Simberloff D. 2009. The role of propagule pressure in biological invasions. Annual Review of Ecology Evolution and Systematics 40: 81-102.

Thuiller W, Lavorel S, Araújo MB, Sykes MT, Prentice IC. 2005. Climate change threats to plant diversity in Europe. Proceedings of the National Academy of Sciences 102: 8245-8250.

Trunschke J, Stöcklin J. 2017. Plasticity of flower longevity in alpine plants is increased in populations from high elevation compared to low elevation populations. Alpine Botany 127: 41–51.

Ullah R, Khan N, Rahman A. 2021. Comparison of invaded and non-invaded sites: A case study of rough cocklebur (*Xanthium strumarium* L.) an alien invasive species. Applied Ecology and Environmental Research. 19: 1533-1548.

Van Kleunen M, Schlaepfer DR, Glaettli M, Fischer M. 2011. Preadapted for invasiveness: do species traits or their plastic response to shading differ between invasive and non-invasive plant species in their native range? Journal of Biogeography 38: 1294–1304.

Wang C, Cao G, Wang Q, Jing Z, Ding L, Long R. 2008. Changes in plant biomass and species composition of alpine Kobresia meadows along altitudinal gradient on the Qinghai-Tibetan Plateau. Science in China Series C: Life Sciences 51: 86-94.

Watermann LY, Hock M, Blake C, Erfmeier A. 2020. Plant invasion into high elevations implies adaptation to high UV-B environments: a multi-species experiment. Biological Invasion 22: 1203-1218.

Wijewardene L, Wu N, Qu Y, *et al*. 2021. Influences of pesticides, nutrients, and local environmental variables on phytoplankton communities in lentic small water bodies in a German lowland agricultural area. Science of The Total Environment 780: 146481.

Wilson JB, Rapson GL, Sykes MT, Watkins AJ, Williams PA. 1992. Distributions and climatic correlations of some exotic species along roadsides in South Island, New Zealand. Journal of Biogeography 19: 183–193.

Xiao HF, Schaefer DA, Lei YB, *et al*. 2013. Influence of invasive plants on nematode communities under simulated CO2 enrichment. European Journal of Soil Biology 58: 91-97.

Xiong X, Yang X, Liu Y, Zhang Y, Wang P, Wang J. 2013. Chinese herbal formulas for treating hypertension in traditional Chinese medicine: perspective of modern science. Hypertension Research 36: 570-579.

Yusof KM, Isaak S, Abd Rashid NC, Ngajikin NH. 2016. NPK Detection Spectroscopy on Non-Agriculture Soil. Jurnal Teknologi 78: 227-231.

Zuur A, Ieno EN, Smith GM. 2007. Analyzing ecological data. Series statistic for biology and health, Springer Publisher New York and London.