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Invasive Species as Rivals: Invasive Potential and Distribution Pattern of *Xanthium strumarium* L.

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Abstract: Xanthium strumarium L. is a globally successful invasive herb that has had significant negative ecological, economic and social impacts in many world regions. The present study was therefore conducted to evaluate the invasive potential and spatial distribution patterns of X. strumarium in heavily invaded plant communities of the semiarid regions of northern Pakistan. Investigations were based on data from 20 plants grown in the Herbarium at the University of Malakand, and from observations in 450 plots distributed across 45 stands representing habitats across Northern Province including open fields, hillocks and abandoned areas in both urban and rural areas. Multivariate analysis identified elevation, organic matter and organic carbon as the environmental variables most associated with communities invaded by X. strumarium. Increased soil silt was positively associated and available water was negatively associated with X. strumarium-invaded communities. These key environmental characteristics allowed us to identify four main associations: Group I: X. strumarium-C. sativa, Group II: X. strumarium-P. hysterophorus, Group III: X. strumarium-A. aspera and Group IV: X. strumarium-C. sativa. Other invasive species were observed, either exotic, such as P. hysterophorus, or indigenous, such as C. sativa and D. inoxia, often co-occurring and responding similarly to these factors. The results suggest that high temperature with drought stress could be a determinant of increasing population at lower elevations, whereas colder climates with adequate moisture are related to reduced populations at higher elevations, near the species' upper range limits. It is recommend that the inclusion of appropriate, additional soil and climatic variables in species distribution models be implemented in order to better explain species' ecological niches and help guide conservation and protection plans for native plant communities.

Keywords: X. strumarium; community structure; co-occurrence; invasive species; plant invasions

1. Introduction

The recent era has witnessed enhanced trade, transport and tourism, industrial expansion, technological advancement and increasing urbanization [1]. These factors place natural ecosystems under increasing pressure to provide ecosystem services to rapidly expanding human populations and further facilitate the introduction and invasion of exotic species [2]. The rapid increase in human transport and goods globally has facilitated the spread of non-native species and their expansion into new habitats such that invasive species are now considered to be among the leading causes of species extinction and biodiversity loss worldwide [3]. Invasive plants affect evapotranspiration, causing severe threats



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). to sustainable water supply [4] and ecosystem services such as resources and energy [5]. Invasive species may have deleterious impacts on native populations via several mechanisms, including genetic effects, pathogen introduction and habitat degradation [6,7]. The ability to colonize new habitats and resist disease and pests have been identified in traits which promote the spread of invasive plant species, including effective propagule production and dispersal via seeds and vegetative structures, early flower and seed formation and other phenological advantages relative to native species [8,9]. When exotic species out-compete native plants for nutrients, light and shelter, they form monotypic stands that pre-empt native flora [10] and contribute to altered ecosystem structure and function [11].

Invasive plant species spread rapidly across roads, railway tracks, cities and rural areas, resulting in community homogenization [12]. Biotic homogenization changes diverse populations within an area to become progressively similar and is a significant factor that affects the establishment of invasive species in an ecosystem [13]. They are regarded as a valuable resource because of their capacity to adapt to new environmental factors and disturb native plant communities [14]. An invasive species may be able to invade and spread in a new habitat despite biotic and abiotic filters [15]. Environmental parameters of any area, notably temperature, relative humidity, day length, and geographical and topographic variables, operate as an abiotic filter for newly invading invaders [16]. The filter's efficiency is determined by how closely environmental conditions correspond to the species' native and invaded habitats [17]. An alien species' phenology does not change if the new range's environment and habitat are identical to its original range [18]. The alien species will produce seeds, propagules and vegetative growth, and flower as it does in its native range [17]. In the opposite situation, the invasive species' success is determined by a combination of the environmental fluctuations and the species' inherent capacity to flower [19].

Research outlining the impacts of invasive species on ecological systems will be critical in protecting and conserving native species and essential ecosystem processes [20]. Pakistan has unique and valuable biodiversity owing to its particular geography, in terms of, for example, topography, climate and soils. This geography includes features such as a tropical marine coastline in the south, a strong elevation gradient inland due to mountain orogeny, and the influence of the moisture-bearing South Asian Monsoons and the Drier Westerly winds [21]. However, being a largely agricultural nation, Pakistan struggles socio-economically with low crop productivity and inefficiencies in its agricultural system [22]. It is vulnerable to limits on its ability to supply food crops to its inhabitants. Invasive species have thus been introduced to meet the increasing demand for foodstuffs, causing pressure on native plant communities [23].

More than 6000 vascular plants have been reported from Pakistan, of which 5600 have been described [24]. In addition, 700 invasive plant species have also been listed as posing a severe threat to native plant communities [25]. In Khyber Pakhtunkhwa, 16 invasive species were reported, including Trianthema portulacastrum L. Amaranthus viridis L. Carthamus oxyacantha M.B., Fl. Taur. Cauc. Conyza bonariensis L. Cronquist in Bull. Tor. Bot. Club, Xanthium strumarium L., Asphodelus tenuifolius Cavan. L., Asphodelus tenuifolius Cavan. L., Eucalyptus camaldulensis Prosopis juliflora L., Avena fatua L. and Imperata cylindrica (L.) Phragmites australis (Cav.) Trin. ex Stued. Gallium aparine L. [26]. Xanthium strumarium L. (Asteraceae), also known by many vernacular names (e.g., cocklebur, lampourde glouteron, ditchbur, clot bur, noogoora bur, sheep bur [27,28]), is an emerging invasive species in various districts of Khyber Pakhtunkhwa (KP), Pakistan [26]. Xanthium originates from South or Central America, from where it spreads into North America, invading riverways and shorelines and dispersing widely via hydrochory (via water) and zoochory (via animals) [29]. In North America, anthropogenic activity has added Eurasian strains, and cross-breeding has resulted in polyploidy and genetic variability in populations of different regions. The highest genetic variability in *Xanthium* is in Northeast North America, where tetraploid populations occur uniformly across the region [29].

The invasion of the *X. strumarium* in KP has significantly increased with the movement of Afghan refugees in the 1970s and has had pronounced effects on plant communities in general and crop plants, e.g., maize, soya bean, cotton and groundnuts, in particular [30]. The species is now a common weed across the region, affecting biodiversity. Its success is due to its invasive traits, i.e., adherent burs that spread via animals along waterways and land, prolonged seed viability and photo-intensity and production of allelopathic compounds [31,32].

In Pakistan, *X. strumarium* was first reported by Baloch et al. [33] as a host for insect pests. Since then, many studies have reported it as a weed species among different crops in Khyber Pakhtunkhwa, Pakistan [26,27]. A limited number of studies address the invasive potential of *X. strumarium* and its impacts on biodiversity [20,34–36], and even less attention to the associated environmental and topographic variables that facilitate invasion. The present research evaluated *X. strumarium* potential to impact the native communities of the area. In addition, we also considered the relationship of communities with environmental variables to investigate which factor sustains these communities. Therefore, this research examines *X. strumarium-dominated* plant communities and characterizes the environmental variables and soil parameters associated with the species to better understand the factors responsible for maintaining its populations and affecting invaded communities. The aim is to inform and address efforts to control and eradicate *X. strumarium*.

2. Materials and Methods

2.1. Study Area

2.1.1. Zones and Distribution Points

KP is one of the five administrative provinces of Pakistan (Figure 1). KP Environmental Protection Agency classified the province into four agro-ecological zones based on climate, precipitation, temperature, altitude and topography. Zone A includes mountainous areas of district Swat, Shangla, Dir, Bunir and Chitral. Zone B lies within the eastern mountainous sub-humid and wet mountainous regions and includes the districts of Abbottabad, Batagram, Mansehra, Kohistan, Haripur and Torghar. Zone C includes the central plain valley of Peshawar, Charsadda, Hangu, Karak, Nowshera, Bannu, Mardan, Kohat and Swabi.



Figure 1. Study region in the Khyber Pakhtunkhwa province, Northern Pakistan, showing agroecological zones and distribution of *Xanthium strumarium* in the province.

Zone D includes the Piedmont plains and Suleiman, within the districts of Bannu, Lakki Marwat, Tank, Karak and Dera Ismail Khan [37]. Zones C and D are characteristically plains with agricultural fields that contribute to the livelihood of local people, adding 20% in GDP and 44% in labor potential to the province [37]. *X. strumarium* heavily invades and affects these two zones' agricultural fields and vegetation [25]. The sites sampled were located between 34.29° to 34.55° N and 71.84° to 72.62° E (Elev. 390 to 981 m asl; see Figure 1), covering the commonly invaded roadsides and abandoned areas in both urban and rural vicinities.

2.1.2. Climate, Physiography and Vegetation

The climate of the study area is temperate, with mean annual rainfall ranging from 384 mm to about 639 mm (Figure 2) and mean monthly temperatures ranging from 34.96 ± 1.36 °C to 0.67 ± 0.97 °C [38].



Figure 2. Important climate variables of the sampling sites.

The regional geology is classified into thirteen substrate types, aggregated from thirtyseven sub-categories based on bedrock lithology [39]. For example, the northern Indus suture zone mixes igneous, mafic and ultramafic rock sequences. The northern portions of the study area are predominantly volcanic rocks; the Khyber-Hazara zone is dominated by metamorphic complexes with rich mineral material, including diverse mineral ores [40].

Ecological communities in the region are complex and quite dissected partly due to the rugged and variable topography in this massive mountainous region of the Himalayan Hindu Kush (HHK) [37]. A variable and biodiverse flora and fauna are thus supported [41]. Plant communities include montane, alpine forests, dry coniferous forests and sub-tropical forests. Montane forests occur at elevations of 4000 m in Swat, Chitral and Hazara; Alpine and dry coniferous forests occur throughout, at elevations ranging from 3350–3360 m for alpine and 1525–3660 m for dry forests. Sub-tropical forests occur on south-facing slope aspects at elevations of 900–2000 m in the Himalayan range within the districts of Swat and Hazara [42].

2.2. Study of Invasive Characteristics

X. strumarium invasive traits were studied under experimental and field conditions. *X. strumarium* (n = 20 plants) was grown in the Botanical Garden of the University of Malakand Khyber Pakhtunkhwa, Pakistan. Characteristics known to predict invasiveness of a species were recorded, including the number of days required for seedling emergence, % age of seed germination, vegetative growth period and reproductive output, i.e., number of seeds and fruits produced per plant. In addition, important climatic indicators such as daily temperature, humidity, day length and precipitation, daily dew point temperature in °C and daily ultraviolet index were recorded. *X. strumarium* dried fruits were collected to assess seed characteristics including average number of seeds per fruit (n = 2), determined by counting the seeds, and mean seed mass of 100 seeds (n = 50), determined by weighing the seeds on an electronic weighing balance. These data were also used to calculate a Discriminant Function [43], which serves as a basic screening tool to assess the invasive potential of woody species. The following formula calculates it:

Discriminant Function =
$$19.77 - 0.51 \sqrt{M} - 3.14 \sqrt{J} - 1.21S$$
 (1)

where, M = mean seed mass (mg); J = minimum juvenile period (years); S = mean interval between large seed crops (years).

2.3. Field Studies of Invasive Species Distribution and Inter-Species Associations

Our field studies involved regular visits to collect vegetation data within 15 districts (Figure 1), where 45 *X. strumarium* dominated stands were randomly sampled. The data-set consists of 450 phytosociological plots ($45 \times 10 = 450$ plots), taken strictly according to floristic and structural homogeneity within sampled vegetation stands to provide a reasonable survey of the whole study area. The size of the plots varied between 3 m² to 5 m², with differences by a factor of up to five, considered acceptable by [44]. In each plot, density, frequency, cover, relative density, relative frequency, relative cover and Importance Values were calculated following the standard procedure [45]. Edge effects were reduced by excluding a 10-m buffer zone from the stand boundaries [46]. All plants were identified following Flora of Pakistan [47], following the nomenclature of plant taxa according to [48].

2.4. Ecological Impact Assessment

An additional survey was conducted to assess the ecological impact of *X. strumarium* on floristic composition and species diversity. Soil characteristics were further investigated to evaluate impacts of the species invasion on soil variables.

2.4.1. Impact on Diversity

A total of 450 plots invaded at varying intensities by *X. strumarium* were established, and the number of species (richness), density, cover, height and volume were calculated to compare species diversity and invasion effects. An inventory was prepared to list the species name, plant family and other floristic attributes following Flora of Pakistan [47]. Herbarium vouchers were deposited in the Department of Botany, University of Malakand Khyber Pakhtunkhwa, Pakistan. Three important ecological diversity indices, Species richness (*S*), Shannon-Wiener diversity index (*H'*) and evenness index (*E*), were calculated based on species density following [49]. Species richness was measured as a simple count of species in the stand. The *H'* and *E* indices are calculated as:

$$H' = \sum_{i=1}^{5} pi \operatorname{In} pi$$
 (2)

$$E = \frac{H'}{\ln S} \tag{3}$$

where pi = proportion of the species (*i*) to total number of species, In = natural logarithm of pi and InS = natural logarithm of species richness.

2.4.2. Soil Assessment

To characterize soil properties in each *X. strumarium*-invaded stand, we obtained 3-kg soil from two opposite corners and at a mid-position of each stand with auger borings. Soil top layer is generally rich in nutrients, and consequently, samples were obtained over a depth of 0–30 cm [50], bulked, and mixed thoroughly to reduce heterogeneity following standard pedological procedures [46,51]. Soil pH was immediately determined in soilwater suspension (1:5 ratio) in the field by a digital pH meter and electrical conductivity by EC-meter. The physiochemical and textural nature of the soil was determined by air-drying and sieving the samples through a 2-mm sieve following U.S.D.A. methods [52].

Walkley–Black method was used to estimate organic matter, whereas total and organic carbon were determined by wet combustion with chromic acid digestion followed by dry combustion [53]. Total nitrogen was obtained by the micro-Kjeldahl technique, and available phosphorus (P^{2+}) and exchangeable potassium (K^+) were determined following [54]. Lime (Calcium carbonate; %) was calculated using the geometric method and CO₂ evolution was measured geometrically following [52]. We assessed additional soil parameters including available water (AW), field capacity (FC), wilting and saturation point (WSP), conductivity (μ s/cm) and bulk density (BD) following [55] using an online calculator (https://www.nrcs.usda.gov (accessed on 15 October 2021)). The hydrometer method determined textural properties such as sand, clay and percent silt distribution [56] at Swat Agriculture Research Institute (SARI).

2.5. Data Analysis

Phytosociological data of species in the 45 stands and corresponding environmental variables were data-banked for analysis and interpretation. The relative phytosociological attributes were transformed into importance value index (IVI) according to [57]. The classification of vegetation communities was evaluated using Ward's agglomerative method by opting for Euclidean distance in the package PC-ORD ver. 6.0 [58]. After the numerical classification, the species occurring in each stand were assigned to a phytosociological group. To evaluate whether a certain species could be considered as diagnostic of a class, we consulted recent literature [45,59–62]. Then, we summed the transformed relative phytosociological values of the diagnostic species in plots to provide a suitable phytosociological locus to the stand's groups obtained by numerical classification. The non-parametric Kruskal–Wallis test analyzed the indices of phytosociological stands from the resulting clusters.

The relationship between floristic variation within *X. strumarium* dominated vegetation and environmental variables were analyzed using Canonical correspondence analysis (CCA). Four topographic variables and 16 soil properties were used in CCA, whereas post hoc interpretation of the CCA ordination axes was statistically evaluated using a Monte Carlo permutation test. In addition, vectors of the significant variables were visualized in the ordination diagram. Finally, the weighted average values of significant variables in the different vegetation types were represented by box plots. All quantitative vegetation and environmental data analyses were performed in Graph Pad Ver 7.0 [63] and MS Excel (2010).

3. Results

3.1. Invasive Potential of X. strumarium

The germination percentage of *X. strumarium* was found to be 78%, indicating a high reproductive capacity and potential for invasion. The plant produces two seeds per bur, where the seed mass was found to be 14.9 ± 5.76 g per 100 seeds. The other phenological and climatic parameters are summarized in Table 1.

The seeds are typically dispersed exozoochorously by animals, in this case by domesticated sheep and goats, assisted by sticky burs (Figure 3). Species' discriminant Function, an index of invasion potential, was determined to be 12.02 ± 0.52 .

Climatic Parameter	DLH	ADH (%)	ADR	ADMNT	ADMXT	ADMT	ADDP	DUVI
	$12{:}14\pm0.06$	53.13 ± 13.93	0.43 ± 0.6	7.9 ± 4.02	34.1 ± 4.7	23.6 ± 3.83	8.23 ± 4.23	3 ± 2.56
Trait Parameter	DE	DI	DD	NI/P	NB/P	BB/50	SB/100	SBB/50
	5.1 ± 0.78	41.65 ± 3.73	131 ± 12.78	400 ± 66	324 ± 56	25.5 ± 5	14.9 ± 5.76	39.45 ± 10.5

Table 1. Climatic parameters and different associated traits of *Xanthium strumarium* related to invasive behavior.

Note: DLH (Day length in hours); ADH % (Average daily humidity in percentage); ADR (Average daily rainfall in millimeters); ADMNT (Average daily minimum temperature in °C); ADMXT (Average daily maximum temperature in °C); ADMT (Average daily mean temperature in °C); ADDP (Average daily dew point in °C); DUVI (Daily ultraviolet index); DE (Days to emergence); DI (Days to inflorescence); DD (Days to drying); NI/P (number of inflorescence per plant); NB/P (number of burs per plant); BB/50 (bur biomass in g/50); SB/100 (seed biomass in g/100); SBB/50 (Seeds and bur biomass in g/50).



Figure 3. Phenological phases and invasive spreading of *Xanthium strumarium* i.e., (**A**) (Burs for germination); (**B**) (First emergence); (**C**) (Vegetative phase); (**D**) (reproductive phase i.e., inflorescence and burs); (**E**) (collected burs); (**F**) (Empty burs); (**G**) (Developed seeds); (**H**) (Nomads and domestic animals); (**I**) (Sheep having sticky burs); (**J**) (Goat with sticky burs).

3.2. Local Distribution of X. strumarium

X. strumarium-invaded communities were classified into four ecologically meaningful groups depending on varying habitats and species composition (Figure 4). These relatively discrete phytosociological groups have substantial floristic bulk of species composition, which differs significantly in the average Importance Value percentage (*F*-value 6.47; p < 0.01; Table 2). Nevertheless, the four groups differed significantly in terms of the IVI of diagnostic species (*F*-value 53.79; p < 0.001) (Table S1). In particular, the second most dominant species in all the four groups are different unusually except in group I and IV which are similar but slightly different in terms of IVI, i.e., 12.94 ± 1.14 and 18.22 ± 3.01 , respectively for *Cannabis sativa*. In addition, the floristic assemblage in group-I is characterized by *Xanthium-Cannabis*, with a real predominance of *Chenopodium album* (7.22%) and *Cynodon dactylon* (Table S1). The distinction of this cluster was further supported by the significant prominence of an exotic invasive species, *Parthenium hysterophorus* (6.15%), and native invasive *Silybum marianum* (3.90%) as well as *Amaranthus viridis* (4.43%), respectively.



Figure 4. Cluster dendrogram of the 45 stands (450 plots) dominated *X. strumarium*. Note: Group I (community of *X. strumarium-C. sativa*); Group II (community of *X. strumarium-P. hysterophorus*); Group III (community of *X. strumarium-D. metel*); Group IV (community of *X. strumarium-C. sativa*); G (Group); St. (Stand number).

Communities	Species	AH/Plant	AIVI (%)	H-Index	J-Index	AD/st.	AC/st.	AV/st.
Group-I	29	26.4 ± 4.3	$39.7\pm1.42~\mathrm{a}$	$1.67\pm0.11~\mathrm{a}$	0.78 ± 0.02	34.8 ± 4 a	$65\pm27~\mathrm{a}$	$24.9\pm2~a$
Group-II	28	23.6 ± 1.2	45.4.94 ab	$1.44\pm0.04~\mathrm{ab}$	0.74 ± 0.01	38.3 ± 2.0 a	38.6 ± 3 ab	25.5 ± 1.9 a
Group-III	26	31.9 ± 6	$47.0\pm2.3\mathrm{b}$	$1.42\pm0.09b$	0.67 ± 0.04	$48\pm7.8~\mathrm{ab}$	95.4 ± 43 a	$39\pm7.4\mathrm{b}$
Group-IV	18	32.0 ± 4.1	$50.9\pm3\mathrm{b}$	$1.19\pm0.11~{ m c}$	0.71 ± 0.03	$50\pm7.8~\mathrm{ab}$	$84.4\pm38~\mathrm{a}$	$31.6\pm4.4b$
F-value	-	1.1584	6.47	5.45	2.30	3.184	2.512	2.920
<i>p</i> -value	-	0.3402	0.001	0.0029	0.090	0.03	0.05	0.04

Table 2. Vegetation characteristics following a period of *X. strumarium* invasion and potential stabilization in Khyber Pakhtunkhwa, Pakistan.

Note: AH (average height); A.IVI (average importance values index); H-index (Shannon-wiener diversity); J-index (Equitability or Evenness index); AD/ha (average density ha^{-1}); AC/ha (average cover ha^{-1}); AV/ha (average volume ha^{-1}). Column wise different letters indicate significant difference at p < 0.05

The group-II was distinguished by the overwhelming presence of invasive exotic P. hysterophorus (13.61%) and C. album (4.66%), respectively, with several other new individuals from their preceding and proceeding groups, i.e., groups I, III and IV. The group-III is an X. strumarium-D. innoxia community based on IVI dominance (Table 2). None of the species except Carthamus oxycantha showed IVI greater than 5%, while Eryngium coereculum and Amaranthus spinosus (IVI \leq 3%) were distinct species of the group. Surprisingly, the vegetation belongs to group-IV, a community of X. strumarium and C. sativa that seems similar to group-I. However, owing to the floristic heterogeneity (Table S1), the syntaxonomical position of stands in group-IV should not be mixed with the first phytosociological group and is perhaps a distinct vegetation type. Group-IV, hereafter reported as a community of X. strumarium and C. sativa, showed a distinct floristic composition, having IVI of D. innoxia (9.78%), Verbascum thapsus (5.29%) and P. hysterophorus (2.56%). The group-IV could be tentatively placed in a separate category based on its floristic composition, as recently proposed by [64] and subsequently accepted by [65]. The floristic composition of the X. strumarium and C. sativa community (Group-IV) differed from the former groups owing to the reduced IVI of some constituent species and the prominence of new species such Tagetes minuta (IVI = 2.98%) and Justicia adhatoda (IVI = 1.26%).

3.3. X. strumarium Invasion Impacts on Diversity

Overall, 50 species distributed in 48 genera and 25 families were recorded in 45 vegetation stands (Table S2). The dominant angiosperm families were Asteraceae (13 species, 13 genera) and Amaranthaceae (4 species, 3 genera), whereas the contribution of Solanaceae, Brassicaceae and Fabaceae were 3 species and 3 genera each, altogether accounting for 60% of the total genera and species respectively. Based on habit, the community consists of 54% annuals that were significantly more common than perennials (36%) and accounted for 90% of the total plant species. The remaining 10% of species were biennial, annual/biennial or annual/perennial, but were not homogeneously distributed in the region. Analysis of the historic geographical spectrum or origin of plants shows a high proportion of invasive plant species (42%) relative to native (26%) and naturalized (20%) species, whereas cultivated and casual aliens comprised rare taxa. Generally, the vegetation exhibited wide phytogeographically affinities, predominantly representing species native to South America (22%), Europe (20%), Tropical America and Pantropical (6% each) regions. Eighteen other regions contributed low species richness, including native species (26%) which were more important from a phytogeographical perspective (Table S2). The average species richness, Shannon-Wiener index, coverage, density and volume of the plants within the communities (p < 0.001) decreased with increasing importance values, highlighting the invasiveness of *X. strumarium* and its invisibility in the region (Table 2).

3.4. Impacts on Soil and Associated Environmental Variables

The various environmental factors associated with *X. strumarium* communities are depicted in Table 3. In communities, elevation ranged from 390 m to 981 m (Figure 5b), which differed significantly (*H*-statistic 13.66; *p*-value = 0.0034) and correlated positively

with IVI (r = 0.247; p < 0.05) and density ha⁻¹ (r = 0.281; p < 0.05). Geophysical coordinates also showed marked differentiation of latitude and longitude (Figure 5a,c) that negatively affects *X. strumarium*. Cover/hectare and volume/hectare (r = -0.278; p < 0.05). The soil of invaded communities was relatively thick (up to 90 cm in depth) and generally well drained, with flat to gently sloping terrain. Soil texture was typically sandy-loam or clayloam, with a clay fraction showing a significant increase (*H*-statistic 7.71; *p*-value = 0.0066) with depth in the soil profile.

Table 3. Mean (\pm) standard deviation (SD) of twenty-two different environmental variables for the vegetation stands associated with each of the four clusters. Significant difference was computed by using a non-parametric Kruskal-Wallis test followed by Tukey HSD.

E. d. m	Groups I	II	III	IV		n Values
Factors	$\mathbf{Mean} \pm \mathbf{SE}$	$\mathbf{Mean} \pm \mathbf{SE}$	$\mathbf{Mean} \pm \mathbf{SE}$	$\mathbf{Mean} \pm \mathbf{SE}$	H-Values	<i>p</i> -values
Elev	390.4 ± 87	843.1 ± 70	614.3 ± 123	981.1 ± 146	13.663	0.0034 **
N°	34.29 ± 0.11	34.16 ± 0.06	34.3 ± 0.16	34.55 ± 0.05	11.972	0.007 **
E°	71.84 ± 0.17	72.62 ± 0.12	71.9 ± 0.19	72.3 ± 0.15	12.538	0.005 **
AA	142.27 ± 32	193.72 ± 22	129.2 ± 37.9	126 ± 37.67	3.885	0.274
Cl%	24.76 ± 3.7	32.70 ± 2.5	21.7 ± 1.44	26.07 ± 3.43	7.17	0.066 *
Si%	35.88 ± 5.9	36.33 ± 3	44.3 ± 4.89	43.2 ± 6.31	4.497	0.212
Sa%	39.35 ± 5.4	30.96 ± 3	33.8 ± 4.77	30.7 ± 4.36	2.195	0.532
pH1:5	6.74 ± 0.13	6.91 ± 0.09	6.7 ± 0.092	6.71 ± 0.15	2.3041	0.511
OM%	1.02 ± 0.18	1.67 ± 0.1	1.48 ± 0.23	1.18 ± 0.16	5.672	0.128
OC%	0.57 ± 0.10	0.97 ± 0.1	0.85 ± 0.13	0.68 ± 0.09	2.458	0.076
L%	10.02 ± 1.0	8.22 ± 0.4	8.33 ± 0.95	8.82 ± 1.88	2.905	0.406
TC%	1.5 ± 0.10	1.98 ± 0.1	1.87 ± 0.13	1.7 ± 0.09	2.458	0.076
Ν	0.030 ± 0.007	0.064 ± 0.01	0.052 ± 0.019	0.030 ± 0.009	1.082	0.781
Р	4.72 ± 0.33	4.65 ± 0.2	5.11 ± 0.59	5.04 ± 0.43	1.034	0.793
Κ	114.18 ± 9.4	102.2 ± 8.8	110.87 ± 14.81	100.2 ± 13.4	1.580	0.663
EC	249.72 ± 36	324.2 ± 20.67	308.62 ± 32.75	334.1 ± 13.3	7.759	0.051 *
TDS	159.82 ± 23	207.50 ± 13.2	197.52 ± 10.96	213.8 ± 8.53	7.759	0.051 *
WP	0.153 ± 0.017	0.18 ± 0.012	0.13 ± 0.0058	0.154 ± 0.01	7.651	0.053 *
FC	0.28 ± 0.018	0.328 ± 0.01	0.27 ± 0.008	0.301 ± 0.013	7.232	0.064 *
BD	1.38 ± 0.02	1.32 ± 0.016	1.38 ± 0.014	1.35 ± 0.017	6.481	0.090 *
SP	0.47 ± 0.009	0.49 ± 0.006	0.47 ± 0.0054	0.487 ± 0.0066	6.388	0.094 *
AW	0.13 ± 0.008	0.14 ± 0.005	0.14 ± 0.0085	0.14 ± 0.0068	1.686	0.639

Note: Elev (Elevation in m); N° (North); E° (East); Cl% (Clay percentage); Si% (Silt percentage); Sa% (Sand percentage); pH (Protenz Hydrogen); OM% (Organic Matter percentage); OC% (Organic carbon percentage); TC% (Total carbon percentage); L% (Lime percentage); N (Nitrogen percentage); P (Phosphorus in mg/Kg); K (Potassium in mg/kg) EC (Electrical conductivity μ s/cm); TDS (Total dissolve solid ppm, 640 Scale.); WP (Wilting Point at 1500 kPa); FC (Field capacity at 33 kPa); BD (Bulk Density in g/cm3); SP (Saturation at 0 kPa); AW (Available water); * (p < 0.05); ** (p < 0.01).

Soil pH was slightly acidic in all the vegetation types, probably due to similar parental material (Igneous rocks). The values of organic matter, carbon contents and total carbon decreased from species-rich to species-poor vegetation types and correlated positively (r = 0.297; p < 0.05) with density ha⁻¹. The soil percent lime varies (8.22–10.02%) and exhibited negative relationships with Importance values (r = -0.214; p < 0.05), density (r = -0.427; p < 0.01) and volume ha⁻¹ (r = 0.295; p < 0.05), whereas the nitrogen and potassium content showed a similar trend and negatively affecting vegetation density (r = -0.251; p < 0.05) in the communities (Table S3). The available phosphorus was comparatively very high, which was always below the detection limit of 2.5 mg/kg in other natural plant communities, as reported by [42] for *Dodonaea viscosa*. Several soil properties, such as electric conductivity, total dissolved salt, wilting point, field capacity, bulk density and saturation point, showed statistically significant differences and decreased from species-rich to species-poor vegetation types (Table 2) but did not appear to play a major role in shaping these or other vegetation characteristics.



Figure 5. Box-plot diagrams for the significant indicator values (**a**) (latitude in degree: N°); (**b**) (elevation in meters from sea level); (**c**) (longitude in degree: E°); the four vegetation communities; Different capital letter indicate significant difference at p < 0.0.5, the details and abbreviations are given in Tables S1 and S2.

3.5. Pattern of Communities' Composition

The CCA analysis revealed 21.3% of the variance on the three axes, in which the axis contributes 8.7, followed by axis 2 and 3 (Table 4). The Eigenvalues were 0.28, 0.22 and 0.19 for the three axes.

Table 4. Summary statistics for the first three axes obtained from CCA-ordination.

Total Variance ("Inertia") in the Species Data: 3.3001	Axis 1	Axis 2	Axis 3
Eigenvalues	0.28	0.22	0.19
% of variance explained	8.7	6.8	5.9
Cumulative % explained	8.7	15.4	21.3
Pearson Correlation, Spp-Envt *	0.769	0.924	0.926
Kendall (Rank) Corr., Spp-Envt	0.453	0.697	0.762

The floristic variation in the four assemblages was predominately structured and influenced by topographic and soil gradients (Figure 6). Six indicator values of the twenty-two environmental variables investigated were found to significantly influence the vegetation and be reflected in the main ordination axes (Table 5).



Figure 6. CCA-biplot ordinations of the 45 stands and 50 plant species associated with *X. strumarium*. Acronyms of the species and stands are presented in Table S2. Arrows indicate the vectors of significant indicator values. Elev (elevation); OM% (organic matter percentage); OC (organic carbon capacity); AW (available water) Si% (silt percentage); Sa (sand percentage) TC% (total carbon percentage) L% (Lime percentage); Species codes are the same as that mentioned in Table S2.

The first ordination axis had significant positive correlations with organic matter (r = 0.307), organic carbon (r = 0.307) and total carbon, (r = 0.307), respectively. The second axis explained 6.8% of the floristic variation and can be interpreted as an elevation gradient showing a negative effect (r = -0.288) on *X. strumarium*-dominated vegetation. In contrast, silt content showed a relatively weak positive relationship (r = 0.231) and potentially favored populations of this invasive species. The CCA-ordination biplot did not separate the vegetation types, with species and stands tending to be scattered, and some compactly in the center along axis 1 and 2, respectively indicating homogenization of the lower right whereas, major exotic and native invasive species such as *X. strumarium*, *C. sativa, P. hysterophorus* and *D. metal* were positioned in the upper left in ordination configuration (Figure 6).

N/	Inter-Set Cor	relations *		Biplot Scores			
variable -	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3	
Elev	0.174	-0.609 ***	-0.339 **	0.093	-0.288 *	-0.149	
N°	0.298	-0.191	0.338 **	0.159	-0.09	0.149	
E°	0.119	-0.241	-0.052	0.064	-0.114	-0.023	
AA	-0.039	-0.26	-0.255	-0.021	-0.123	-0.112	
Cl%	0.163	-0.21	0.022	0.087	-0.099	0.01	
Si%	-0.284	0.489 **	-0.056	-0.152	0.231	-0.024	
Sa%	0.194	-0.386	0.045	0.104	-0.182	0.02	
P1:5	-0.019	-0.218	0.084	-0.01	-0.103	0.037	
OM%	0.574 ***	-0.429 **	-0.221	0.307 *	-0.203	-0.097	
OC%	0.574 ***	-0.429 **	-0.221	0.307 *	-0.203	-0.097	
L %	-0.273 *	0.35 **	0.359 **	-0.146	0.165	0.158	
TC%	0.574 ***	-0.429 **	-0.221	0.307 *	-0.203	-0.097	
Ν	0.367 **	-0.274 *	0.06	0.196	-0.13	0.026	
Р	0.114	0.051	-0.206	0.061	0.024	-0.091	
Κ	0.032	0.201	0.337 *	0.017	0.095	0.148	
EC	0.013	0.12	-0.246	0.007	0.057	-0.108	
TDS	0.013	0.12	-0.246	0.007	0.057	-0.108	
WP	0.155	-0.19	0.006	0.083	-0.09	0.003	
FC	0.029	0.021	-0.009	0.016	0.01	-0.004	
D	-0.071	0.059	-0.024	-0.038	0.028	-0.01	
SP	0.07	-0.056	0.023	0.037	-0.026	0.01	
AW	-0.286 *	0.459 **	-0.034	-0.153	0.217	-0.015	

Table 5. Intra-set correlations and biplot scores of the twenty-two environmental variables associated with *Xanthium* dominated communities. The first three axes of CCA ordination are computed in the analysis.

Note: *p* < 0.001 for ***. *p* < 0.01 for ** and *p* < 0.05 for *.

4. Discussion

4.1. Invasive Potential of X. strumarium

The exact pathway and year of *X. strumarium* invasion in the region is not known, but is reported for the first time by [33] in Pakistan and neighbouring countries. In the last two decades, *X. strumarium* has been introduced unintentionally into multiple regions of Khyber Pakhtunkhwa during the movement of Afghan refugees, and it has subsequently established, naturalized and propagated widely, with adverse effects on biodiversity and agriculture [26].

Invasive plant species introduced into new environments may either propagate or perish depending on their adaptability in new environments. It is difficult to predict whether the exotic species will establish and become naturalized in the new environment [66,67]. When successful, invasive plants often proliferate, changing vegetation composition and environmental parameters [68–70], and consequently threaten native flora and fauna [20,71]. Our study supported earlier claims that *X. strumarium* has successfully displaced native plant species and spread throughout the semi-arid regions of Pakistan to become one of the prominent invasive species in the region, and furthermore provided insight into the mechanisms that enabled this. We found that seed germination rates of this species (75%) exceed those of other invasive plants, for example those reported for *B. papyrifera* (25%) [49]. Furthermore, the results of our discriminant analysis indicated higher sexual propagation and seed and fruit formation in this species compared to other regional invasive species as reported by [9,49,65].

4.2. Local Distribution of X. strumarium

Cluster analysis identified four distinct vegetation assemblages dominated by *X. strumarium*. These communities were found to be dominated by other invasive species, either naturalized or native, including *Cannabis sativa*, *Parthenium hysterosporus* and *Acyranthus aspera*. These particular invasive species have been found to be co-dominant in other communities' according to other authors. For example, [34] reported *P. hysterosporus* growing alongside *X. strumarium* in communities in the Boren zone, Ethiopia, as did [72] in Punjab Pakistan. *Xanthium-Cannabis* communities were reported by [73] from Lucknow, India. In the present study, most of the species we found to have a strong association with *X. strumarium* L. were invasive, including *C. sativa* L., *P. hysterosporus* L. and *Datura innoxia* L. Only one species associated with it, *A. aspera* L., was non-invasive. The invasive species with which it was associated were all noxious weeds which pose high risk and threat to future biodiversity. This finding supports the hypothesis of reduction in biodiversity as a result of the arrival of invasive plants, as reported by many authors, including [74–78]. Our findings also provide further evidence for the observation that *X. strumarium-P. hysterosporus* support each other in community invasion [79], and the findings of a *X. strumarium-D. innoxia* association as reported by [80] from Soon Valley, Pakistan.

In terms of plant association, the Group II community had twenty plant species forming a species rich stand. The community was found at an elevation of 843.1 ± 70.3 m and had the highest organic matter and Nitrogen contents ($1.67 \pm 0.19\%$ and $0.064 \pm 0.014\%$, Table 3), which may be a possible reason for its diversity compared to community I, III and IV. Community IV was found to have the lowest diversity of all four communities and a high IVI for *X. strumarium* L. of 50.93 ± 3.00 . This matches the information in the literature pertaining to the role of invasive plants in reducing biodiversity and leading to biotic homogenization of native plant communities [81]. Seedling establishment of *X. strumarium* is known to be promoted in native communities subject to human disturbance, a finding our study supports, and one reported for other exotic species in the region, such as *Dodonaea viscosa* in Malakand as reported by [59,82], and for *P. hysterosporus* in the same region by [65].

4.3. X. strumarium Invasion and Environmental Variables

We recorded 50 plant species in 45 different stands covering wide ecological habitats. These species belonging to 25 families, of which Astereacea, Amaranthaceae, Solanaceae, Brassicaceae and Fabaceae were species-rich families, whereas the remaining families were species poor. In addition to low species richness, this association between particular plant families and *X. strumarium* in invaded areas has been reported worldwide. For example, [34] reported that the major families of *X. strumarium*-dominated communities were *Asteraceae*, *Poaceae*, *Amaranthaceae* and *Fabaceae*, and similar findings were reported by [20,35,72]. While our results matched these findings, discrepancies in taxonomic associations at the family level may be due to geography and climate [83].

The communities were dominated by annual and perennial plant species which relate to contrasting views expressed in the literature that (1) annuals in particular have pronounced effects on community homogenization [84] and (2) perennials promote communities disturbance and thus invasion [85,86]. Compared to *X. strumarium*, *P. hysterosporus* and *C. sativa* are small herbs which may be at a disadvantage given the tendency for smaller herbs to be progressively replaced by larger plants [79]. We also found that the invasion of *X. strumarium* was prominent on the roads/wastes field sides in herb or shrub-dominated communities, and in agricultural fields. Similarly, [87–91] have predicted road pavements, railway tracks, and agricultural lands are most susceptible to plant invasion. Moreover, low diversity indexes were recorded which may correspond to the high invasion rate in the measured sites. Low diversity indexes in *X. strumarium*-invaded sites were also reported by [20,34,35]. Shannon-diversity and IVI indicated a decreasing trend along elevational gradients in *X. strumarium*-dominated communities. The explanation for this elevational impact is not clear since the effects of an increase in non-native plants is poorly understood on spatial and temporal scales of this sort [92].

As to the environmental influences on species invasion, we found that these may include climate and soil characteristics, similar to [93]. Soil and climate may be more important than believed and warrant further evaluation alongside the commonly implicated invasion factors of anthropogenic disturbance and propagule production [94]. In

addition, road networks may be a dominant spreading factor for exotic species, particularly in highland environments [95].

Elevation is considered a predominant factor and explanatory variable in determining community structure and invasion susceptibilities. [96] were of the view that plant invasion is mainly restricted to lowland areas because climatic conditions become harsher with increasing elevation. We found that vegetation characteristics (i.e., Average Importance value percentage, Average cover, height and density) varied across elevation, indicating its prominent role. These variations occurred in conjunction with environmental variables such as latitude, longitude, Nitrogen percentage, Potassium (mg/Kg), Electrical conductivity (μ s/cm), Total dissolved solids (ppm) and Lime (CaCO₃) percentage. In contrast, soil texture was sandy-loam or silty-loam throughout the study sites, having less or no variation in soil composition. Similar results have also been reported by [97,98] while evaluating vegetation structure and its key drivers, with differences in community composition and factors intensity attributed to differences in species identity, climate and vegetation composition among sites.

4.4. Pattern of Communities' Composition

Gradual changes in plant communities across environmental gradients are prominent and important for vegetation invaded by alien invasive species [99]. In CCA results, topographic and soil factors, i.e., elevation, soil texture, organic matter%, organic carbon%, total carbon%, Lime% and available water, are important factors that can affect X. strumarium communities' structure and composition. Organic matter promotes soil structure, porosity and water availability [100,101]. We found increased organic and total carbon in X. strumar*ium*-dominated communities, which was significantly contributing to its invasion [80], also indicating the importance of organic matter in sustaining *Xanthium-Datura* communities. Xanthium species tend to grow well in marshy places, as reported by [29], suggesting that available water is a significant contributing factor in determining the growth and reproduction of the species in communities, while [102] describe the importance of water for X. sibricum seed germination and growth production. Elevation in inter-set correlation (Table 5) shows a significant negative correlation on axes 2 and 3, indicating the rapid spread of this plant species along altitudinal gradients. This may be due to its propagule transport by nomadic peoples and their domestic animals (sheep and goats) as they travel from the plains to the highlands during their seasonal migration. The elevation gradient brings a distinct pattern of vegetation and floristic composition due to changes in micro- and macro-climate along the gradient [103], a trend strengthened by several recent studies [59,60,104,105].

In summary, our study suggests that the focal species has wide ecological amplitude with respect to elevation. It was found throughout the area surveyed and affected the native plant populations negatively. Its pattern of expansion with respect to altitude was in accordance with other studies [36,106].

5. Conclusions

In the study, we found that the focal plant species represents a strong ruderal component in the study region. It is sensitive to elevation and produces taller, larger plants at low altitudes and even at higher altitudes at ruderal sites having a higher importance value index. The species was also found to negatively impact the diversity of communities, facilitated by effective propagation and invasive potential, a feature that relates to its high germination percentage. Additionally, phytosociological characteristics of *X. strumarium* changed across the elevation gradient, suggesting a shift in phenological, morphological, and biomass allocation that was recently reported by [107] in the invaded habitats of the same region. Given its strong invasion potential, we recommend eradicating the species through ecological management, for example, by introducing native plant crops that can compete equally with this species. Alternatively, carefully managing soil nutrients and water characteristics in cultivated fields may be necessary to prevent yield decreases if the invader cannot be eradicated and persists.

However, it is worth noting that the vigorous spread of the species in the region may have benefited economically and implications for medicinal plant markets. The plant may be efficiently utilized as biomonitoring tools, as recently reported by [108], while biomass production may be utilized for biofuel and medicinal purposes. This may warrant further exploration into its potential uses. However, its invasive behavior may offset such advantages, and this would therefore need to be considered carefully, with management plans in mind. Integrated approaches that involve awareness of the human communities may be needed, for example, through surveys of farmers in particular, and by making decisions in consultation with the governmental organizations such as regional agriculture and livestock departments, and by local non-governmental organizations as proposed by [109]. In addition, for efficient control, exploration of invasive species' morphological, phenological, phytochemical, physiological and reproductive biology assessment is required [110]. Invasion may be monitored qualitatively using species inventories (seasonally) and quantitatively using phytosociological approaches and mapping utilizing ground-based methods (through map overlays or GPS), remotely sensed pictures (aerial photos, high resolution multi-spectral digital data). Plant invasions in new places change the dynamics of indigenous communities, reduce species diversity, disrupt ecosystem processes and result in massive economic and ecological imbalances. The construction of a plant detection network in the study area and communication connections between taxonomists, ecologists and land managers are required to monitor and control invasive species. Moreover, we recommend that this invasive species' positive and negative aspects be considered in a broader, systematic framework to overcome the potential tensions between its risks and benefits, as revealed by [111].

Supplementary Materials: The following supporting information can be downloaded at: https:// www.mdpi.com/article/10.3390/su14127141/s1, Figure S1: Status, Habitat, Family and Native country wise distribution of plants found in association with *Xanthium strumarium* dominated communities of KP Pakistan. Table S1: Indices of phytosociological attributes for fifty plant species in the four clusters of *X. strumarium* dominated communities. Values are means (mean importance values (\pm SE) of the importance values obtained from the relative scores of frequency, density and cover data. The symbol (hyphen –) refer to species absent in a particular group. Table S2: Taxonomic, ecological and phyto-geographical details of plant species associated with *X. strumarium* in Khyber Pakhtunkhwa, Pakistan. Table S3: Correlation coefficients among different environmental variables influencing the distribution of *X. strumarium* in Khyber Pakhtunkhwa, Pakistan.

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