INVASIVE PLANT SPECIES AFFECT SODOM APPLE (*CALOTROPIS PROCERA* (AITON) W. T. AITON) AND ASSOCIATED PLANTS BY ALTERING SOIL PHYSIOCHEMICAL CHARACTERISTICS IN NORTHWEST PAKISTAN

FAISAL, S.[1] – KHAN, N.[1*]

Department of Botany, University of Malakand, Chakdara Dir Lower (18800), Khyber Pakhtunkhwa Pakistan

**Corresponding author*
e-mail: nasrullah.uom@gmail.com

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Abstract. Invasive plant species have deliberately affected and reduced the populations of native species, thereby increasing their risk of extinction. The objective of this research was to compare the effects of the two most aggressive invasive alien species currently invading habitats of *Calotropis procera* "an ecologically and medicinally important native plant" in northwest Pakistan, most probably by altering vegetation and soil physical and chemical characteristics. We sampled invaded and non-invaded vegetation of exotic invasive and native species in diverse habitats with corresponding environmental and soil parameters for multiple comparisons like species richness, diversity, and total plant coverage to assess the invasion effects. Our results exposed that soil physiochemical characteristics significantly differed among the native and exotic invaded-derived soils. Principal component analysis revealed that *C. procera* dominated vegetation was strongly affected by the invasion of *X. strumarium* and *P. hysterophorus* as a result of altered soil characteristics, thereby offering strong evidence that invasion of both exotic invasive species alter soil chemistry and ecology, which may create conditions favourable for invasive plants over native plants. Likewise, results showed that invasive species have completely dominated invaded sites (IV ≥45%) whereas in native vegetation both species are overlapping (IV ≥15%) and might be the strong competitors affecting community structure in the future.
Keywords: *plant invasion, native plants, alpha diversity, soil characteristics, importance values*

Introduction

Invasive plant species are considered the second highest global threat to ecosystems worldwide, decreasing biodiversity, increasing net primary productivity, and modifying the invaded environment (Vila et al., 2011; Pysek et al., 2012; Chen et al., 2015; Seifu et al., 2017). Biological invasion poses severe threats to agriculture and other natural resources, thereby affecting native communities, making them endangered, and causing extinction worldwide (Joshi, 2001; Kathiresan et al., 2005; Pysek et al., 2012). Nowadays, ecologists pay special attention to exotic species due to their adverse impacts on biodiversity loss, ecosystem invasibility, and especially disturbance of native flora (Grotkopp et al., 2002; Lake and Leishman, 2004). These exotic species cause economic loss worldwide; therefore, ecologists focus on the factors that enable their successful invasion (Schmidt and Drake, 2011). In this sense, soil characteristics play a fundamental role in the growth of plants by providing essential elements and nutrients for reproduction and development in specific habitats (Sperry et al., 2006). Soil nutrient availability and physicochemical properties play a crucial role in the establishment of exotic species, as invasive plants may alter various biotic and abiotic soil characteristics such as pH, nutrient pool, and moisture to promote invasion (Elgersma and Ehrenfeld, 2011; Osunkoya and Perrett, 2011; Lazzaro et al., 2014; Majewska et al., 2015). Many exotic

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plant invaders are known to interfere with native plants by changing soil physical and chemical properties such as moisture, temperature (Zavaleta, 2000; Belnap and Phillips, 2001; Hawkes et al., 2005; Sperry et al., 2006), soil pH (Kourtev et al., 1998; Timsina et al., 2011), amount of soil organic matter, aggregation (Windham and Ehrenfeld, 2003; Koutika et al., 2007), nitrogen, and phosphorus (Ehrenfeld, 2003; Chapuis-Lardy et al., 2006; Koutika et al., 2007; Dassonville et al., 2008). Likewise, soil microbial, micro-fauna behaviors (Belnap and Phillips, 2001; Evans et al., 2001; Chacon et al., 2009) and allelopathy intervention (Murrell et al., 2011) may also be key drivers in promoting successful invasion of many tropical plants.

Many studies emphasize that invasive species-dominated communities severely affect native plant species in biotic and abiotic factors (Allen et al., 2003; Yu et al., 2005; Stinson et al., 2006). Because of the change in phosphorus, nitrogen, and other element balances, the plants in disturbed soil have a significant impact on the local plant populations, leading to invasive species biomass expansion, that is greater than native vegetation (Dassonville et al., 2008; Liao et al., 2008; Castro-Díez et al., 2014). Pakistan has a long history of invasion and so far, about 700 invasive species have been reported (Nasim and Shabbir, 2012), of which *Xanthium strumarium* L., and *Parthenium hysterophorus* L., of the Asteraceae family, pose severe threats to native flora (Hussain and Zarif, 2003; Nasim and Shabbir, 2012). *P. hysterophorus* is an annual exotic ephemeral herb that is commonly known as white-top weed, which originated in the neo-tropical region but has expanded its populations now in the pan-tropical region as well (Navie et al., 1996; Mahadevappa, 1997; Jayasuriya, 2021). *P. hysterophorus* is spreading in rocky crevices, aggressively colonizing wastelands, along roadsides and water channels, as well as in agricultural lands (Shabbir, 2002). This plant has got international level importance because of its rapid propagation and invasion (Ali et al., 2018) due to its severe negative impacts on human health, crop production, and biodiversity (Chippendale and Panetta, 1994; McFadyen, 1995; Shabbir, 2002). Similarly, the genus *Xanthium* comprised of more than sixteen species (Dekker, 2011), in which *X. strumarium* (cocklebur) a monoecious annual herb, appeared to be one of the most noxious weeds in Pakistan and had been introduced due to the Russian-Afghan dispute in 1980s, thereby posing negative impacts on plant biodiversity, livestock and the economy (Rezene and Taye, 2014). This species was first described in Europe and later originated in North America (Stesevic and Petrovic, 2010; Dekker, 2011). Since then, a few sparse studies on both the species have been conducted with special emphasis on their invasive successes and ecological determinants in Pakistan (e.g., Khan et al., 2020; Ullah et al., 2021).

According to Shinwari and Qaiser (2011), about 7.8% species are endemic, and several are endangered in Pakistan. Among the native flora, *C. procera* (family Ascelpiadaceae) is one of the most important and critical native species distributed in the arid and semiarid regions of Pakistan (Abbas et al., 1992; Hassan et al., 2015). This Giant Milkweed is well-known for its allelochemicals or secondary metabolites like cardiac glycosides, sterols, flavonoids and triterpenes (Heneidak et al., 2006). It has been studied due to its photo-pathogenic properties (Kareem et al., 2008) and bio-controlling agents for plants and animals (Ahmad, 2005; Iqbal et al., 2005). Besides containing three toxic glycosides in its milky latex, it also has steroidal heart poisons (Zeng et al., 2008) and is also widely recognized for its adverse effects on the germination inhibition of several plants (Al-Zahrani and Al-Robai, 2007; Yasin et al., 2012). This evergreen shrub is native to Pakistan and distributed in diverse habitats (i.e., riverside, forests, roadsides, and areas

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containing ruderal species) at an elevation ranging from 200 to 1500 m (Hussain, 2020). Despite its adverse effects, this plant provides various ecosystem services, benefiting different stakeholders and hosting many beneficial insects (Bidak et al., 2015; Hassan et al., 2015). However, in the last few decades, this species has been severely exploited and therefore, under severe threat and at the risk of becoming endangered, primarily due to anthropogenic activities like explicit harvesting for medicinal and burning purposes by the local inhabitants. Secondly, the most severe and unnoticed threat to this vital plant and its associated vegetation is possibly due to the rapid invasion of two aggressive noxious invasive species *i.e. X. strumarium* and *P. hysterophorus,* which deliberately change the vegetation structure and soil composition, making the environment suitable for their invasion (Khan et al., 2020; Ullah et al., 2021). Here we hypothesized that *C. procera* native populations faced severe threats due to the rapid invasion of these invasive species through the interference of soil physical and chemical characteristics, thereby setting the objectives for this study 1) to compare the selected biotic and abiotic factors between *X. strumarium* and *P. hysterophorus* (invaded) and *C. procera* dominated vegetation (non-invaded sites), 2) to investigate the effects of abiotic variables on community structure and, 3) to assessing the factors that have a significant impact on the *C. procera* populations in their native range.

Materials and methods

Study site

The study was conducted in the selected administrative divisions (i.e., Malakand, Peshawar, and Hazara) of Khyber Pakhtunkhwa, Pakistan (34.11 to 34.59 N and 71.44 to 73.14 E) located in the northwest of the country (*Fig 1*). The climate is varied, with annual average rainfall ranging from 347 to 600 mm and an average annual temperature of 27°C, respectively. The province's topography ranges from rocky outcroppings in the south to forests and green plains in the north (Marwat et al., 2010). The province's elevation ranges from 327 to 7708 meters above sea level, whereas the sampling was conducted between 350 to 1456 meters across the current distribution ranges of the species. This province is situated at the crossroads of the Iranian plateau and the Eurasian land plate, where the Hindukush Mountains and the Eurasian land plate meet (Shad et al., 2011). It has a wide range of climatic conditions, with warm summers and bitterly cold winters in the mountainous north. On the other hand, the southern regions have very different climatic patterns, with topography ranging from dry, rocky areas in the south to woodlands and fertile plains in the north (Marwat et al., 2010).

Sampling design

A total of sixty-nine study sites (twenty-three each for *Xanthium*, *Parthenium*, and *Calotropis*) were randomly selected during the summer of 2019 in Khyber Pakhtunkhwa, Pakistan, for vegetation analysis and collection of soil samples after extensive field survey. All the sites were moderately or purely dominated by the investigated species where sampling was performed by following standard procedure (Vanderhoeven et al., 2005). At each study site, ten plots of 3×3 m were established along a 100 m transect and data was recorded in 345 plots, including two invasive (i.e., *X. strumarium* and *P. hysterophorus*) and one native *C. procera* dominated vegetation. The invaded and native plots were chosen as close as possible to elaborate the effect of invasion on

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vegetation populations, and at least 10m from the edge of a patch to minimize any effects of contrasting vegetation such as shading or litter-fall. The phytosociological variables recorded in each plot include the frequency, density, and cover of each plant species to calculate the importance value index (IVI) following Mueller-Dombois and Ellenberg (1974) and Brower et al. (1998). All the plants were identified following the Flora of Pakistan (Nasir and Ali, 1995) and also confirmed through various pictorial guides. Likewise, three biodiversity indices were calculated to compare vegetation between invading and non-invaded sites, i) species richness (*S*), referring to the number of species per plot in each vegetation group, ii) Shannon diversity index *H*' was computed using the formula H' = -∑*pi* ln *pi*, where pi = frequency of (*i*th) species per plot. Furthermore, the Evenness index (J') was calculated to estimate the relative abundance of different species in a particular community type using the formula given:

$$\text{Evenness index (J')} = \frac{\text{H}'}{\ln S} \qquad \text{(Eq.1)}$$

where *H*' is Shannon-Wiener index and *S* is the total number of species in each site (Mahdavi et al., 2013; Zhang et al., 2017).

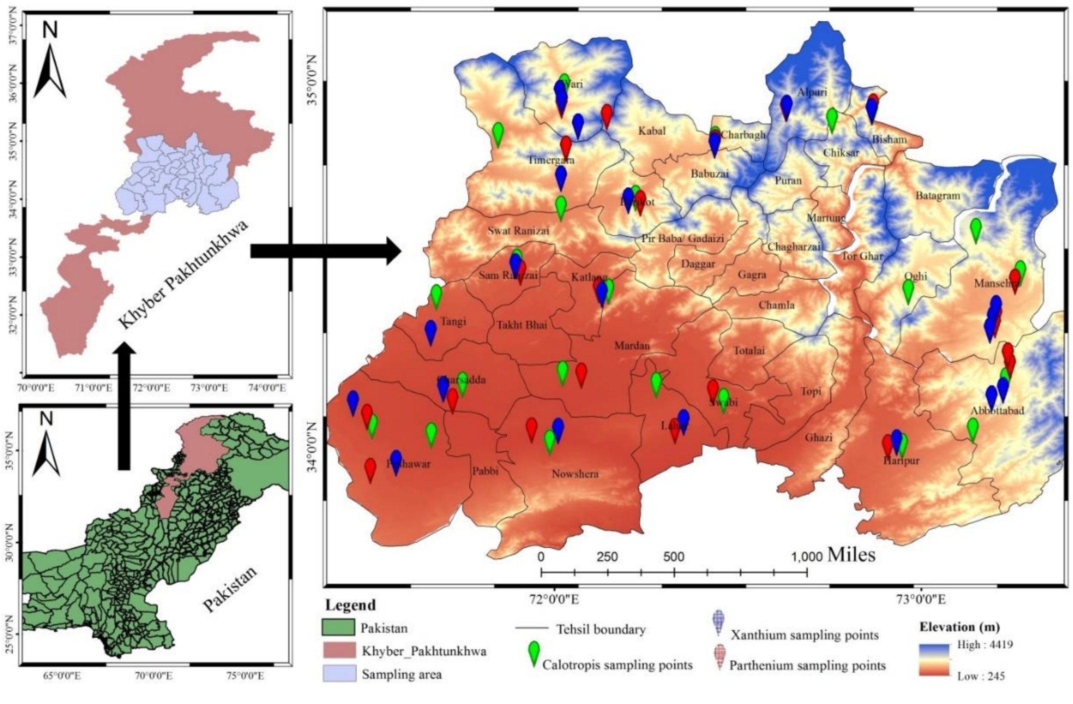

Figure 1. *Location of the sampling points of the investigated species in Khyber Pakhtunkhwa, Pakistan showing invaded and non-invaded sites*

Spatial parameters of the sites were recorded using a Geographic Positioning System (GPS), altimeter, and clinometer. Likewise, soil bulked samples (3 replicates per stand) were collected during the peak season at a depth of 20cm following a completely randomized design. All samples were air-dried, and visible fine roots were removed before physiochemical analysis in the laboratory. All the collected samples of native and invasive species were brought to the Agriculture Research Institute Swat (ARIS) for further analysis.

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Soil physiochemical analysis

We measured fourteen soil physical and chemical properties, namely soil texture (sand, silt and clay), pH, organic matter, nitrogen (N^{3-}), phosphorus (P^{3-}), potassium (K^{1+}), total carbon (TC) and soil hydraulic properties (i.e., EC μS/cm, wilting point (mm/m), field capacity (mm/m), saturation point (%) and available water (%). Soil samples were sieved (2-mm mesh) by mechanical sieve shakers before determining dry weight and moisture content after drying treatment at 105 °C overnight. We followed the hygrometer procedure proposed by Bouyoucos (1936) for soil texture properties. Although, this is a simpler and quicker analytical method, it is considered to be less accurate than other methods, like Pipette and Bouyoucos_M-T methods (Beretta et al., 2014; de Oliveira Morais et al., 2019). Soil pH was measured within 15 minutes by preparing 1:5 soil-water suspensions using a digital pH meter (McLean, 1983). Soil organic matter (SOM %) was determined by the loss-on-ignition method following Salehi et al. (2011), whereas nitrogen, phosphorus, and potassium (NPK) were determined using an optical transducer method recently introduced by Masrie et al. (2017). Likewise, we estimated different soil hydraulic properties (SHP) that included electrical conductivity (EC), wilting point (WP), field capacity (FC), saturation point (SP) and available water (AW) following the methods outlined by Marandi et al. (2013), Assi et al. (2019) and Mbah (2012), respectively.

Statistical analysis

Descriptive statistics of the data coupled with inferential parameters were used to describe and interpret the information obtained from the data. Analysis of variance (ANOVA) was performed on log-transformed (for data uniformity and better results) data, followed by a post-hoc Tukey HSD test for inter-group differences in environmental and soil parameters. All soil and vegetation variables were subjected to Principal component analysis (PCA) to distinguish relationships between them and produce a limited number of principal components (PCs) that represented the key sources of heterogeneity in the soil dataset. To achieve a better understanding of invasion effects on soil (some PCs can be difficult to read if loadings on all variables within a PC are not very high), we performed split-plot ANOVA to use all PCs and variables with the highest loading on each PC as dependent variables. The split-plot ANOVA was used in order to investigate the impact of site types (3-site types, with *C. procera*, *X. strumarium* or *P. hysterophorus*, including both native and invasive plots; whole plot factor), locations (2-locations –dominated by native and invasive species; whole-plot factor) and invasion (2-plot types within sites– invaded vs. native; split-plot factor), and their interaction on soil physiochemical characteristics, density (D. ha^{-1}) and cover per hectare (C. ha^{-1}) respectively (Stefanowicz et al., 2017). In addition, contrast analyses were also performed for the comparison of invaded and non-invaded sites separately to measure the effects of invasion at each level. All the statistical analyses interpreted in this research paper were performed using the software's PAST (ver. 2.17) and SPSS (ver. 22).

Results

The floristic diversity revealed a total of 105 species belonging to 43 families at invaded and non-invaded study sites. Species richness was found higher in invaded sites comprising of 52 different species as compared to native vegetation (40 species). Species

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distribution was uneven among the families e.g., Asteraceae (17 species) followed by Poaceae (11 species), Fabaceae (7 species), Euphorbaceae and Amaranthaceae each with 6 species were the families of high species richness. Brassicaceae and Solanaceae were represented by 5 species, Polygonaceae and Lamiaceae with 4 species and Rhamnaceae and Moraceae with 3 species each. However, the remaining families contributed only two or one species. The invaded communities were dominated by *P. hysterophorus* and *X. strumarium* with a mean importance value of 47.49 ± 3.52% and 46.36 ± 1.5% respectively. In contrast, *C. procera* occurred with very low importance values (0.16 ± 0.15% and 0.59 ± 0.43%) in these invaded sites. The non-invaded communities in all the studied sites were dominated by *C. procera* with a mean importance value of 22.71 ± 3.81%, where the invasive species were found in all sites almost competing with 17.23 ± 1.56% and 15.38 ± 1.78% of importance values. The invasive *Xanthium* and *Parthenium* were overlapping with the native species in all the non-invaded sampling sites (*Table 1*). The vegetation study also revealed that the selected invasive species were completely dominating in the invaded area (IVI ≥45%). In contrast, in the native sites, these are the strong competitors of the native species and were found overlapping in the vegetation affecting the whole structure of the communities.

Similarly, we also observed clear differences in soil physiochemical characteristics between native and invaded sites (*Table 2*). Among the environmental variables, elevation and aspect angle did not differ significantly between the invaded and non-invaded sites. Concerning soil texture properties, the invaded sites represent the highest percent of sand (31.4 ± 3.3 and 41.1 ± 2.6 for *X. strumarium* and *P. hysterophorus* respectively) and silt (42.8 ± 4.1 and 35.1 ± 2.1 for *X. strumarium* and *P. hysterophorus* respectively) compared to clay content (25.7 ± 2.4 and 27.9 ± 2.4 for *X. strumarium* and *P. hysterophorus*, respectively) which shows a significant variation ($p < 0.05$) between invaded and non-invaded sites. Invaded sites exhibited higher levels of phosphorus (P), organic matter (OM %), total carbon (TC), and electrical conductivity (EC) than native sites showing significant variations ($p < 0.05$), suggesting that invaded areas are nutrient-rich and may better sustain plant development. In contrast, some soil nutrients i.e. Potassium (K) and Nitrogen (N) show less variation among the invaded and non-invaded sites. In soil hydraulic properties saturation point (SP) and available water show significant differences among the invaded and non-invaded sites ($p < 0.05$) as revealed in figure (2).

Plant species composition differed significantly between the invaded and non-invaded sites i.e. species richness (S) having $F = 3.838$, and $p = 0.0287$ indicating a smaller number of species in native sites compared to invaded site. Similarly, Shannon index (H') and evenness index (J') also show the same significant pattern of distribution (F = 20.099, P = 1.597E-7: F = 34.649, P = 2.15E-18) between the invaded and non-invaded sites (*Fig. 2*). Moreover *X. strumarium* and *P. hysterophorus* (invaded) characterized by high density and cover per hectare when compared to the *C. procera* (non-invaded) dominated communities indicating abundance of species in the invaded sites. Three Principal Components (PCs) were selected as a representation of soil properties based on a screen plot and the strength of correlations between the PCs and original variables. The PCs explained 76.6% of the total variance in the soil data (*Table 3*). PC 1 correlated strongly with Clay, Wilting point, Field capacity, sand, and organic matter. PC 2 correlated mainly with species richness, sand, Shannon index, and available water, whereas, PC 3 correlated with available water, silts, and percent sand content.

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Table 1. *Comparison of species importance values between the non-invaded and invaded communities*

Species name	A	S	Capr (Mean±SE)	Xast (Mean±SE)	Pahy (Mean±SE)
Calotropis procera (Aiton) W.T.Aiton	Capr	N	22.81±0.72	0.59±0.43	0.16±0.15
Xanthium strumarium L.	Xast	I	15.38±1.78	46.36±1.5	2.61±0.56
Parthenium hysterophorus L.	Pahy	I	17.23±1.56	8.33±1.3	47.49±3.52
Acacia nilotica L.	Acni	Nt	0.33±0.24	-*	0.13±0.09
Aloe barbadensis Mill	Alba	C	0.11±0.11	-*	-*
Amaranthus viridis L.	Amvi	I	2.09±1.19	3.76±0.85	2.31±0.84
Artemisia absinthium L.	Arab	N	2.01±1.38	-*	-*
Asparagus gracilis Royle	Asar	N	1.38±0.94	-*	-*
Avena sativa L.	Avsa	I	3.21±1.73	-*	-*
Cannabis sativa L.	Casa	I	3.17±1.52	11.23±1.5	6.01±1.2
Carthamus oxycantha M. Bieb.	Caox	Nt	4.06±1.53	0.70±0.70	0.39±0.39
Chenopodium album L.	Chal	I	1.44±1.01	3.63±0.88	0.81±0.76
Conyza bonariensis L.	Cobo	Cs	1.78±1.26	-*	2.23±0.73
Cynodon dactylon L.	Cyda	N	6.99±1.69	2.62±0.77	17.31±2.21
Dodonaea viscosa (L.) Jacq.	Dove	I	1.14±0.81	-*	0.32±0.22
Erigeron canadensis (L.) Cronq.	Erca	I	1.83±1.19	0.48±0.36	0.36±0.35
Eucalyptus camaldulensis L.	Euca	I	1.03±0.57	-*	0.36±0.35
Euphorbia helioscopia L.	Euhe	I	2.31±1.20	-*	0.10±0.09
Ficus carica L.	Fica	N	1.03±0.52	-*	0.11±0.07
Ipomoea purpurea (L.) Roth	Ippu	I	1.004±0.72	-*	-*
Jasminum officinale L.	Jaof	I	0.61±0.61	-*	-*
Lamium amplexicaule L.	Laam	N	0.30±0.30	-*	-*
Malva sylvestris L.	Masy	I	0.56±0.56	-*	-*
Medicago denticulata L.	Mede	I	4.52±1.79	-*	-*
Melia azedarach L.	Meaz	Cu	2.63±1.15	-*	0.31±0.15
Mentha longifolia (L.) Huds	Melo	Cu	1.61±1.12	0.77±0.58	1.19±0.74
Morus alba L.	Moal	Nt	0.11±0.11	-*	1.04±1.03
Ricinus communis L.	Rico	I	1.95±1.07	-*	-*
Rumex dentatus L.	Rude	N	2.28±1.27	-*	1.15±0.49
Silybum marianum (L.) Geartn	Sima	I	1.43±0.82	2.42±0.80	-*
Solanum nigrum L.	Soni	I	1.99±1.18	0.16±0.15	0.59±0.30
Sonchus asper (L.) Hill	Soas	N	2.04±1.40	0.57±0.33	0.06±0.06
Trianthema portulacastrum L.	Trpo	I	2.52±1.14	-*	-*
Trifolium repens L.	Trre	I	1.49±1.04	2.07±0.89	0.56±0.35
Triticum aestivum L.	Trae	Cu	2.48±1.37	-*	-*
Urtica dioica L.	Urdi	N	2.39±1.35	1.57±0.33	-*
Verbascum Thapsus L.	Veth	I	1.93±0.80	0.72±0.5	0.19±0.11
Zanthoxylum armatum DC. Prodr	Zaar	N	1.31±0.91	-*	-*
Ziziphus mauritiana L.	Zima	N	1.09±0.76	-*	1.11±0.87
Alternanthera pungens Kunth	Alpu	I	-*	0.84±0.34	-*
Amaranthus spinosus L.	Amsp	I	-*	0.19±0.19	-*
Brassica campestris L.	Brca	N	-*	0.84±0.44	0.53±0.39
Capsella bursa pestoris (L.) Medik	Cabp	I	-*	1.19±0.5	0.18±0.13
Cenchrus ciliaris L.	Ceci	N	-*	0.35±0.34	-*
Calendula arvensis L.	Caar	I	-*	1.81±0.75	-*
Cassia occidentalis L.	Caoc	I	-*	0.70±0.70	-*
Cyprus rotundus L.	Cyro	N	-*	0.41±0.23	1.61±1.05
Chrozophora tinctoria (L.) Raf.	Chti	N	-*	0.21±0.20	-*

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Species name	A	S	Capr (Mean±SE)	Xast (Mean±SE)	Pahy (Mean±SE)
Datura metel L.	Dame	I	-*	3.11±1.3	0.060±0.02
Eclipta alba L.	Ecal	N	-*	0.58±0.41	-*
Helianthus annuus L.	Hean	I	-*	0.13±0.12	-*
Heliotropium curassavicum L.	Hecu	N	-*	0.11±0.10	-*
Justicia adhatoda L.	Juad	N	-*	0.91±0.62	-*
Lepidium sativum L.	Lisa	N	-*	0.88±0.36	-*
Mirabilis jalapa L.	Mija	N	-*	0.77±0.58	0.09±0.08
Oxalis corniculata L.	Oxca	Nt	-*	0.14±0.14	2.33±0.7
Polygonum aviculare L.	Poav	I	-*	0.16±0.16	0.11±0.11
Physalis minima L.	Phmi	N	-*	2.42±0.80	-*
Tagetes erecta L.	Taer	I	-*	1.42±1.08	-*
Tagetes minuta L.	Tami	Nt	-*	1.04±1.03	0.56±0.35
Tribulus terrestris L.	Trte	N	-*	0.10±0.09	0.05±0.04
Zea mays L.	Zema	Cu	-*	0.17±0.17	1.11±0.87
Achyranthes aspera L.	Acas	N	-*	-*	0.06±0.05
Ailanthus altissima L.	Alal	I	-*	-*	2.31±0.84
Broussonetia papyrifera (L.) L'Herit. ex Vent	Brpa	I	-*	-*	0.22±0.20
Centaurea cyanus L.	Cecy	N	-*	-*	0.53±0.39
Cirsium arvense (L.) Scop.	Ciar	I	-*	-*	0.81±0.76
Convolvulus arvensis L.	Coar	N	-*	-*	0.41±0.32
Cucurbita pepo L.	Cupe	Cu	-*	-*	0.18±0.13
Desmostachya bipinnata L.	Debi	I	-*	-*	0.044±0.02
Dichanthium annulatum (Forssk).	Dian	I	-*	-*	1.61±1.05
Dysphania ambrosioides	Dyam	I	-*	-*	0.52±0.37
Eichhornia crassipes (Mart.) Solma in DC	Eicr	I	-*	-*	0.33±0.14
Eragrostis minor L.	Ermi	N	-*	-*	0.060±0.02
Eryngium coeruleum M.Bieb.	Erco	N	-*	-*	0.36±0.35
Euphorbia hirta L.	Euhi	N	-*	-*	0.076±0.07
Euphorbia prostrate L.	Eupr	I	-*	-*	1.03±0.73
Fragaria indica L.	Frin	N	-*	-*	0.24±0.16
Hackelia virginiana L.	Havi	I	-*	-*	0.13±0.13
Hibiscus mutabilis L.	Himu	I	-*	-*	0.69±0.34
Indigofera gerardiana L.	Inge	N	-*	-*	0.08±0.06
Mangifera indica L.	Main	N	-*	-*	0.06±0.01
Mallotus Philippines L.	Meph	I	-*	-*	0.09±0.08
Mentha spicata L.	Mesp	I	-*	-*	0.07±0.06
Narcissus tazetta L.	Nata	I	-*	-*	0.14±0.11
Nasturtium officinale W.T. Aiton	Naof	Nt	-*	-*	0.15±0.11
Persicaria maculosa S.F.Gray	Pema	N	-*	-*	0.15±0.07
Phragmites karka (Retz). Trin. Ex Saud	Phka	I	-*	-*	2.33±0.77
Pinus roxburghii L.	Piro	N	-*	-*	0.11±0.11
Poa annua L.	Poan	N	-*	-*	0.21±0.13
Populus nigra L.	Poni	I	-*	-*	0.06±0.06
Prosopis juliflora (Sw.) DC.	Prju	I	-*	-*	0.21±0.16
Robinea pseudoacacia L.	Rops	I	-*	-*	0.20±0.11
Rubus fruticosus L.	Rufr	N	-*	-*	1.15±0.49
Rumex hestatus L.	Ruhe	I	-*	-*	0.04±0.02
Salvia moorcroftiana Wall. Ex. Benth	Samo	N	-*	-*	0.16±0.11

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Species name	A	S	Capr (Mean±SE)	Xast (Mean±SE)	Pahy (Mean±SE)
Sisymbrium officinale (L.) *Scop.*	Siof	I	-*	-*	0.76±0.64
Solanum melongena L.	Some	N	-*	-*	0.15±0.12
Solanum xanthocarpum (SX). Schard and Wendl.	Soxa	N	-*	-*	0.042±0.01
Sonchus oleraceus L.	Sool	N	-*	-*	0.59±0.30
Taraxicum officinale Weber	Taof	I	-*	-*	0.06±0.06
Trianthema portulacastrum L.	Trpo	I	-*	-*	0.065±0.06
Triticum aestivum L.	Trae	N	-*	-*	0.05±0.04
Ziziphus nummularia (Burm.f.). Wight and Arn.	Zinu	I	-*	-*	0.047±0.01
Zizipus oxyphyla L.	Ziox	I	-*	-*	0.047±0.02

Note: A (Acronyms): S (Status): N(Native): Nt (Naturalized): I(Invasive): Cu (Cultivated): values are presented as mean and standard error: -* (absence of species)

Table 2. *Comparison of environmental drivers and diversity indices in the plant communities of invaded and non-invaded sites*

Factors	*Capr*	*Xast*	*Pahy*	F-value	P-value
	Mean+SE	Mean+SE	Mean±SE		
Lat	34.33±0.06[a]	34.20±0.45[a]	34.708±0.03[a]	1.684	0.194
Long	72.382±0.22[a]	71.81±0.51[a]	72.100±0.36[a]	0.502	0.684
Elev	841±42[a]	822±50[a]	802±37[a]	1.376	0.3011
AA	160±20[a]	159±20[a]	190±24[a]	0.98	0.3806
Cl%	24.7±2.6[a]	25.7±2.4[a]	27.9±2.4[a]	1.998	0.14
Si%	32.5±2.4[ab]	42.8±4.1[a]	35.1±2.1[b]	3.1395	**0.051***
Sa%	36.56±2.4[b]	31.4±3.3[b]	41.1±2.6[a]	3.01	**0.0484***
pH	6.7±0.1[a]	6.71±0.08[a]	6.93±0.11[a]	1.029	0.3631
OM%	0.91±0.1[c]	1.08±0.1[b]	2.29±0.23[a]	18.912	**3.27E-7*****
N	0.12±0.02[ab]	0.04±0.09[b]	0.127±0.1[a]	4.006	0.0911
P	4.48±0.2[a]	4.5±0.23[a]	3.30±0.51[b]	3.8413	**0.0327***
K	90.5±7.9[b]	108±8.4[a]	104.7±8.0[a]	1.607	0.2083
EC	281±14[c]	294±20[b]	365±1.8[a]	11.1	**6.94E-05*****
TC	1.49±0.07[b]	1.88±12[a]	0.985±0.12[c]	18.028	**4.64E-01*****
WP	0.17±0.07[a]	0.15±0.01[a]	0.15±0.02[a]	2.9981	0.3741
FC	0.30±0.08[a]	0.31±0.01[a]	0.28±0.01[a]	1.5971	0.2744
SP	0.49±0.01[b]	0.48±0.05[a]	0.47±0.07[a]	2.9981	**0.051***
AW	0.13±0.04[b]	0.14±0.06[a]	0.039±0.03[b]	3.398	**0.038***
S'	5.603±0.22[c]	7.52±0.56[b]	9.12±0.88[a]	3.838	**0.0287****
H	1.769±0.03[a]	1.39±0.05[b]	1.18±0.08[c]	20.099	**1.597E-7*****
J	0.94±0.05[a]	0.71±0.09[b]	0.56±0.03[c]	34.649	**2.15E-18*****
D/ha	3067±807[b]	11697±55[c]	8193±230[a]	8.522	**0.0029****
C/ha	3866±785[c]	12318±68[a]	7282±128[b]	11.113	**2.41E-02*****

Note: Native plots were compared with invasive plots of *Xanthium strumarium* and *Parthenium hysterophorus* using One-way ANOVA followed by Tukey's HSD test. A significant difference was shown by different letters. SD (standard deviation), Lat (latitude), Long (longitude), Ele (elevation), AA (aspect degree), OM (organic matter), N (nitrogen), P (phosphorous), K (potassium), EC (electrical conductivity), TC (total carbon), WP (welting point), SP (saturation point), AW (available water), S' (species richness), H' (Shannon index), J' (evenness index), D/ha (Density ha^{-1}), C/ha (Cover ha^{-1})

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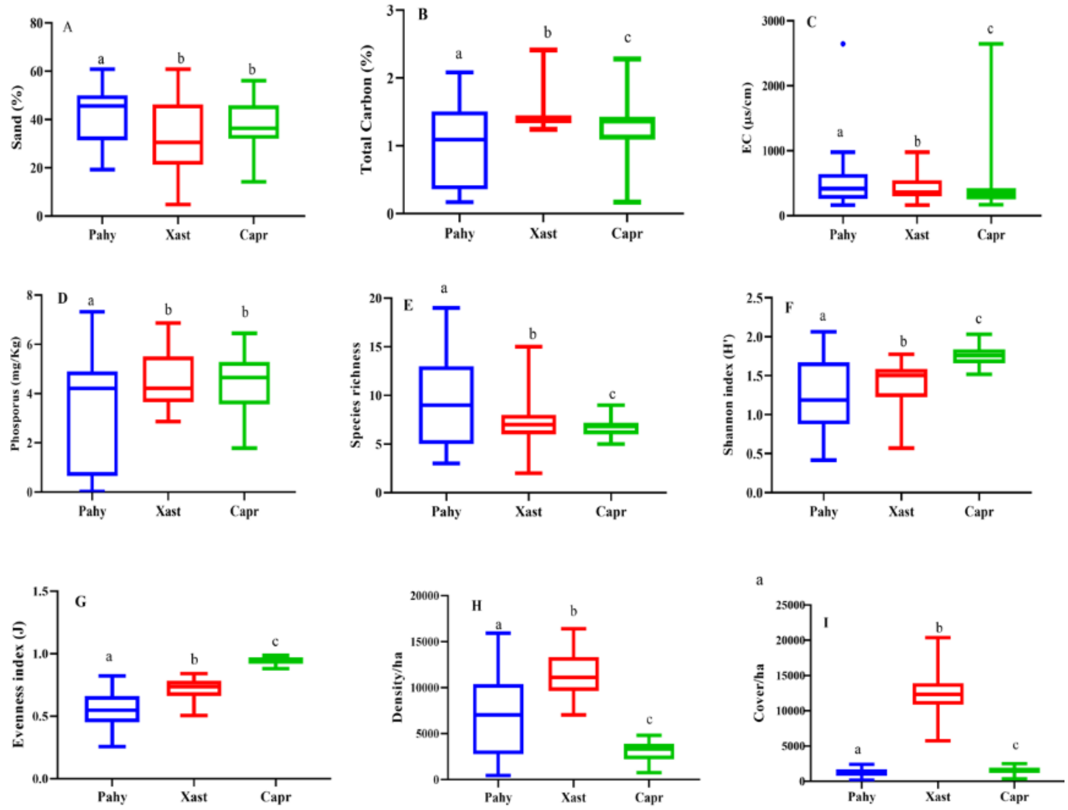

Figure 2. *Box-plot diagrams for comparison of significant values among the native and invasive plots. Different letters indicate significant difference between the native and invasive species (Calculated through post-hoc Tukey HSD test at p<0.05). A. % of Sand: B. % of Total Carbon: C. Electrical conductivity (µs/cm): D. Phosphorus (mg/Kg): E. Species Richness: F. Shannon index (H'): G. Evenness index (J): H. Density ha*$^{-1}$*: I. Cover ha*$^{-1}$

Table 3. *Three major axis of principal components with its explained variance and loading value of associated environmental variables*

Factors	% variance explained	Variables loading (with value >0.6)
PC-1	37.0	Clay (0.979), WP (0.965), FC (0.864), SP (0.932), Sand (-0.796), EC (-0.829), OM (-0.733)
PC-2	15.7	S (0.897), Sand (0.858), H' (0.832), Ele (0.723), AW (-0.788), Si (-0.721), J' (0.785), D ha^{-1} (0.727), OM (0.635), N (0.666), C ha^{-1} (-0.788)
PC-3	23.5	AW (0.965), Sand (-0.920), Silt (0.899)

Cumulative variance percentages along the PCA axis are presented in *Table 4*, showing that 22 axes cumulatively show 100% variance of the entire axis and can be considered for variation. The 1st four axes showed the highest cumulative variance (44.5%) having 9.2-13.3% of variance for a single axis. Plant invasions strongly reduced the number of native plant species and their cover while total plant cover remained unaffected (*Tables 3 and 4*). As far as soil properties are concerned, most variables were unaffected by invasion or by its interaction with site type or location (*Tables 5*). PCA diagram showed that *C. procera* vegetation was strongly affected by *X. strumarium* and

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P. hysterophorus community invasions along with the soil characteristics (*Fig. 3*). Plant invasion effects on PC2 and available water depended on invasive species as indicated by significant invasion × site type interactions (*Table 5*). Contrast analysis revealed that PC2 changed due to the invasion compared to control. Still, the direction of the changes differed between species (a decrease in the former species and an increase in the latter). The only significant invasion × location interaction was observed in PC2 (*Table 5*), but contrast analysis did not confirm that the invasion impact differs between locations.

Table 4. *Percentage cumulative variance and the axes of Principal Component (PC) of different environmental variables associated with invaded and non-invaded communities*

Axis	**Eigen-values**	**% variance explained**	**Cumulative variance**	**Broken-stick Eigenvalues**
X_1	151.8	13.3	13.3	141.1
X_2	137.6	12.1	25.4	106.6
X_3	112.8	9.9	35.3	89.3
X_4	104.5	9.2	44.5	77.8
X_5	94.6	8.3	52.8	69.2
X_6	83.9	7.4	60.2	62.3
X_7	74.3	6.5	66.7	56.5
X_8	65.3	5.7	72.4	51.6
X_9	57.9	5.1	77.5	47.3
X_{10}	45.2	4.0	81.5	43.5
X_{11}	43.7	3.8	85.4	40.0
X_{12}	32.4	2.8	88.2	36.9
X_{13}	29.3	2.6	90.8	34.0
X_{14}	24.2	2.1	92.9	31.3
X_{15}	22.3	2.0	94.8	28.9
X_{16}	18.8	1.7	96.5	26.6
X_{17}	11.5	1.0	97.5	24.4
X^{18}	9.9	0.9	98.4	22.4
X_{19}	8.9	0.8	99.2	20.5
X_{20}	4.5	0.4	99.6	18.7
X_{21}	3.5	0.3	99.9	16.9
X_{22}	1.6	0.1	100.0	15.3

Table 5. *The effects of site type, location, invasion, and their interactions portrayed by the first three principal components (PCs), soil and vegetation characteristics using split-plot analysis of variance (ANOVA)*

Variables	Site type		Location		Invasion		Site type ×Location		Invasion ×Site type		Invasion ×Location		Invasion ×Site type ×Location	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
PC 1	**34.00**	**<0.00**	1.50	0.31	0.16	0.24	6.30	**0.00**	0.94	0.64	0.85	1.74	1.92	0.3
PC 2	1.35	0.106	**6.03**	**0.00**	**2.30**	**0.01**	1.43	0.45	**7.23**	**0.00**	0.44	0.391	1.85	0.26
PC 3	0.293	0.232	0.4	0.51	0.30	0.36	**5.30**	**0.00**	0.22	1.43	0.80	0.421	1.90	0.31
Clay	**5.87**	**0.004**	0.54	0.4	1.34	0.26	**8.96**	**0.00**	16.8	3.08	**6.70**	**0.008**	1.31	0.54
S	0.487	0.044	1.48	0.09	**11.3**	**<.01**	1.33	0.11	0.38	1.06	1.47	0.810	**15.1**	**<.01**
Aw	1.97	0.24	0.93	0.57	0.49	0.14	1.08	0.40	**5.87**	**0.00**	0.97	0.567	**10.5**	**0.00**
D h^{a-1}	**12.54**	**<0.00**	0.74	0.24	**30.4**	**<.00**	1.99	0.64	2.49	1.91	**2.87**	**0.010**	0.42	0.82
C ha^{-1}	**4.65**	**0.015**	1.65	0.19	0.40	0.70	1.76	0.97	0.56	0.46	0.93	0.579	0.47	0.88

Note: Soil physiochemical variables were chosen based on the principal component analysis. Significant values (p<0.05) are shown in bold

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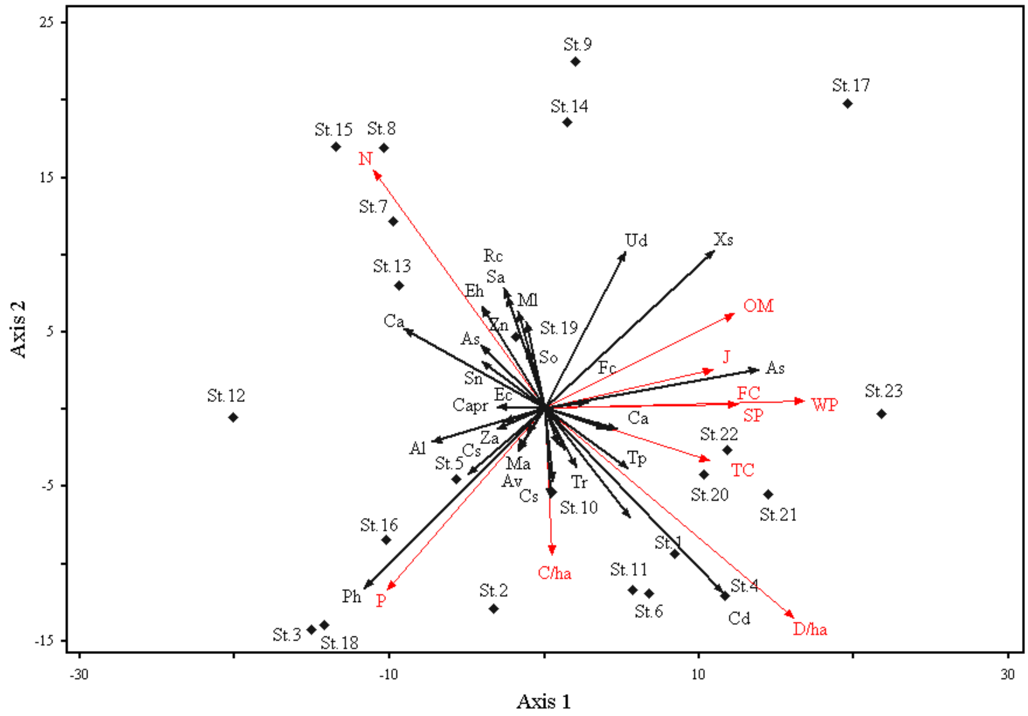

***Figure 3.** PCA-ordination showing the effect of environmental variables on plant communities*

Discussion

Invasion of plant species causes significant changes in soil physio-chemistry and vegetation characteristics, and invasive species are frequently found to disrupt native vegetation quickly and intensely (Ullah et al., 2021) The invaded sites demonstrated low IVI of *C. procera* and species diversity revealing that invasive species have negatively affected the native plant communities. These results were in compliance with the findings reported by Grice (2006); Mc Fadyen (1992); Sakai et al. (2001) for Australia, India, and Ethiopia, where the native and associated species were found to be strongly influenced by the invasion of invasive species. Invaded sites have a more diversified floristic composition (70 plant species) compared to non-invaded areas (45 plant species) presumably because invasion improves soil nutrient retention, which supports various heterogeneous populations. Physiochemical soil characteristics supported these impacts, showing that invaded areas had greater nutrient levels than non-invaded sites. Similarly, diversity indices are linked and reflect the theory proposed by Bimova et al. (2004), Dunbar and Facelli (1999), Kohli et al. (2004), and Pysek and Pysek (1995) in their findings. Our target invasive species was discovered near other invasive species such as *Silybum marianum* and *Amaranthus caudatus*, complementing Timsina et al. (2011), that disturbed communities' overlap, increasing the threat to native plant species. Changes in the physiochemical properties of soils caused by invading species are also considered feedback mechanisms with various eco-physiological manifestations, increasing the chances of invasive species establishment and propagation, and eventually posing a threat to native species (Stefanowicz et al., 2017). These changes illustrate how introduced plants will expand, disturb the natural ecology, and promote their role in causing human health and economic disruption (Charles and Dukes, 2008). In contrast, some authors are of the view that invasive species may be economically and ecologically beneficial in

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proportion to their negative consequences (Mack et al., 2000). Therefore, it is difficult to assume that the modification by invasive plants of the physiochemical characteristics of soil will always be lethal, harmful, or just beneficial, depending on the circumstances (Pimentel et al., 2000). The rise of nutrients in the soil area would also encourage many more plant species to thrive and spread with invasive plants that increase their diversity.

The findings clearly show significant disparities in soil properties between invaded and non-invaded locations, indicating that invasive species alter soil qualities in order to establish themselves in a new region, disrupting native plant populations and allowing invaders to expand rapidly. Similarly, Soti and Jayachandran (2016) reported that *lygodium microphyllum,* an invasive species in Florida, has changed and increased soil nutrients in areas of three different locations. Plant growth and productivity require organic matter in the soil (Lehmann and Kleber, 2015). Invaded sites with *X. strumarium* and *P. hysterophorus* had more organic matter than non-invaded sites compared to the native species *C. procera*, which might be due to quicker decomposition of soil litter in the root region caused by invasive plants, as advocated by Duda et al. (2003). Due to the differences in eco-physiological processes between invasive and non-invasive plants, invasive sites exhibit greater levels of nitrification and nitrogen mineralization than non-invaded sites (Hibbard et al., 2001; Ehrenfeld et al., 2001; Windham and Ehrenfeld, 2003). Our findings showed that invaded sites had higher nitrogen concentrations than non-invaded sites, owing to a faster decomposition rate and the process of nitrification may possibly enhance the process in the root region which might possibly cause increased soil fertility (Borken and Matzner, 2009). Changes in carbon and nitrogen concentrations would have a major influence on recycling, which may be as a consequence of invasive plant species' invasion and establishment in new habitats (Horgan et al., 2014). Hughes and Uowolo (2006) record a higher rate of invasion and a different mode of plant growth in an environment invaded by invasive plant species compared to the non-invaded area, which is in compliance with the findings of our results.

The pH differences between invaded and non-invaded sites were found to be inconsistent. According to Osunkonya and Perret (2011), invaded sites have a higher pH than non-invaded sites, which is consistent with our findings in urban and rural areas. Higher pH was found to increase cation concentration, allowing nutrient absorption for faster plant growth and production (James et al., 2005). The shift in pH of roadside soil, on the other hand, is in the opposite direction, confirming Kuotika et al. (2011). Many scholars, including Dassonville et al. (2007) for *Fallopia japonica*, Herr et al. (2007), Scharfy et al. (2009), and Quist et al. (2014) for *Solidago gigantean* and Maurel et al. (2010) for *Reynoutria japonica*, have documented this inconsistency of pH changes. Gordon (1988) reported that invasive species have greatly altered soil organic matter and texture, which ultimately have an effect on soil pH that can be easily detected.

Conclusion and recommendation

The study revealed higher species diversity in invaded areas than non-invaded sites and a higher concentration of nutrient and physiochemical characteristics. Thus, the increase in nutrients leads to an increase in the density and population of invasive species, thereby decreasing the native population of *Calotropis procera,* which is progressively be reduced in the region and may be endangered shortly. Many other factors may also contribute to threatening the native plant species, which may be the environmental conditions that may be better utilized by the invasive species and the plant's internal

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genetics, which is essential irrespective of the environmental conditions. The study recommends that the local and federal governments conduct extensive monitoring and preventive measures to eradicate and control these invasive species, which will not only flourish native species but also regulate the other ecosystem services. Here we recommend further detailed studies on the invasive behaviors of *X. strumarium* and *P. hysterophorus* and their impacts on soil micro-biota, nutrient pools in plant biomass and the content of secondary metabolites in both homogenous (in control environment) and heterogeneous environments. Likewise, we suggest investigating the seasonal changes in the influence of plant invasion on soil, which might be helpful, especially on the range of impacts of these invasive taxa and the overall magnitude of their increasing threats.

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