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Paired plot experiments to assess impact of invasive species on native floral diversity in Pakistan

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The threat of invasion is growing globally and endangers biodiversity. Exotic invasive plants are putting a harm to the vegetation of Pakistan's Pothwar region, which is a biodiversity hotspot. In the current study, the effects of *Broussonetia papyrifera*, *Parthenium hysterophorus, Xanthium strumarium*, and *Lantana camara* on the local flora in the Pothwar area were examined. Two categorical groups (invaded and non-invaded) were used in a dichotomized experimental design to collect data. Using the software PRIMER-7 and IBM SPSS v. 21, different diversity indices including Margalef's index of species richness, Shannon index of diversity, and Simpson index of dominance were measured and compared between invaded and control plots. In comparison to the experimental plots, the control plots had an average of more individuals and diversity. On a multivariate scale, non-metric multidimensional scaling (nMDS) and analysis of similarity (ANOSIM) revealed higher species richness in control plots. The invasion effect of *L. camara* was the highest, followed by *X. strumarium, P. hysterophorus,* and *B. papyrifera.*

KEYWORDS

invasion impacts, diversity indices, multivariate analysis, primer, conservation

Introduction

Biological invasion can be defined as "distribution of species to previously inhospitable habitats, followed by their proliferation, spread, and persistence, as well as detrimental impacts on biodiversity, health, and/or the economy". One form of biological pollution is biological invasion. Biopollution is the result of the influence of non-native species on the ecosystem, including habitat degradation and modifications, the spread of infections, competition with and extinction of native species, and population genetic changes (Holm et al., 1991; Alpert, 2006). Exotic fauna, flora, insects and other living things can all be categorized as biological pollutants, but because of their massive biomass, plants offer the largest concern (Florece and Baguinon, 2011). Plants make up 32 of the top 100 invasive species worldwide (Holzmueller and Jose, 2009). Global economic activities including employment and travel are speeding up the spread of invasive species (Pysek and Hulme, 2005).

Invasive plants diminish agricultural production, alter native flora, endanger human and animal health, disturb ecosystem processes (hydrology, soil nutrient composition), and spread vector-borne diseases (Etana, 2013; Qureshi et al., 2014). Invasive species are the second leading cause of biodiversity loss after habitat degradation because of their ability to outcompete and displace native species (Gaertner et al., 2009).

Seventy-three vascular plant species have been identified as invasive in Pakistan (Qureshi et al., 2014). Some of the most significant invaders in Pakistan include *Parthenium hysterophorus*, *Lantana camara*, *Broussonetia papyrifera*, *Eucalyptus camaldulensis*, *Xanthium strumarium*, *Prosopis juliflora*, *Eicchornia crassipes*, *Leucanea leucosephala*, and *Salvinia molesta*. The goal of this research was to see how much of an impact the world's top four invaders had on diversity indices in Pakistan's Pothwar region. Exotic invaders included *P. hysterophorus, L. camara, B. papyrifera*, and *X. strumarium*.

Materials and methods

Research study area

Between the Indus and Jhelum Rivers, the Pothwar Zone is located between latitudes 32.5°N and 34.0°N and longitudes 72°E and 74°E. The cities of Jhelum, Islamabad, Attock, Rawalpindi, and Chakwal are included in the Pothwar zone. The Pothwar area has a harsh environment, with scorching summers and chilly winters. In this location, the average annual rainfall is 812 mm. The mean maximum temperature is in June (37°C) and the coldest month being January (14–18°C).

Experimental design

Calculations and comparisons of diversity indices for selected invaders were made in the five regions, individually (Attock, Chakwal, Jhelum, Islamabad, and Rawalpindi). Six paired vegetation plots, each measuring 3.16 m × 3.16 m, were randomly selected from each district and were labelled as either invaded or uninvaded. The "treatment" consisted of an invaded vegetation plot (referred to as "invaded plot") where the invader demonstrated dominance, whereas the "control" consisted of an uninvaded vegetation plot (referred to as a "non-invaded plot") where the invader displayed no dominance. Using the Flora of Pakistan (https://www.eflora.com), the collected plant specimens were identified from the selected plots. World flora online (http://www.worldfloraonline.org/) was used to locate the right scientific names, and APG IV 2016 was used to determine the right family name. The data from five districts were pooled and presented as a whole.

Data analyses

With the use of the PRIMER-7 program, diversity indices such as Margalef's index of richness, Shannon-Weaver index of diversity, and Simpson index of dominance were created and compared for control and invaded plots. To verify if sampling was adequate in each area, PRIMER was used to create rarefaction curves (Clarke and Warwick, 2001). Both univariate and multivariate methods of data analysis were applied, including the non-metric multidimensional scaling method. Districts and invasion status were taken into account when conducting ANOVA on the diversity variables using SPSS. The significance of dissimilarities among invasion and control plots between diversity catalogues for each of the five districts was examined separately. The analysis of species collections was carried out using PRIMER software and non-metric multidimensional scaling in two to three dimensions with invasion status as factor. We were able to determine the range of clustering of sites and locations in response to invasion using analysis of similarity (ANOSIM) and similarity percentage (SIMPER). The mean difference of species between and within sites is used by ANOSIM to calculate the global statistic. According to SIMPER, the species that were most prevalent were also the ones that contributed the most to the average community dissimilarity (invaded and control plots).

Results

To gauge sample completeness, rarefaction curves, were developed with the results indicating that sampling was satisfactory (Figures 1A–D). For four of the analyzed invaders, control plots had better average species diversity and richness per plot (Figures 2A–D).

Comparison of diversity indices revealed significant difference between districts and invasion status. In the case of *P. hysterophorus*, control plots had an average of 6.033 ± 1.75 species. This was higher than the invasion plots (5.133 ± 1.83). In the control and invaded plots, a total of 181 and 154 individuals were recorded, respectively. Similarly, abundance in the control and invaded plots differed by 3.7 ± 3.83. Diversity indices for *L. camara* indicated variation across locations and invasion status. Control plots harbored on average 13.90 ± 3.50 species. This was by 1.734 ± 0.14 more than invaded plots. In total, 212 and 139 individuals were recorded in control and invaded plots respectively. Similarly, abundance in control and invaded plots differed by 2.3 ± 1.80. Control plots exhibited higher values of species richness by a difference of 0.15 ± 0.41, Shannon index of diversity by 0.20 ± 0.40 and Simpson index

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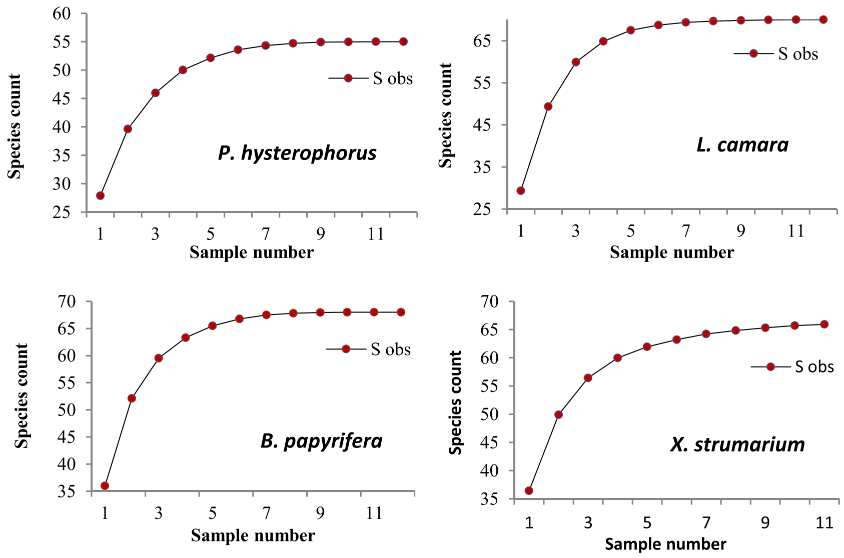

FIGURE 1
A rarefaction curve illustrates the total number of species observed.

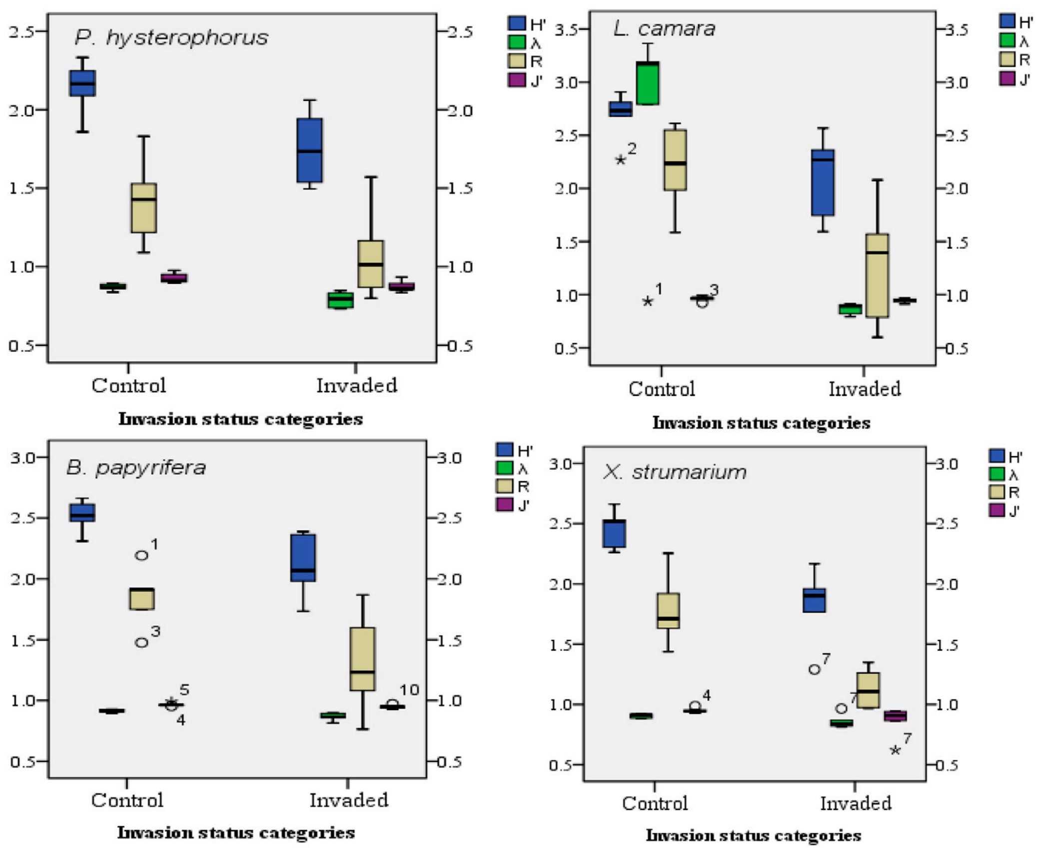

FIGURE 2
R = Margalef's index of species richness; H' = Shannon index of diversity; J' = Species evenness; λ = Simpson index of dominance.

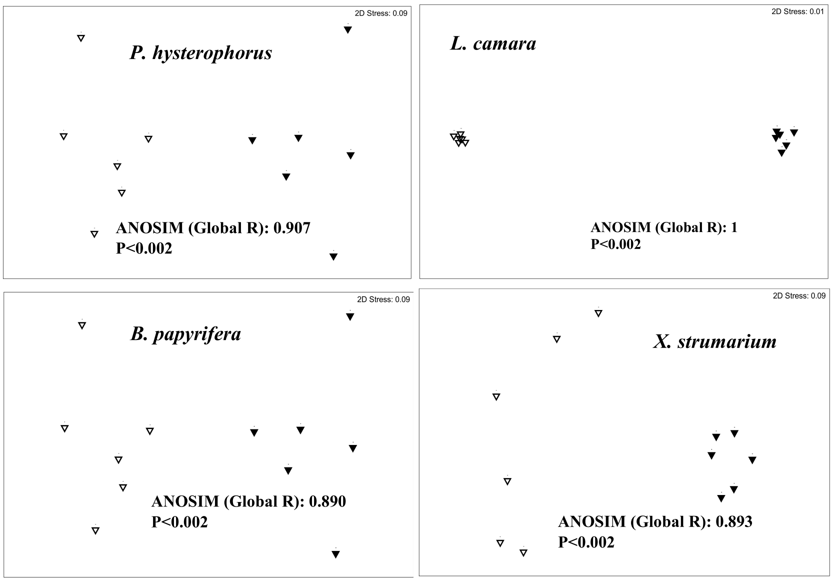

FIGURE 3
Invasion status data multidimensional scaling (MDS) ordination and assessments of resemblance (ANOSIM) effects for the Pakistani region of Pothwar (open symbols are for control, uninvaded plots, and closed symbols are for invaded plots).

of dominance by 0.22 ± 1.27. In case of *B. papyrifera*, comparisons of diversity indices showed differences in invaded and control plots across sites. Control plots harbored on average 9.07 ± 2.50 species. This was by 3.54 ± 2.08 more than invaded plots. In total 298 and 156 individuals were found in control and invaded plots respectively. Similarly, abundance in control and invaded plots differed by 2.97 ± 3.96 and the difference was significant. Control plots exhibited higher values of species richness by a difference of 0.89 ± 0.53; Shannon index of diversity by 0.5 ± 0.29 and Simpson index of dominance by 0.081 ± 0.042. For *X. strumarium*, comparisons of diversity indices showed difference across sites and invasion status. Control plots harbored on average 10.86 ± 2.50 species. This was by 2.86 ± 2.07 more than invaded plots and the difference was significant. In total, 226 and 140 individuals were found in control and invaded plots respectively. Similarly, abundance in control and invaded plots differed by 2.97 ± 3.93; species richness by 0.89 ± 0.53, Shannon index of diversity by 0.90 ± 0.29 and Simpson index of dominance by 0.18 ± 0.09.

Significant magnitude variations between the species composition of the invaded and control plots were shown at the multivariate scale by ordination (nMDS) and ANOSIM (Figure 3).

The species that are primarily responsible for the average variation between the control and occupied ploats were identified by calculating similarity percentage. The top species in Table 1 that distinguish invaded plots from non-invaded plots are listed.

Discussion

P. hysterophorus is annual herb native to Mexico, the southern United States, and South and Central America. Due to its global presence, it is ranked among the top 10 worst weeds on the planet (Tamado and Milberg, 2000; Khan et al., 2014; Qureshi et al., 2018). In the current study, analyses of the diversity indices between the invaded and control plots showed noticeable changes in the diversity of the plants. These findings are in line with those of Riaz and Javaid (2011), Shabbir and Bajwa (2007), Ojija et al. (2021) and Ojija & Lutambi (2022) all of which report changes in the vegetation composition of the invaded plots. Crop output, biodiversity, and human and animal health are all impacted by parthenium (Shabbir, 2013). By displacing native species and forming massive monocultures, *P. hysterophorus* significantly alters natural habitats. Using the ordination (nMDS) and ANOSIM techniques, significant differences were identified between invaded and control plots at each of the five study locations, but Jhelum revealed the most. According to Upadhyay et al. (2013), *P. hysterophorus* plants have been found to thrive in more salinized soil, which is detrimental to many native plant species. Because of this, Jhelum's salinity-rich soil may be to blame for the increasing invasion impacts there. When compared to control plots, invading plots had lower levels of species dominance, according to SIMPER analysis.

TABLE 1 SIMPER analysis of district-level data for P. hysterophorus invaded and control sites

Average dissimilarity = 60.14%					
Average abundance					
Species	Control	Invaded	Av. Diss	Diss./SD	Contribution (%)
Poa annua	2.94	0.00	2.38	8.06	3.95
Lathyrus aphaca	0.00	2.69	2.18	5.82	3.63
Solanum miniatum	2.47	0.00	2.00	7.85	3.32
Ricinus communis	2.19	0.00	1.77	2.05	2.95
Convolvulus arvensis	1.80	1.79	1.49	1.32	2.48
Taraxacum officinale	1.77	0.00	1.40	2.07	2.32
Rosa damascena	1.82	0.18	1.38	1.41	2.29
Tribulus terrestris	1.62	0.00	1.31	2.03	2.18
Fumaria indica	2.35	1.15	1.31	1.55	2.18
Tephrosia purpurea	0.00	1.63	1.29	1.36	2.15
District-level SIMPER analysis for L. camara invaded and control sites. Average dissimilarity = 65.56%					
Average abundance					
Species	Control	Invaded	Av. Diss	Diss/SD	Contribution (%)
Stellaria media	3.04	1.71	1.38	7.99	2.10
Oxalis corniculata	2.98	0.00	1.35	9.94	2.06
Cynodon dactylon	2.81	1.82	1.27	9.48	1.94
Digitaria ciliaris	2.74	0.00	1.24	6.40	1.89
Malva parviflora	2.70	0.00	1.22	7.69	1.86
Croton tiglium	2.65	1.77	1.20	9.38	1.83
Eclipta prostrata	2.65	0.18	1.19	12.44	1.82
Clematis grata	2.54	1.62	1.15	6.88	1.76
Chenopodium album	2.46	2.35	1.12	4.51	1.71
Calotropis procera	2.43	0.01	1.11	5.98	1.69
District-level SIMPER analysis for B. papyrifera invaded and control sites.Average dissimilarity = 57.19%					
Average abundance					
Species	Invaded	Control	Av. Diss	Diss/SD	Contribution (%)
Tribulus terrestris	2.90	0.00	1.63	7.24	2.85
Malvastrum coromandelianum	2.57	0.00	1.46	4.14	2.55
Cynodon dactylon	2.44	0.00	1.36	1.91	2.38
Silybum marianum	0.81	2.69	1.12	1.54	1.97
Calotropis procera	2.02	0.00	1.12	1.89	1.96
Datura innoxia	1.98	0.00	1.04	1.33	1.82

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TABLE 1 (*Continued*) **SIMPER analysis of district-level data for P. hysterophorus invaded and control sites**

Average dissimilarity = 60.14%					
Average abundance					
Species	Control	Invaded	Av. Diss	Diss./SD	Contribution (%)
Digeria muricata	1.94	0.00	1.02	1.36	1.79
Kochia indica	1.40	3.12	1.01	1.22	1.77
Desmostachya bipinnata	2.96	1.25	1.00	1.29	1.74
Swertia paniculata	2.30	0.85	0.97	1.53	1.70
District-level SIMPER analysis for *X. strumarium* invaded and control sites.					
Average dissimilarity = 53.90%					
Average abundance					
Species	Control	Invaded	Av. Diss	Diss/SD	Contribution (%)
Solanum nigrum	2.93	1.03	1.73	5.58	3.21
Cynodon dactylon	2.94	1.55	1.72	9.37	3.21
Parthenium hysterophorus	2.69	1.61	1.58	9.91	2.93
Dodonaea viscosa	2.59	1.35	1.51	4.16	2.81
Tamarix aphylla	2.94	1.51	1.43	2.33	2.66
Ajuga bracteosa	2.41	1.34	1.42	5.80	2.63
Rumex dentatus	2.16	1.48	1.25	2.17	2.32
Typha domingensis	2.70	1.44	1.13	2.57	2.10
Withania somnifera	1.95	0.90	1.12	3.28	2.09
Lantana camara	1.89	0.52	1.11	1.95	2.06

Values represent average abundance ranking (rare, common, very common, >4-dominant, and so forth).
The rankings of average profusion (rare, common, extremely common, >4-dominant, and so forth) are represented by the values.

L. camara is a medium-sized, fragrant Neotropical perennial shrub. It has spread to more than 60 countries and is ranked among the ten worst weeds (Qureshi et al., 2014). This study found that there had been substantial changes in the study area based on diversity indices comparisons between invaded and control plots. These results support past research on this invasive weed, which found that the invader has a considerable impact on natural resources (Shabbir and Bajwa, 2007; Riaz and Javaid, 2010; Riaz and Javaid, 2011). The values for diversity indices of the control and invaded plots were considerably different, as shown by the ordination (nMDS) and ANOSIM approaches. The alteration was substantial across the five study sites. The main invasive plant in the Attock region was previously recognized as *L. camara* along with two other species, *Prosopis juliflora* and *Xanthium strumarium* (Malik and Husain, 2006). SIMPER analysis revealed a total dissimilarity of 65.56 percent between the invading and control plots. The most severely impacted by the *L. camara* invasion were found to be herbs.

The dioecious, deciduous *Broussonetia papyrifera* is a common tree in tropical and subtropical areas and is endemic to East Asia, is one of Pakistan's six deadliest plant invaders because of its detrimental effects on local vegetation (Malik and Hussain, 2007). *B. papyrifera* has a number of harmful consequences on the environment and people, including a decrease in biodiversity, poor effects on anthropological health, obstruction of city sewer systems, and a rise in caw inhabitants that spread seeds (Huston 1979; Hsu et al., 2008). The species groupings of the invasion and control plots showed notable magnitude of differences when employing the ordination (nMDS) and ANOSIM techniques. Invaded plots had lower levels of species dominance than control plots.

The annual herb *X. strumarium* is native to North and South America. It has turned into a common weed in orchards, cultivated areas, and inhospitable surroundings (Hashim and Marwat, 2002). Ordination and ANOSIM showed that the invaded and control plots' diversity indices differed significantly from one another. *X. strumarium* invasion was previously recognized as the most invasive plant in the Rawalpindi district, along with two other species, *Prosopis juliflora* and *Lantana camara* (Malik and Husain, 2006). The SIMPER analysis found that there were overall changes of 53.90 percent between the invaded and control plots.

Plants' ability to successfully colonize unnatural surroundings, quick growth and reproduction, short lifespan, mass seed production, vegetative proliferation, early flowering and sowing, phenology that differs from inhabitants, and pest and infection tolerance are all elements that help them to be invaders. Secondary metabolites have recently been implicated in the ecological dominance of invasive species (Balezentiene, 2015). For resources like space, light, and nutrients, invasive plants outcompete endemic species (Tilman, 1997). It is believed that the absence of evolutionary connections between native and invasive species causes invasion.

Conclusion

Plant invasions cause huge ecological and economic imbalances by reducing species diversity, changing indigenous population composition, and disrupting ecosystem processes in new places. Invasive species research has previously demonstrated that invasion impacts are complicated, and that they can always vary community role and organization, as well as trigger local extinction and fluctuations in environmental procedures. Alien plant invasions change ecosystem dynamics and structure on a large scale, which can significantly affect ecosystem services. The current study's drop in ecological diversity indices between attacked and control locations showed that floral groups were little resilient as a result of the invaders assessed, posing a risk to plant diversity in invaded areas. Strong control methods, comprising the use of verified natural control managers, are immediately essential to contest this plant in Pakistan.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Design, experiments, analysis-HQ, editing-TA, helped in field visits-MM, SM and RY, helped in formal analysis-UH, LM and AH, helped in writing-MI.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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