Pol. J. Environ. Stud. Vol. 29, No. 4 (2020), 2825-2831
DOI: *10.15244/pjoes/111320* ONLINE PUBLICATION DATE: 2020-03-27

Original Research

Invasion Impact Analysis of *Broussonetia papyrifera* in Pakistan

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Received: 5 February 2019
Accepted: 25 July 2019

Abstract

Phytosociological studies help to understand extent of biological invasion. The current study assessed impact of *Broussonetia papyrifera* invasion on native vegetation in Pothwar region of Pakistan. The approach used for study was random samplings with two categorical factors; invaded and non-invaded (control) under same habitat conditions. Differences in number of species (S), abundance (N), species richness (R), evenness (J'), Shannon diversity index (H') and Simpson index of dominance (λ) were compared between invaded and control plots by t-test series. Control plots harbored by average of 1.28 more species per 10 m^2. The control category was diverse (H' = 2.15) than invaded category (H' = 1.65). The higher value of species richness in control plots shows heterogeneous nature of communities and vice versa in invaded plots. The lower value of index of dominance in invaded plots shows less sample diversity than control ones. This decrease in number of species directly affects α-diversity in invaded plots. At multivariate scale, ordination (nMDS) and ANOSIM showed significant magnitude of differences between invaded and control plots in all sites. The decrease in diversity indices in invaded indicated that plant communities become less productive due to Paper mulberry invasion. This makes *B. papyrifera* candidate of consideration for appropriate control measures.

Keywords: biological invasion, non-parametric multidimensional scaling, analysis of similarity, diversity indices, conservation

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Introduction

Species introduced to regions beyond their native range, established in wild and spread substantially from their point of introduction are referred as invasive species. Invasive species threaten global biodiversity [1], cause ecological problems [2], introduce diseases or incur economic costs [3]. Exotic plants, animals, insects and other living organisms are biological pollutants, among which plants are probably the worst one attributed to their huge biomass [4]. According to the 'Invasive Species Specialist Group', out of 100 worst invasive species in the world, 32 are plants [5]. Due to their potential to outcompete and replace native species, invasive species are second leading cause of biodiversity loss after habitat destruction [6]. Studying the community level impacts of invader identify its potential effects and provide valuable information for management and nature conservation strategies [7].

Broussonetia papyrifera (Paper mulberry) is medium to large, deciduous, dioecious tree, native of East Asia, common in China and Japan and widespread in tropical & subtropical regions. Due to adverse effects on native vegetation, it is listed amongst six worst plant invaders in Pakistan [8, 9]. In Pakistan the tree was intentionally introduced during 1960s in Islamabad and Rawalpindi as an avenue tree, but in less than a 30 year period, has become highly invasive in many localities [10]. Adverse effects of *B. papyrifera* on ecosystem include damaged ecosystem services, reduced natural biodiversity, negative effects on human health, choking of sewerage lines in urban set-up and increased crow population acting as seed dispersal vectors. Its pollens cause rhinitis and asthma [11, 12, 13]. Adaptability to different habitats, rapid growth rate, strategy of vegetative regeneration, effective dispersal by birds and allelopathy contribute to its invasion [14].

Paper mulberry is one of the worst weeds presently known in Pakistan. No previous study is reported from Pothwar region regarding its ecological impacts. Current study was carried out to find out (1) what is the effect of *Broussonetia papyrifera* weed on ecological diversity indices in different districts of Pothwar region (assuming each district as 'site'); (2) do the effects on diversity differ between different sites (districts) in the area?

Experimental

The Pothwar is north-eastern plateau in Pakistan, making the northern part of Punjab. It edges Azad Kashmir (the western parts) and Khyber Pakhtunkhwa (southern parts). Pothwar Zone extends from 32.5°N to 34.0°N Latitude and 72°E to 74°E Longitude and lies between Indus and Jhelum River. The plateau expanses from salt range northward to the foothills of Himalayas. The Pothwar region embraces Jhelum (32.9405°N, 73.7276°E), Islamabad (33.73°N, 73.09°E), Attock (33.76°N, 72.36°E), Rawalpindi (73.04°E, 33.59°N), and Chakwal (72.85°E, 32.93°N) districts. Total area of Pothwar region is 28488.9 Km^2. Pothwar region has an extreme climate with hot summers and cold winters. Weather is divided into four seasons; Cold (December-March); Hot (April-June); Monsoon (July-September) and Post-Monsoon season (October-November). This area practices an average annual rainfall of 812 mm, about half of which occurs in the Monsoon months (July-September). The mean maximum temperature rises till the month of June and then falls appreciably with advent of rains being coldest in January (14.62-18.7ºC). Average temperatures range from 14ºC in January to 37ºC in June (Pakistan Meteorological Department University Road Karachi, Pakistan). The region has broadly four types of soil; loess, river alluvium, residual and piedmont alluvium. Due to dynamic climate and combination of hills and plains, Pothwar region is rich in biodiversity. *Albizia lebbeck* (L.) Benth., *Acacia modesta* Wall., *Abies pindrow* (Royle ex D. Don) Royle, *Cassia fistula* L., *Cedrela toona* Roxb. ex Rottler, *Dalbergia sissoo* Roxb., *Dodonaea viscosa* Jacq., *Ficus religiosa* L., *Ficus benghalensis* L., *Melia azedarach* L., *Olea cuspidata* Wall. Ex G. Don., *Zizyphus jujuba* Mill. and *Zizyphus nummularia* (Burm.f.) Wight & Arn. are principle species in the region [15].

The study was carried out during July-August, 2016. The effect of invasion was studied in each of five districts (Attock, Chakwal, Jhelum, Islamabad & Rawalpindi). Ecological indices for selected invaders were calculated and compared at various sites. The sampling technique was random. For each district six invaded and six non-invaded paired vegetation plots (each 3.16×3.16m in size) were sampled. Plot of invaded vegetation where the invader showed dominance was considered as 'treatment' and a second vegetation plot, usually 0.5-1 km apart from treatment, where invader has no dominance ('non-invaded plot') was considered as the "control". The estimated density of the weed in the area across locations was 6/m^2. In all, a total of 60 vegetation plots were sampled. Within each randomly chosen plot, all vascular plant species in control and invaded plots were identified.

Species frequency data were created and invasion impacts of *B. papyrifera* on local flora were assessed by calculating and comparing ecological indices including Margalef's index of richness, Shannon-Weaver index of diversity, Simpson index of dominance and index of evenness for control and invaded sites. These parameters were calculated as:

(i) $$\text{Margalef's index of richness (R)} = \frac{S-1}{\ln N}$$

…where N = total number of individuals and S = total number of species.

(ii) $$\text{Shannon-Weaver index of diversity } (H') = -\sum_{i=1}^{S} \left(\frac{n_i}{N} \times \ln \frac{n_i}{N}\right)$$

…where N = total number of individuals of all species.
n = Actual number of individuals of one species

(iii) Simpson index of dominance $(\lambda) = 1 - \frac{\sum_{i=1}^{S} n_i(n_i-1)}{N(N-1)}$

N = Total number of individuals of all species
n = Number of individuals of one species

(iv) Index of evenness $(E) = \frac{H'}{lnS}$

…where H' is Shannon's index and S = number of species.

Rarefaction curves were plotted to determine if sampling was adequate in each district using observed, Coleman's, Jackknife, Bootstrap and Chao2 models in PRIMER v. 7. All gave comparable results; consequently only that of real (observed) data are presented. Data were then subjected to univariate and multivariate analyses of non-metric multidimensional scaling procedure. For invasion impact analysis, diversity indices including total number of species (S), abundance (N), species richness (R), species evenness (J'), Shannon index of diversity (H′) and Simpson index of dominance (λ) were calculated for control as well as for invaded plots. The above diversity indices were subjected to analysis of variance (ANOVA) with invasion status and districts as factors using IBM SPSS v. 21. Differences between diversity indices for five districts were individually tested for significance between invaded and control plots by multiple comparisons tests of t-test. Data were further analyzed for species assemblages by non-metric multidimensional scaling (nMDS) in two-three dimensions with invasion status (control, invaded) as factor using PRIMER V.7 software. nMDS was used to ordinate the similarity of data between site categories (invaded, control) based on Bray-Curtis dissimilarity matrix following log-transformation of species abundance data due to zero species count in some plots. The range of clustering of sites and locations in response to invasion were assessed by analysis of similarity (ANOSIM) and similarity percentage (SIMPER).

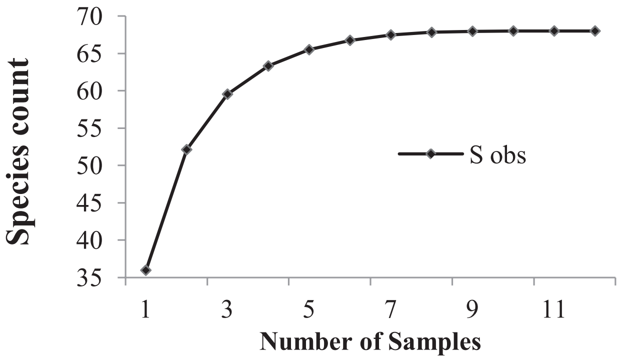

Fig.1. Rarefaction curve showing cumulative number of recorded species.

ANOSIM relates mean difference of ranks between and within groups, generating the Global statistic (R). Global R-values range from -1 to +1. Values near 0 and negative values demonstrate similarity among groups. Values impending +1 indicate a strong dissimilarity among groups. SIMPER identified species contributed most to average dissimilarity between groups (invaded and control plots). Values of percentage similarity between groups range between 0 to 100, with 100 stating maximum similarity [16].

Results and Discussion

To assess sampling completeness, rarefaction curves plotting cumulative number of species as a function of sampling effort were used which indicated that sampling was reasonably complete (Fig. 1).

A total of 65 plant species from 60 genera were documented during the study. A total of 65 species were recorded in control plots compared with to infested plots. Mean species diversity and richness/quadrat was higher in control plots (Fig. 2). Comparisons of ecological indices showed significant differences for all ecological indices in invaded and control plots across sites while differences were significant across invasion status except species evenness. *B. papyrifera* exhibited

Table 1. Summary ANOVA of invasion impacts and site on diversity indices of local plant community

Diversity index	SUMMARY ANOVA			Mean (±SD)	
	Site (S)	Invasion status (IS)	S×IS Interaction	Control (30)	Invaded (30)
No. of species (S)/10 m²	*	***	**	6.81±2.50	5.53±1.65
Abundance (N)/10 m²	*	***	**	22.1±3.81	19.13±4.12
Species Richness (R)	NS	***	*	2.58±0.59	1.69±0.47
Species evenness (J')	NS	NS	NS	0.0077±0.005	0.0073±0.007
Shannon index (H')	*	***	**	2.15±0.27	1.65±0.32
Simpson index (λ)	*	***	**	0.203±0.075	0.122±0.033

S×IS = Site and invasion status interaction effect

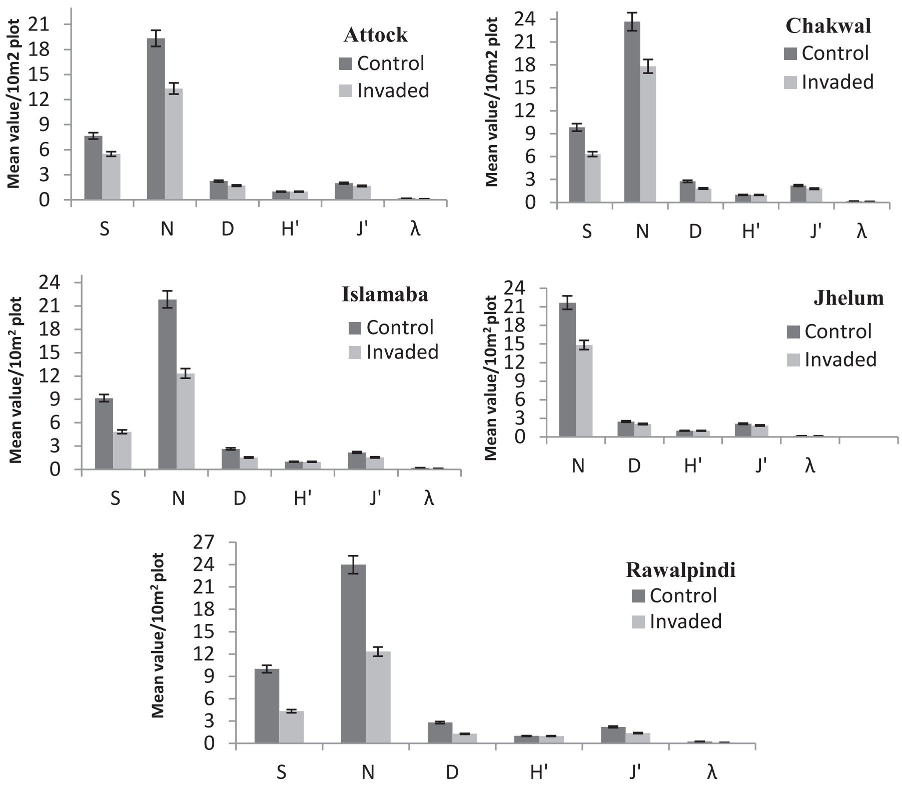

Fig. 2. Mean values/10 m² for diversity indices of invaded vs control plots in different sites (S = Number of species; N = Frequency; D = Species richness; H' = Shannon index of diversity; J' = Species evenness; λ = Simpson index of dominance).

variable impacts in five sites by reducing species number per plot (S) and abundance (N) by a maximum of 48% in Islamabad. Control plots harbored on average 9.07±2.50 (mean±SD, n = 30) species. This was by 3.54±2.08 more than invaded plots and the difference was marginally significant (t = 2.09, df = 29, p = 0.045). In total 298 and 156 individuals were recorded in control and invaded plots respectively. Similarly, abundance in control and invaded plots differed by 2.97±3.96 (mean±SD, n = 30) and the difference was significant (t = 3.34, df = 29, p = 0.00). Control plots exhibited higher values of species richness by a difference of 0.89±0.53, species evenness by 0.0004±0.006, Shannon index of diversity by 0.5±0.29 and Simpson index of dominance by 0.081±0.042 (Table 1). For individual sites, *B. papyrifera* invasion had significant impacts on all ecological indices except species evenness (J') at site 1 (Attock). For site 2, (Chakwal) species abundance and Simpson index of dominance was not affected significantly. For site 3 (Islamabad) invasion impacts on species evenness were not significant. Species richness, evenness (J') and Simpson index of dominance was non-significant for site 4 (Jhelum) while for site 5 (Rawalpindi) species evenness was not affected significantly (Table 2).

The ordination (nMDS) and ANOSIM showed significant magnitude of differences between species composition of invaded and control plots in all sites with global R values of 0.740 (p = 0.002), 0.302 (p = 0.002), 0.813 (p=0.002), 0.730 (p = 0.002) and 0.691 (p = 0.002) for Attock, Chakwal, Islamabad, Jhelum and Rawalpindi (Fig. 3). The greatest dissimilarity

Table 2. Student's t-test for significance of differences between control and invaded plots at different sites

Site	Number of species (S)	Abundance (N)	Species Richness (R)	Species Evenness (J')	Shannon index (H')	Simpson index (λ)
Attock	**	**	**	NS	***	***
Chakwal	**	NS	**	**	*	NS
Islamabad	**	**	**	NS	**	**
Jhelum	*	*	NS	NS	*	NS
Rawalpindi	**	**	**	NS	**	**

*** P≤0.001; ** P≤0.02; * P≤0.05; NS (not significant) P>0.05

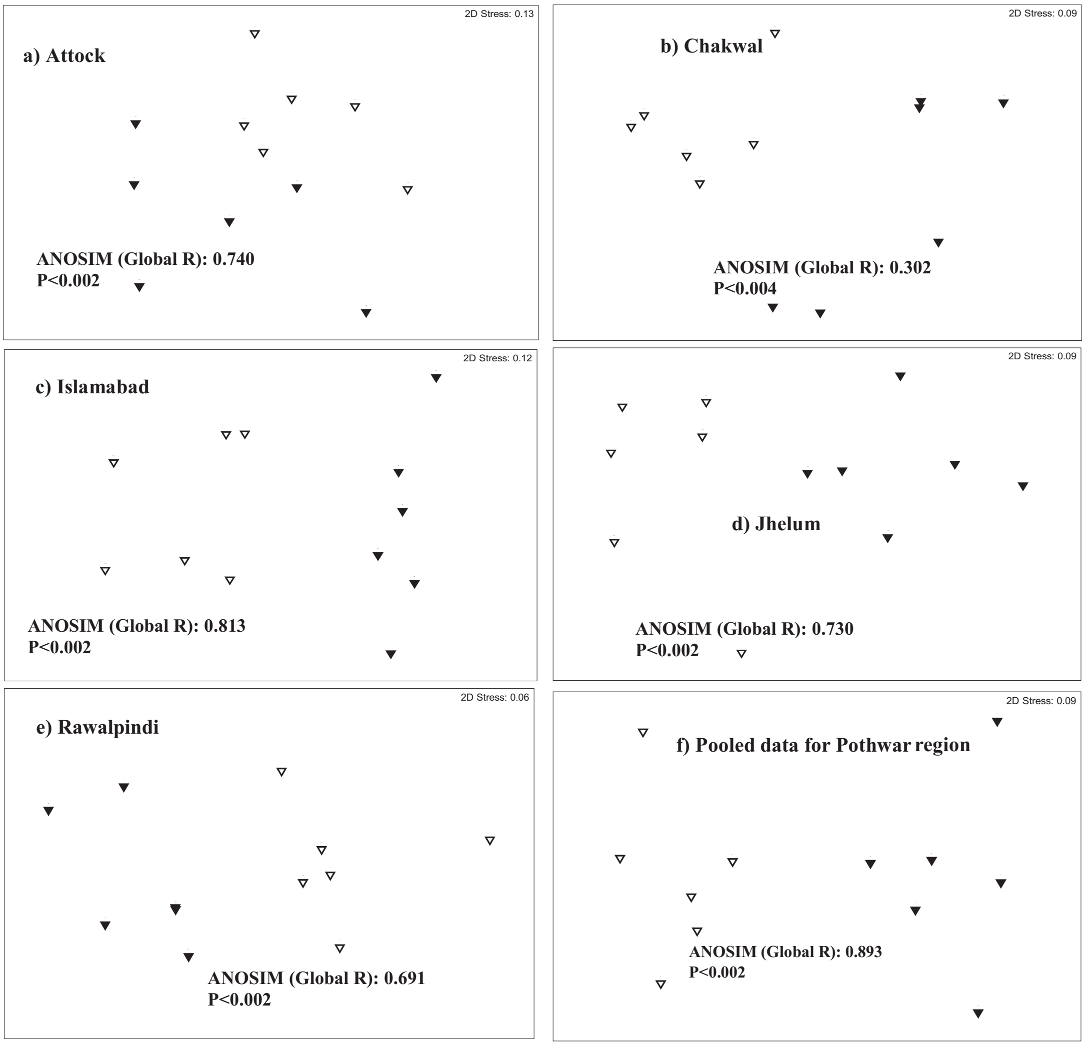

Fig. 3. Multidimensional scaling (MDS) ordination and analyses of similarity (ANOSIM) results of invasion status data for Pothwar region, Pakistan (open symbols are for control, uninvaded plots, and closed symbols are for invaded plots).

between invaded and control plots was noticed by Islamabad. Similarity percentage (SIMPER) analysis of data suggested species contributing most to average dissimilarity between control and invaded groups. This analysis also computed average contribution of species causing dissimilarity. Few top species separating invaded plots from non-invaded plots (control) for analysis are enlisted in Table 3.

Stellaria media, Oxalis corniculata, Cynodon dactylon, Digitaria ciliaris, Malva parviflora, Croton tiglium, Eclipta prostrata, Clematis grata, Chenopodium album, Calotropis procera, Medicago sativa, Achyranthus aspra, Solanum nigrum, Datura stramonium and *Sonchus asper* were top contributing species causing difference between control and invaded plots in Pothwar region. *B. papyrifera* exerts significant impacts on natural communities by displacement of native species and hence, forming large monocultures. In present study, comparisons of ecological indices across invaded and control plots indicated significant differences in study area. These findings are in-line with studies on other alien invasive weeds, which indicated strong effects of the invader on ecosystem properties [17, 18, 19]. The results show modifications in vegetation composition of invaded and control plots. Analysis of variance among invaded and control plots showed significant decrease in ecological indices across site and invasion status. These results are consistent with studies on invasive species indicating their negative effects on bio-diversity and ecosystem properties [3, 14, 20, 21, 22]. Adaptability to different habitats, rapid growth rate, strategy of vegetative regeneration, effective dispersal by birds and allelopathy contribute to its invasion success [23]. Allelopathy especially plays important role in invasion of this weed. The major allelopathic compounds found in *B. papyrifera* are, Broussonin A, Broussonin B, (+)-Marmesin, Broussochalcone A, (2S)-euchrenone a7, Broussoflavonol F, Naringetol, Albanol A, Moracin N, Isogemichalcone C, Chushizisin H, Broussoflavonol E, Broussoflavonol G, Broussoflavonol C, Broussoflavonol D, Chushizisin I; Broussoflavonol B, Broussoflavonol

Table 3. SIMPER analysis of *Broussonetia* invaded and control sites in Pothwar region, Pakistan

Average dissimilarity = 57.19%					
Average abundance					
Species	Invaded	Control	Av. Diss.	Diss/SD	Contribution (%)
Tribulus terrestris L.	2.90	0.00	1.63	7.24	2.85
Malvastrum coromandelianum (L.) Garcke	2.57	0.00	1.46	4.14	2.55
Cynodon dactylon (L.) Pers.	2.44	0.00	1.36	1.91	2.38
Silybum marianum (L.) Gaertn.	0.81	2.69	1.12	1.54	1.97
Calotropis procera (Aiton) W.T.Aiton	2.02	0.00	1.12	1.89	1.96
Datura innoxia Mill.	1.98	0.00	1.04	1.33	1.82
Digeria muricata L. (Mart.)	1.94	0.00	1.02	1.36	1.79
Desmostachya bipinnata (L.) Stapf	2.96	1.25	1.00	1.29	1.74
Swertia paniculata Wall.	2.30	0.85	0.97	1.53	1.70
Euphorbia helioscopia	1.77	0.00	0.96	2.14	1.68
Solanum surratensis Burm. F.	2.28	0.88	0.95	1.29	1.66
Melilotus indica L.	2.09	0.62	0.93	1.53	1.63
Alternanthera pungens	1.62	0.00	0.93	1.27	1.62
Corchorus depressus (L.) Stocks	1.74	0.00	0.92	1.36	1.62
Stellaria media	2.32	0.76	0.92	1.57	1.62
Fumaria indica	1.73	0.00	0.92	1.33	1.61
Ranunculus muricatus	1.77	0.46	0.92	1.43	1.61
Cenchrus bifloris	1.60	1.98	0.86	1.17	1.50
Anagallis arvensis	1.45	2.44	0.86	1.17	1.50

*Values are average abundance ranking (1-rare; 2-common; 3-very common; >4-dominant)

A, Broussoflavan A, Broussoflavonol F, Kazinol A, Kazinol B, Gancaonin P, Uralenol, Isolicoflavonol, Chushizisin C, Chushizisin D, Chushizisin E, Chushizisin B, Chushizisin A, Chushizisin F, Broussochalcone A, Broussoaurone A, Chushizisin G, Broussinol, Isobavachalcone, Broussochalcone B, Broussonin C, Broussonin F, Broussin, Broussonin E [24, 25, 26]. These allelochemicals are supposed to reduce native seed germination, allowing the weed to pre-empt space and establish monocultures.

B. papyrifera invasion reduced the values for diversity indices compared to control in each of five studied districts. The trend of decrease in values of ecological indices in invaded plots is similar to invasion studies on *B. papyrifera* from Australia, Argentina, Carolina, Columbia, Florida, Georgia, Louisiana, Maryland, North Carolina, Oklahoma, Pennsylvania, South Tennessee, Uganda and Virginia [27, 28]. The most effected site by *B. papyrifera* invasion was Islamabad followed by Attock, Jhelum, Rawalpindi and Chakwal. The most invasion impacts in Islamabad compared to other sites are probably because of its initial introduction in Capital territory during 1960_s with objective to make capital city green [6]. The ordination (nMDS) and ANOSIM showed significant magnitude of differences between species assemblages of invaded and control plots. The difference was significant for all of five study sites but the greatest dissimilarity between invaded and control plots. In current study, we noticed negative effects of *B. papyrifera* on all of ecological indices in invaded over control plots. The highest impact is noticed in Islamabad. SIMPER analysis showed dominance of few species in invaded plots than in control. Reduction in grass density due to *B. papyrifera* invasion is reported earlier [2].

Conclusions

In the current study the higher value of species richness in control plots shows heterogeneous nature of communities and *vice versa* in invaded plots. This decrease in number of species directly affects α-diversity in invaded plots. At multivariate scale, ordination (nMDS) and ANOSIM showed significant magnitude of differences between invaded and control plots in all sites. The decrease in diversity indices in invaded indicated that plant communities become less productive due to Paper mulberry invasion. The increased occurrence of invasion around the world poses a major threat to indigenous diversity. Plant invasions in novel areas deplete species diversity, alter indigenous community composition, affect ecosystem process and thus cause huge ecological and economic imbalance. Invasive species studies in the past revealed that the effects of invasion are complex and can permanently alter the function and structure of communities, cause local annihilations and changes in ecosystem processes. The decrease in ecological diversity indices in invaded over control sites in present study indicated that plant communities become less productive due to *B. papyrifera* invasion hence a threat to plant diversity of invaded areas. There is urgent need of appropriate control measures.

Conflict of Interest

The authors declare no conflict of interest.

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