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Impacts of Invasion by *Ageratina adenophora* on Soil Properties and Plant Diversity

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Abstract

Ageratina adenophora, one of the most noxious invasive plants globally, is found to have escaped into novel ecological ranges in Bhutan. As such, this study was an attempt to assess the impacts on soil properties, community abundance, plant diversity, and invasion intensity within the study area spanning the Punakha and Wangdue Phodrang districts. The study was conducted along the roadsides with sampling intervals of 5 kilometres. Two circular plots were drawn at each sampling sites to record invaded and uninvaded areas; and plant and soil samples were collected from both the plots. Soil properties measured were Nitrogen, Phosphorus, Potassium, pH, Organic matter, and Organic carbon. While there was no clear impact of invasion on soil properties ($p > .05$), the diversity index and abundance clearly showed that invasion had reduced the diversity ($U = 2335$, $p = .001$) and abundance ($H_{(1)} = 6.173$, $p < .05$) at the lower community level, particularly at the herb level. The invasion intensity analysis also showed that the study area has favourable environmental conditions for higher intensity of invasion. In sum, the study implied that this species if not managed, would lead to loss of other resident plants, particularly at the lower community levels due to its cosmopolitan characteristics.

Keywords: Abundance, cosmopolitan, diversity, invasion, soil properties

Introduction

Invasive species pose a significant threat to established patterns of biodiversity and ecosystem functions. *Ageratina adenophora* (Spreng.) King & H. Rob., which is commonly known as Crofton weed, is native to Mexcio and Central America; and is a blooming perennial plant belonging to the Asteraceae family (Kluge, 1991). Currently, it is a typical vicious invasive weed worldwide (Kumar *et al.*, 2020) invading farm lands, grasslands, and forests (Parsons *et al.*, 2001). This plant has the ability to regrow vegetatively and produce huge amounts of tiny seeds for reproduction. *A. adenophora* is considered as one of the most noxious invasive species in Bhutan spreading to 17 dzongkhags (BBS, 2018).

Ageratina adenophora is known to have profound ecological impacts such as changing soil properties and reducing plant diversity. Soil

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properties are found to become toxic to non-invasive or indigenous species of plants by lowering soil pH and raising the amount of organic matter, total nitrogen, phosphorus, and potassium thereby favouring the growth of *A. adenophora* while inhibiting the growth of native plants (Niu *et al.*, 2007; Darji *et al.*, 2021). This ultimately leads to displacement of native species and establishment of monoculture (Niu *et al.*, 2007; Poudel *et al.*, 2019; Wu *et al.*, 2020). Moreover, *A. adenophora* has an allelopathic mechanism that affects native plants (Wan *et al.*, 2010) such as the leachates from fresh leaves and roots of *A. adenophra* being phytotoxic to the growth and development of native shrubs such as *Osbeckia stellata* and *Elsholtzia blanda* (Darji *et al.*, 2021).

Previous studies have shown that aerial parts and roots of *Ageratina adenophora* also have negative effects on the physiology and morphology of some crops such as rice, weeds such as *Lolium perenne, Trifolium repens, Galinsoga parvifora*, and *Medicago sativa* and native trees such as *Schima wallichii* and *Alnus nepalensis* (Zhang *et al.*, 2012; Thapa *et al.*, 2016; Thapa *et al.*, 2020b). *A. adenophora* is known to have negative effects on understory species and functional diversity, abundance, and plant community composition (Fu *et al.*, 2018). Thapa *et al.* (2020a) reported that this species has reduced native species richness in the invaded plots in Nepal where the invaded plots exhibited a 35 percent reduction in total native plant density compared to the uninvaded plots. There is no such a scientific study carried out in Bhutan, particularly the impacts of invasion by *A. adenophora* based on comparisons between the invaded and uninvaded plots. Such a study is imperative for Bhutan as a signatory to Convention on Biological Diversity (CBD) that requires any member to prevent introduction, control or eradication of invasion according to Article 8 of CBD. Also, according to Hejda *et al.* (2017), comparison of biodiversity between invaded and uninvaded plots at the community level provides crucial information for community management and biodiversity conservation. This study therefore aims to assess the impacts of *A. adenophora* on soil properties and associated lower community vegetation diversity to serve as baseline information.

Materials and Methods

Study area

Punakha and Wangdue Phodrang districts located within 27°18'50''N, 89°31'0''E and 27° 45'0''N, 90°49'30''E were selected as the study area (Figure 1). The average altitude of Punakha is 1242 metre above sea level (m asl) and 1273 m asl for Wangdue Phodrang with hot and humid summers, and cool to mild winters (Chhogyel *et al.*, 2016). These districts represent an ideal area for acquiring samples for the species since they encompass both suitable ecological range of *A. adenophora* (David, 2012; CABI, 2019; Changjun *et al.*, 2021) and dispersal corridors with the road connectivity to different parts of the country.

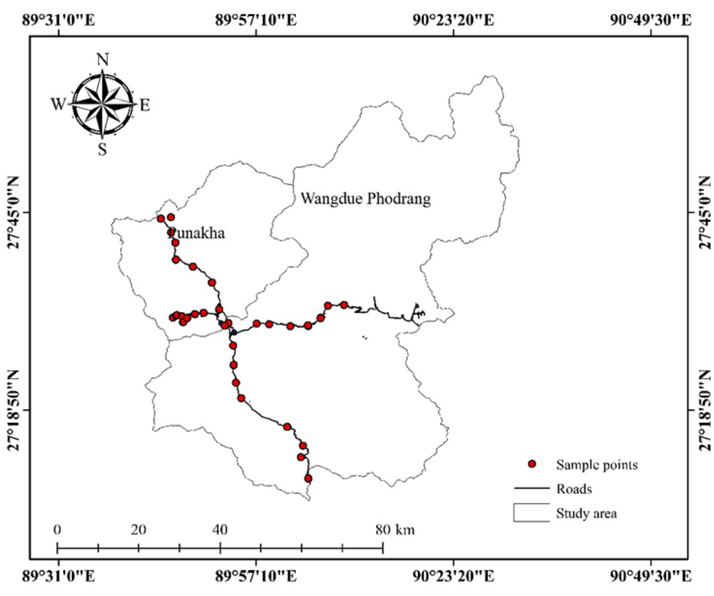

Figure 1: Map showing sampling points

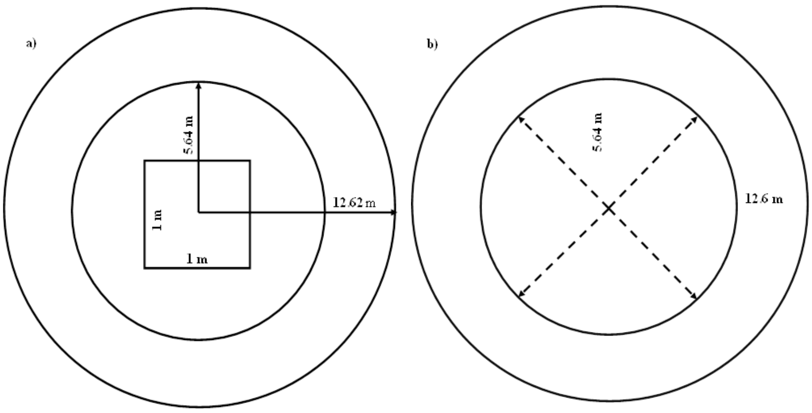

Figure 2: a) Vegetation sample plot; b) Soil sample plot

Sampling design

Sample points falling on human structures and other natural features that are not plausible for sampling were subtracted from the potential sampling areas. Sample points were created along the roads after every five kilometres. The sample points created on the ArcGIS was uploaded to GPS handheld unit for tracking locations on the ground. The distance of species search from the point was maintained at 100 m on either side of the point. Four corresponding circular plots, two above and two below the roads were used – one representing the invaded plot and the other as uninvaded plot totalling up to 116. Figure 2a represents the sampling design for collecting vegetation data collection and Figure 2b represents the sampling design for collecting soil samples.

Vegetation data collection

Vegetation samples (Figure 2a) were collected as follows: trees were counted within the radius of 12.62 m, shrubs were counted within the radius of 5.64 m, herbs were counted within the 1 x 1 m squared plot.

Soil data collection

Soil samples (Figure 2b) were collected as follows: samples were collected at the depth of 1 to 30 cm using soil auger, four sub-samples were collected within the radius of 5.61 m, samples were made into a composite sample by mixing all four sub samples from each plot, and samples were air-dried prior to analysing them in the laboratory.

Data analysis

The samples were analysed at three stages — first, the soil samples were analysed for the properties; second, the diversity indices were derived using Shannon Diversity formula along with species abundance analysis; and third, invasion intensity was analysed.

Laboratory soil analysis

The following soil properties were analysed in the laboratory using laboratory manual guide (Moktan *et al.*, 2020).

Soil pH determination

With the use of Soil pH meter, the soil acidity or basicity on a scale of 0 to 14 was measured in soil-water suspension ratio of 1:5. The pH meter was standardised with buffer tablet which was dissolved in 100 ml distilled water.

Nitrogen, Phosphorus, and Potassium analysis

Available Nitrogen (N) was measured or derived from organic matter using Loss on Ignition (LOI) method. Available Phosphorus (P) was determined using Bray molybdenum blue method in photo spectrometer and available Potassium (K) was determined using Ammonia acetate method using flame photometer.

Moisture content (MC %)

Soil moisture content was measured in grams with gravimetric method oven dried at 105°C for 24 hours as shown below (Eqn. 1):

$$\text{Eqn. 1: MC\%} = \frac{Initail\ soil\ wt\ (g) - Oven\ dried\ soil\ soil\ wt\ (g)}{Initial\ soil\ wt\ \ (g)}\ X\ 100$$

Organic Matter % and Organic Carbon %

Organic matter (OM) and organic carbon (OC) were calculated using LOI method (Hoogsteen *et al.*, 2018). The difference in weight before and after ignition provides organic matter (Eqn. 2), which when multiplied by constant 0.58 provides organic carbon since 58% of the mass of soil organic matter exists as soil organic carbon (Eqn.3).

$$\text{Eqn. 2: OM\%} = \frac{Oven\ dried\ soil\ wt\ (g) - Incinerated\ soil\ wt\ (g)}{Initial\ soil\ wt\ (g)}\ X\ 100$$

$$\text{Eqn. 3: OC\%} = OM\%\ X\ 0.58$$

Shannon diversity index analysis

The Shannon diversity index (H') was used to find the plant diversity in each plot using the following formula (Eqn. 4);

Eqn. 4: H'= - $\Sigma(Pi * ln(pi))$

Where: H' is the Shannon diversity index; Σ = sum across all species; Pi = proportion of individuals belonging to the i^{th} species; ln = natural logarithm function.

Community-level abundance analysis

To assess the effect of invasion by *Ageratina adenophora* among three community levels of vegetation – trees, shrubs and herbs. Abundance was computed with the Eqn. 5 where invasion refers to the plots either invaded or uninvaded, community types refers to trees, shrub, and herb.

Eqn. 5: *Abundance ~ invasion + community type + invasion * community type.*

Mapping invasion intensity using Co-Kriging interpolation

To estimate invasion intensity with environmental covariates such as elevation, slope steepness and distance to road, simple Co-Kriging, a geostatistical interpolation algorithm (Eqn. 6a and 6b) was used. This algorithm is useful when the samples are sparse to predict missing values of variables of interest.

Eqn. 6a: $Z_1(s) = \mu_1 + \varepsilon_1(s)$

Eqn. 6b: $Z_2(s) = \mu_2 + \varepsilon_2(s)$,

Where: μ_1 and μ_2 are unknown constants; $\varepsilon_1(s)$ and $\varepsilon_2(s)$ are random errors; $Z_1(s_1)$ and $Z_2(s_2)$ are variables and covariates to be predicted respectively.

Statistical test for significance

Kruskal-Wallis test, Mann-Whitney *U* test, and linear regression were conducted to compare community-level abundance, diversity indices between invaded and uninvaded plots, and analyse influence of environmental factors on plant diversity respectively. These tests were performed in an open statistical software called Jeffreys Amazing Statistics Programme (JASP 0.16.3.0).

Results and Discussion

Comparison of community level abundance between invaded and uninvaded plots

Vegetation types were lesser in invaded plots (Trees = 67, Shrubs = 72, and Herbs = 110) than in uninvaded plots (Trees = 75, Shrubs = 101, and Herbs = 148). Comparison of abundance of trees, shrubs and herbs between the invaded and uninvaded plots showed a statistical significance in herbs; ($H_{(1)} = 6.173$, $p < .05$), indicating that the abundance of at least one of the three variables differed significantly between the invaded and uninvaded plots (Table 1). Therefore, a pairwise comparison using Mann-Whitney *U* test was conducted between invaded and uninvaded plots of herbs, which revealed that the abundance of herbs was significantly higher in uninvaded plots (*Mdn* = 4) than in invaded plots (*Mdn* = 3); *U* = 44654.5, *p* =.001, indicating that herbs are more vulnerable to invasion than shrubs or trees (*p* > .05) as shown in Table 1 and Figure 3. This stands with the concept of invasive species' ability to outcompete other resident plants particularly the lower community plants, as stated by Prasad (2010) that the tree seedling regeneration was stalled by *Lantana camara* invasion in dry subtropical forest and suppression of top canopy tree seedlings in the invaded area of *Ageratina adenophora* (Fu *et al.*, 2018).

Table 1: Kruskal-Wallis test for community level abundance between invaded and uninvaded plots

Community	Plot	Mean	Median	Chi-Square	*df*	Sig.
Tree	Invaded	287.86	1	0.0576	1	0.81
	Uninvaded	279.46				
Shrub	Invaded	271.08	3	1.1022	1	0.29
	Uninvaded	291.67				
Herb	Invaded	258.88	4	6.1732	1	0.01*
	Uninvaded	300.56				

*Significant $p < .05$

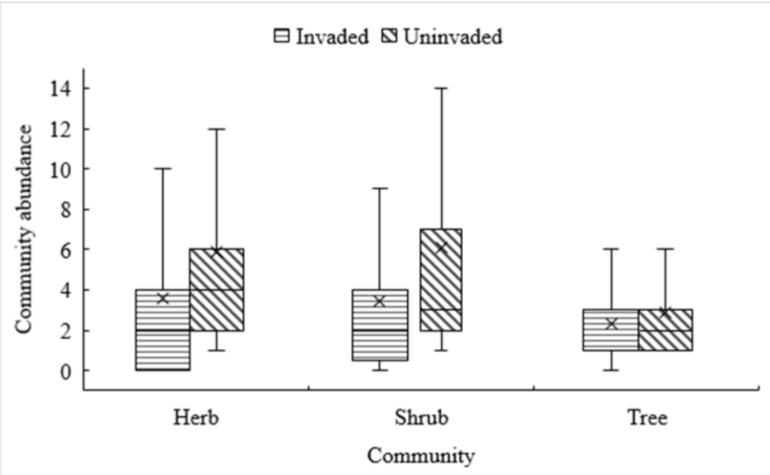

Figure 3: Boxplot showing comparison of abundance

In the lower community (herbs and shrubs) scenario, uninvaded plots had a significantly higher index (*H'* = 2.0) compared to invaded plots (*H'* = 1.7), *U* = 2335, *p* = .001 with medium effect size of 0.389 (Table 2). Similar results was shown in China (Fu *et al.*, 2018) as well as in India (Kumar *et al.*, 2020) where this target species affected understory vegetation composition and diversity due to its higher specific leaf area, leaf nitrogen concentration, and leaf phosphorus concentration as compared to native species. Moreover, allelopathic effect is another characteristic where *Ageratina adenophora* produces phytotoxic chemical that inhibits the growth and reproduction of other plant species in invaded area (Thapa *et al.*, 2020a; Kumar & Garkoti, 2022).

According to Mann-Whitney *U* test, there was no significant difference in soil properties such as pH, N, P, K, MC, and OC between invaded and uninvaded plots (Table 3) which is further illustrated in Figure 4 by less or no differences in terms of data distribution and central tendency. However, it was found that such

Comparison of vegetation diversity and soil properties between invaded and uninvaded plots

Mann-Whitney *U* test of plant diversity showed significant difference between the invaded and uninvaded plots, where uninvaded plots had higher index (*H'* = 2.80) than invaded plots (*H'* = 1.95); *U* = 2318.00, *p* = 0.001 with the medium effect size of 0.379 (Table 2). A similar result was observed by Hejda *et al.* (2009) in that the diversity reduced with increase in cover and height of the invading species. However, there are some results that are contentious on the diversity index between the invaded and uninvaded plots due to microclimatic conditions (Diekmann *et al.*, 2016).

Table 2: Mann-Whitney U test for diversity index between invaded and uninvaded plots

Community types	W	*p*	Rank-Biserial Correlation
H (all vegetation communities)	2318	< .001	0.379
H (lower communities)	2335	< .001	0.389

*Significant at $p < .05$

Table 3: Mann-Whitney *U* test comparing soil properties between invaded and uninvaded plots

Soil parameters	*W*	*p*
pH	1571.5	0.545
N (%)	1949	0.14
P(ppm)	1758	0.675
K (ppm)	1705.5	0.897
MC (%)	1806	0.493
OC (%)	1949	0.14

properties are altered by the *Ageratina adenophora* invasion (Wan-Xue *et al.*, 2010; Wu *et al.*, 2020; Darji *et al.*, 2021). For instance Darji *et al.* (2021) and Niu *et al.* (2007) reported that nitrate nitrogen, ammonia nitrogen, available phosphorus, and potassium contents were significantly higher in invaded plots than in uninvaded plots. This contradiction could be attributed to the differences in methods of the study and geographical locations of study plots. These authors studied *A. adenophora* invasion impacts in the controlled environment as opposed to this study.

Influence of environmental factors on diversity

The overall regression was not significant (R^2 = .047, $F_{(10, 98}$ = .479, p = .9) suggesting that environmental factors do not have influence on the plant diversity (Table 4). However, this could also mean that there are other factors that are more influential relating to diversity, as reported by Niedrist *et al.* (2009) that there are other factors influencing plant diversity, of which the land use was one. The authors found that the number of plant communities along with the number of species decreases significantly with increase in land use intensity. Keeley *et al.* (2005) and Ma *et al.* (2020) pointed out that diversity is also affected by fire incidences and soil desertification, which would lead to oscillation on species richness.

Table 4: Regression analysis showing influence of environmental factors on plant diversity

Variables	Unstandardised	Standard Error	Standardised	*t*	*p*
pH	0.197	0.591	0.034	0.334	0.739
P(ppm)	0.017	0.466	0.004	0.037	0.97
K (ppm)	-0.12	0.543	-0.023	-0.222	0.825
N (%)	0.193	0.634	0.036	0.305	0.761
MC (%)	-0.177	0.579	-0.035	-0.306	0.761
Mean temperature	0.366	0.892	0.064	0.411	0.682
Annual rainfall	-0.86	0.95	-0.141	-0.905	0.368
Aspect	0.081	0.165	0.056	0.492	0.624
Slope	0.068	0.04	0.172	1.711	0.09
Elevation	-0.0003	0.0007	-0.055	-0.483	0.63

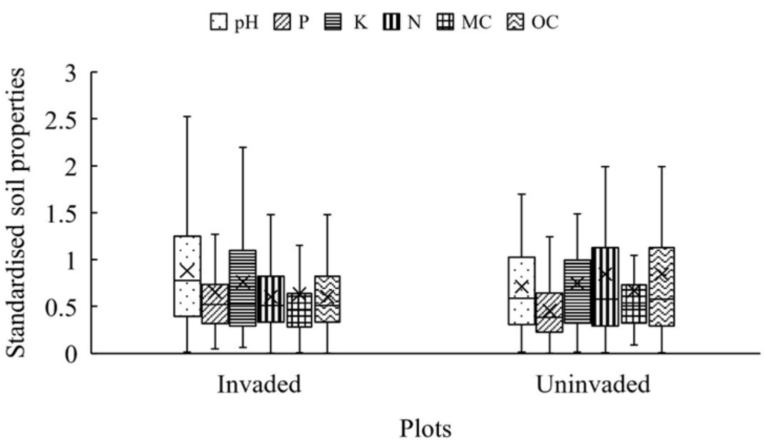

Figure 4: Standardised soil property between invaded and uninvaded plots

Prediction of invasion intensity

Calculation of invasion in-

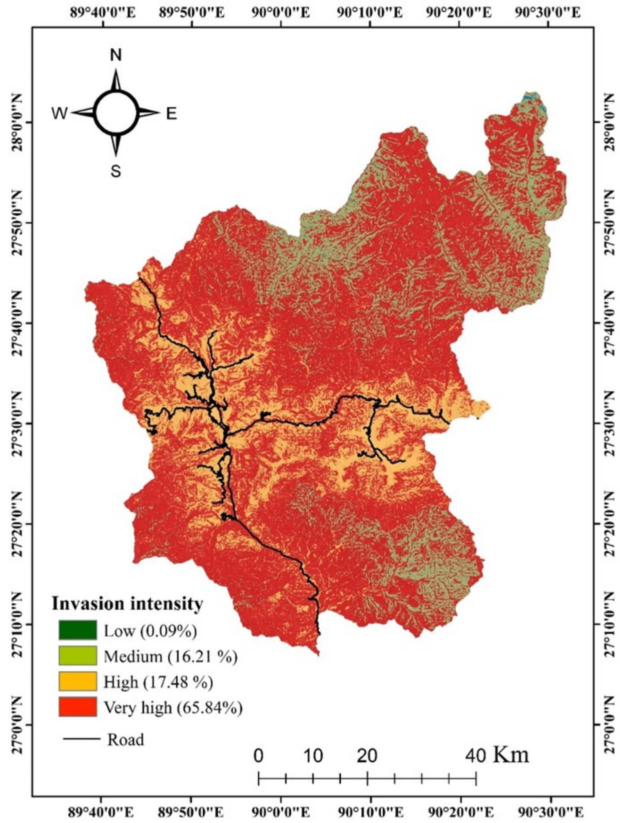

Figure 5: Invasion intensity map

tensity using Co-Kriging algorithm showed a very high invasion intensity of over 65% (Figure 5) in Wangdue Phodrang and Punakha districts. The results also indicated that higher intensities of invasion are in proximity to disturbed environments, such as roads which complies with the report of Ward *et al.* (2020) that such human activities ease pathways for invasion. Zhang *et al.* (2023) also reinforces that biological interactions (agriculture practices, urbanization, forest fire, transportation etc.) amplify the intensity of invasion. Overall, the result suggested that *Ageratina adenophora* is a cosmopolitan species in the study area.

Conclusion and Recommendation

The study found that lower community plants (herbs) are more vulnerable to invasion by *Ageratina adenophora*, while shrubs and trees remained unaffected, which was further confirmed by lower diversity index within the invaded plots of herbs and shrubs as compared with uninvaded plots. However, there was no clear indication of invasion impacts on soil properties since they fluctuated in both the invaded and uninvaded plots. Such unclear impacts of invasion on soil properties could be attributed to the method of the study. This study being cross-sectional in nature, it would not have been able to capture the long-term impacts of invasion. In future, it is recommended that a longitudinal study be conducted for an in-depth understanding of invasion impacts on soil properties. Additionally, influence of environmental factors on invasion was weak, which could be confounded by the lack of other important variables or limited spatial variabilities in landscape and climatic gradients. One of the insights that this study provided was that in the study area, *A. adenophora* was predicted to be a cosmopolitan species according to the interpolation of invasion intensity variables from the plots. In future, such studies demand coverage of wider geographical area and longer temporal period to ascertain variabilities in space and time, thereby assuring the impacts of invasion.

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