ECOLOGY AND IMPACT OF AN INVASIVE WEED *Ageratina adenophora* IN CENTRAL NEPAL

A THESIS SUBMITTED TO THE
CENTRAL DEPARTMENT OF BOTANY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY
NEPAL

FOR THE AWARD OF
DOCTOR OF PHILOSOPHY
IN BOTANY
BY
ANJU SHARMA POUDEL

JUNE, 2021

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TRIBHUVAN UNIVERSITY
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Kirtipur, Kathmandu, Nepal

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DECLARATION

Thesis entitled "**Ecology and impact of an invasive weed *Ageratina adenophora* in Central Nepal**" which is being submitted to the Central Department of Botany, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Dr. Bharat Babu Shrestha, Central Department of Botany, Tribhuvan University and co supervised by Prof. Dr. Pramod Kumar Jha, Central Department of Botany, Tribhuvan University and Dr. Rangaswamy Muniappan, IPM Innovation Lab, Virginia, USA. This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

Date: June, 2021

……………………………
Anju Sharma Poudel

RECOMMENDATION

This is to recommend that **Ms. Anju Sharma Poudel** has carried out research entitled **"Ecology and impact of an invasive weed *Ageratina adenophora* in Central Nepal"** for the award of Doctor in Philosophy (Ph.D.) in **Botany** under our supervision. To our knowledge, this work has not been submitted for any other degree.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.

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On the recommendation of **Dr. Bharat Babu Shrestha**, Associate Professor (Research supervisor), **Dr. Pramod Kumar Jha**, Professor Emeritus and **Dr. Rangaswamy Muniappan** (Research co-supervisors), this Ph.D. thesis entitled **"Ecology and impact of an invasive weed *Ageratina adenophora* in Central Nepal"** submitted by **Ms. Anju Sharma Poudel** is forwarded by the Central Department Research Committee (CDRC) to the Dean, Institute of Science and Technology (IOST), Tribhuvan, University.

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Anju Sharma Poudel
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ABSTRACT

Invasive alien species (IAS) have been increasing at an alarming rate all around the globe with significant negative impacts on the environment, ecosystem services, human health and socio-economy. Therefore, understanding the ecological features of IAS invasion mechanisms, potential shift in climatically suitable regions of IAS under future climate change scenarios, and factors influencing abundance of their biological control agents is an urgent need to better inform the management policies and practices. In this study, ecological features such as distribution (current and future), functional traits variation along the elevation gradient, ecological impacts, and influence of abiotic factor (elevation) on the abundance of a biocontrol agent of a noxious invasive weed *Ageratina adenophora* (Sprengel) R. King & H. Robinson were analyzed in central Nepal.

Maxent model was used to predict the suitable areas of *A. adenophora* in Chitwan Annapurna Landscape (CHAL), Nepal under current and three future climate change trajectories based on three representative concentration pathways (RCPs 2.6, 4.5 and 8.5) in two different time periods (2050 and 2070) using occurrence data of the species in the study region, climatic variables, and topographic features. To analyze the change in area invaded by *A. adenophora* from the year 1990-2018 in CHAL, Landsat images were used and knowledge-based image classification technique of remote sensing was applied assigning seven rules (elevation, landuse, Normalised Difference Vegetation Index (NDVI), digital number, maximum temperature, minimum temperature and rainfall). Furthermore, spatial distribution of *A. adenophora* was assessed in small area of interests viz. Mahadevbesi (Dhading district), Sarangkot (Kaski district) and Ghasikuwa (Tanahun district) using both low spatial and spectral resolution (Landsat) and high spatial and spectral resolution (Worldview-2) images to find out the more accurate multispectral sensors. Six functional traits namely Specific leaf area (SLA), Leaf nitrogen content (LNC), number of floral heads per ramet, total seeds per floral head, seed size and seed mass of *A. adenophora* were taken into consideration for analyzing their variation along the elevation gradient of 240 – 2965 m asl in central Nepal. Changes in species richness, species diversity, species composition and soil chemical characteristics (Organic carbon, Total nitrogen, phosphorus and potassium) due to *A. adenophora* invasion was studied at two sites (Chandragiri and Simbhanjyang) in Middle Mountain region. Role of elevation on the abundance of a biocontrol agent, i.e., *Procecidochares utilis* Stone and on size of gall formed by this gall fly was explored. Furthermore, the effect of this agent on the stem diameter of the weed, and the capacity of the gall to act as nutrient sink was further analysed.

Minimum temperature in the coldest month (bio 6) was the most important variable affecting the distribution of *A. adenophora*. About 38% of the CHAL area is currently climatically suitable for the weed, Middle Mountain being the most suitable one. A predicted increase in suitable area under future climate scenarios ranged from 1-2 % from the current suitable areas. All protected areas and three physiographic regions (Siwaliks, High Mountains and High Himalaya) are likely to gain climatically suitable areas in future climate scenarios. The upper elevational distribution limit of the weed is expected to expand by 31-48 m in future climate scenarios. The area invaded by *A. adeophora* increased from 0.22% in 1990 to 4.55 % in 2018. Multispectral sensors, i.e., Worldview-2 images had higher accuracy in comparison to Landsat images and provided a better scenario of species distribution.

SLA, LNC, number of floral heads per ramet and total seeds per floral head decreased with the increasing elevation along the elevation gradient of 500 – 2500 m asl. However, different trend in functional traits was observed along elevation gradient of 1500-2530 m asl. Seed size and seed mass increased with the increasing elevation at all sites along the elevation gradients. A trade-off was noticed between seed size and seed number indicating the strategy of the weed to adapt to low temperatures at high elevations.

Ageratina adenophora reduced the species richness as well as species diversity in the invaded sites. Shannon's diversity index in non-invaded plots was 1.9 times higher than in invaded plots. Similarly, Simpson's diversity index in non-invaded plots was 1.6 times higher than in invaded plots. Invasion by *A. adenophora* also caused significant change in species composition. Sorensen's similarity index revealed 49% difference in species composition between invaded and noninvaded plots. Furthermore, *A. adenophora's* invasion increased the carbon and nitrogen content in the invaded plots. However, *A. adenophora* invasion had no effect on phosphorus and potassium concentration. Site-specific variation in the impact of *A. adenophora* was noticed.

Elevation had a significant effect on the gall abundance as well as gall size. Both gall abundance and size peaked at mid elevation (1950-2000 m asl). Stem galling by the fly reduced the stem diameter of the weed and all the three nutrients (N, P and K) were higher in galls than in ungalled stems. This study indicates that the elevation is an important abiotic factor that influences the *P. utilis* stem gall abundance and gall size. Furthermore, the galls formed by *P. utilis* act as nutrient sinks.

In conclusion, probability of range expansion and upslope movement as revealed by niche models and functional trait studies suggests for a need to prevent further spread of the weed in the study area. Significant negative impact on plant community and soil chemical characteristics reported in this study also emphasizes for a prompt action and management interventions to be adopted by scientific community and resource managers. As the study revealed that insect gall abundance is affected by elevation and peaks at mid-elevation range, this knowledge on gall dynamics is central to the successful management of the weed through the use of biological control agents.
Keywords: Invasive species, Distribution, Functional traits, Impact, Biocontrol

LIST OF ACRONYMS AND ABBREVIATION

°C	Degree centigrade
ANOVA	Analysis of Variance
AUC	Area Under Receiver Operating Characteristics (ROC) Curve
CHAL	Chitwan Annapurna Landscape
cm	Centimeter
D	Simpson's Diversity Index
DHM	Department of Hydrology and Meteorology
e.g.	as an example
ENM	Ecological Niche Modelling
et al.	and others
etc.	Et cetera
g	Gram
H	Shannon's Diversity Index
IAPS	Invasive Alien Plant Species
KATH	National Herbarium and Plant Laboratories, Godawari
LNC	Leaf Nitrogen Content
m asl	meter above sea level
MaxEnt	Maximum Entropy
mg	Milligram
OC	Organic Carbon
RCP	Representative Concentration Pathway
SDM	Species Distribution Modelling
SLA	Specific Leaf Area
TSS	True Skill Statistic
TU	Tribhuvan University

LIST OF TABLES

Page No.

LIST OF FIGURES

TABLE OF CONTENTS

Page No.

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 1

1. INTRODUCTION

1.1 Background

Biological invasions are a process in which species are not naturally present in the native assemblage but due to human activities cross the geographic range and successfully spread in the new environment (Blackburn *et al.,* 2011). The negative impact of biological invasions on biodiversity, ecosystem services, human health and socioeconomy is substantial and well known (Villa & Hulme, 2017; IPBES, 2019; Rai & Singh, 2020). Native and endemic species extinction, reduction in growth and fitness of native species and change in community structure and species interactions are some of the profound impacts of biological invasion on biodiversity (Vila *et al.,* 2011; Bellard *et al.,* 2016; Pysek *et al.,* 2017). Biodiversity is inevitably related with ecosystem services and human health. In addition to biodiversity loss, biological invasions have also caused losses to different ecosystem services like provisioning, supporting, regulating and cultural (Vila & Hulme, 2017). And these losses to biodiversity and ecosystem services have ultimately affected the human health (Mazza & Tricarico, 2018). The economic impact caused by the biological invasions worldwide has reached a minimum of US $ 1.288 trillion over the past few decades (1970-2017) exhibiting a threefold increase per decade (Diagne *et al.,* 2021). With the increase in globalization of trade, human movement, human population and anthropogenic activities, the number of established alien species is increasing worldwide (Seebens *et al.,* 2017), and by 2050, number of alien species is predicted to increase by 36% (Seebens *et al.,* 2021). Climate change in addition amplifies the threat and loss caused due to invasive species through multiple mechanisms like promoting traits that support invasiveness, removing climatic barrier for establishment and spread, creating more hospitable climates and by facilitating range expansion in new areas (Hellmann *et al.*, 2008; Walther *et al.,* 2009; Bradley *et al.,* 2010). Therefore, for preventing the rate of introduction of alien species, their establishment and for managing them, Parties to Convention on Biological Diversity (2020) emphasize in focusing on priority invasive species and priority sites (https://www.cbd.int/doc/c/abb5/591f/2e46096d3f0330b08ce87a45/wg2020-03-03-en.pdf).

Once Invasive Alien Plant Species become established in a novel area, then their control and eradication become extremely difficult. Therefore, early detection and rapid response (EDRR) at early stages of invasion is the most cost-effective strategy of management of IAPS (Westbrooks, 2004; Simberloff, 2013). And for EDRR to invasive species, knowledge on their spatial distribution is crucial (Westbrooks, 2004; Bhowmik, 2014). To this end, use of Ecological Niche Models (ENMs) for invasive species risk assessment under current and predicted future climate scenarios is proliferating recently (Qin *et al.,* 2016; Shrestha *et al.,* 2018; Thapa *et al.,* 2018; Shrestha & Shrestha, 2019). These ENMs are very helpful for identifying key climatic variables and predicting the potential climatically suitable areas of the invasive species (Franklin, 2009). Alternatively, remote sensing has also gained a considerable interest in detection and mapping of invasive plant species as this technique enables fast, precise and efficient monitoring of species at regional and global scales (Underwood *et al.,* 2003; Matongera *et al.,* 2017). Furthermore, remote sensing techniques provide an opportunity to observe the invasion pattern of the species as date back satellite images are available. Hence, predicting potential and actual distribution of invasive species in current and future climate as well as understanding their invasion pattern since past would be a valuable tool for resource managers, policy makers and scientific community to take timely actions to prevent further spread of the invasive species and prioritize management decisions.

For understanding the mechanism of biological invasions in the face of current and future environmental changes, it is necessary to understand the relationship between plant functional traits and invasiveness (Rejmanek, 2011). Studies suggest that invasive alien plant species (IAPS) have higher trait values for traits related to physiology, leaf area allocation, shoot allocation, growth, size and fitness (van Kleunen *et al.,* 2010; Matzek, 2012). The ability of IAPS to cross environmental barriers and successfully establish in a range of novel environmental conditions can be attributed to differences of their functional traits with respect to natives (Pysek & Richardson, 2007; Mathakutha *et al.,* 2019). However, there is lack of gradient studies assessing the variation in plant functional traits of a species along major environmental gradients like elevation, water availability (Hulme & Bernard-Verdier, 2018). Elevation, being the most powerful gradient for studying ecological responses of plants to geophysical influences (Korner, 2007), knowing how plant functional traits of invasive species vary along elevation gradient would foster our understanding of the adaptation by the species in introduced ranges and their response to global and environmental drivers (Violle *et al.,* 2012; Lavorel *et al.,* 2013). Furthermore, this type of study would also be helpful to predict range limit and climatic niche of the invasive species (vanNuland *et al.,* 2020). Thus, for prediction of spread and management of invasive species under changing environmental scenarios, understanding the role of functional traits is crucial (Drenovsky *et al.,* 2012).

With the growing concern of management of IAPS, biological control, being cost effective and environmentally friendly method, has been adopted by many countries against different IAPS. Among different biocontrol agents known so far, herbivorous arthropods especially gall inducing insects are used in weed biological control and have been found to inhibit the vegetative growth and reproduction of the invasive weeds (Erasmus *et al.,* 1992; Florentine *et al.,* 2005; Bitume *et al.,* 2019). Gall inducers are thought to act as a good biocontrol agent because they have narrow host range, therefore have low probability of harming non-target plants (Harris & Shorthouse, 1996). Interaction among gall inducing insect used as a biocontrol agent, host plant, and the environment determine the population dynamics of the gall inducers on weeds (Price & Hunter, 2015). Therefore, understanding the factors inducing gall dynamics is central to the successful management of weeds (Clerck-Floate & Bourchier, 2000; McEvoy, 2018).

Among 1517 invasive alien species reported globally, 938 are IAPS (Turbelin *et al.,* 2017). *Ageratina adenophora* (Sprengel) H. King & H. Robinson is one of the noxious invasive weeds that is already established in 40 countries from tropical to temperate regions of the globe including Nepal (Poudel *et al.,* 2019). The weed is a native of Mexico and belongs to family Asteraceae (Poudel *et al.,* 2019). It has invaded grasslands, agricultural lands, wetlands and forests causing significant negative ecological as well as economic impacts in the invaded regions (Wu *et al.,* 2004; Baral *et al.,* 2017; Poudel *et al.,* 2019). In Nepal, the weed is the topmost ranked problematic invasive weed that was first reported in 1958 A.D. (Tiwari *et al.,* 2005). It is also one of the invasive weeds that was prioritized by local community for management in one of the important landscapes i.e., Chitwan Annapurna Landscape (CHAL) of central Nepal (Shrestha *et al.,* 2019). Though different management strategies have been applied for the control of this weed in various regions, their efficacy remains poor and the weed thrives to spread in new region. Climate change in addition is thought to amplify the negative consequences of the weed by creating more climatically suitable areas in future (Lamsal *et al.,* 2018; Thapa *et al.,* 2018; Shrestha & Shrestha, 2019). Therefore, for successful management of the weed, a thorough understanding of its invasion mechanism, its spatial distribution under current and future climate, its impacts and the effectiveness of current management efforts would be valuable.

1.2 Rationale of the study

IAPS have been identified as the major driver of global change and they pose serious threats to biodiversity, ecosystem services and human health (Villa & Hulme, 2017; Rai & Singh, 2020). Invasion by alien species is also the most common cause of species extinction (Blackburn *et al*., 2019). Though different management strategies have been identified and implemented, with worldwide trade intensifying, the number of invasive alien species is still increasing and is predicted to increase in future as well (Seebens *et al.,* 2017; Seebens *et al.,* 2021; Essl *et al.,* 2020). About one fifth of the earth's surface including global biodiversity hotspot are at risk due to biotic invasions (IPBES, 2019). While most of the global biodiversity hotspots are situated in developing countries, the majority of research related to invasive species occurs in developed countries (Nunez & Pauchard, 2010; Early *et al.,* 2016). In coming decades, the risks from invasive species are going to be far more in developing countries (Early *et al.,* 2016), therefore, there is a need of additional research on invasive species in developing countries.

A developing country like Nepal is at high risk of plant invasion from wide range of biogeographic areas because of its high dependency on international trade (Shrestha, 2016) and changes in environmental condition due to elevational variation (Bhattarai *et al.,* 2014). Furthermore, due to the lack of expertise and limited resources availability for management, the impact of invasive species is likely to increase in Nepal (Shrestha, 2016). Till date, 27 IAPS have been reported in Nepal and all 27 IAPS are found in central Nepal (Shrestha & Shrestha, 2021; B.B. Shrestha, personal communication, August 23, 2021). In addition, central Nepal also harbors the highest number (118) of naturalized plant species in comparison to eastern and western Nepal (Bhattarai *et al.,* 2014) and all 27 IAPS representing 0.5% of Nepal's flora are already established in this region (Shrestha & Shrestha, 2021; B.B. Shrestha, personal communication, August 23, 2021 for *Mimosa diplotricha*). A recent Ecological niche modeling identified central Nepal as "invasion hotspot" having high proportion of climatically suitable areas for 10 IAPS under current climate (Shrestha & Shrestha, 2021). Therefore, the current and future threats of IAPS is relatively high in central Nepal than in other regions, suggesting a need of additional research particularly in central Nepal. One of the important landscapes in central Nepal is Chitwan-Annapurna Landscape (CHAL), where exceptionally rich biodiversity is threatened by multiple anthropogenic pressures including invasive alien species (WWF, 2013; MFSC, 2016).

Ageratina adenophora is one of the most problematic invasive weeds in Nepal (Tiwari *et al.,* 2005). First time reported in 1958 in Nepal, the weed is found forming monodominant stands in grasslands, forests, roadsides and bare land preventing the regeneration of other species and causing ecological and economic impacts (Tiwari *et al.,* 2005; Poudel *et al.,* 2019). The weed is thus widespread in Nepal currently and has probabilities of further expansion under future climate change scenarios (Shrestha & Shrestha, 2019). Therefore, in this twenty first century, Species distribution modelling studies is an urgently needed task to understand the relationship between climate change and invasive species' distribution which is challenging as well (Bellard *et al.,* 2018; Lenzner *et al.,* 2019). Distribution mapping of IAPS helps in early detection and rapid response system by identifying current potential habitat of invasive species and predicting its spread in future (Wei *et al.,* 2017; Martinez *et al.,* 2020; Beaury *et al.,* 2020). Though, many studies have been conducted to investigate the potential distribution of *A. adenophora* in broader spatial scales like Nepal (Shrestha & Shrestha, 2019), China (Wang & Wang, 2006; Wang *et al.*, 2017), South Africa (Tererai & Wood, 2014) and the Himalaya (Lamsal *et al.,* 2018; Thapa *et al.,* 2018), there is lack of such studies in smaller scales where management strategies are implemented. Hence, predicting suitable habitat of *A. adenophora* in CHAL under current and future climate would identify areas at invasion risk which will allow policy makers and resource managers to take timely action for preventing the further spread of the weed in new areas.

Assessment of impacts of IAPS is a major task in invasion science (Bartz & Kowarik, 2019; Lenzner *et al.,* 2019). Lack of empirical data on impacts of invasive species is one of the main obstacles to advancing invasion theory (Pearson *et al.,* 2016). There are only handful of researches that have quantified the ecological impacts of invasive species in Nepal (Pandey *et al.,* 2020), yet quantifying ecological impacts of IAPS is crucial to prioritize species-specific and site-based management strategies to the most problematic invasive species and affected area (Parker *et al.,* 1999; Shrestha, 2019). Though impacts of *A. adenophora* have been studied elsewhere (Poudel *et al.,* 2019), studies quantifying impacts of *A. adenophora* in Nepal is very limited. Therefore, additional studies would lead to a better understanding of the ecological impacts of *A. adenophora* to inform management decisions.

Plant functional traits reflects adaptation of species to abiotic changes (Lavorel *et al.,* 2007). Therefore, understanding the role of functional traits in invasion success of *A. adenophora* along the wide elevation gradient would help in knowing the probability of future range expansion of the weed and response to climate change (vanNuland *et al.,* 2020). *Procecidochares utilis*, a biocontrol agent for *A. adenophora* has been less effective in several countries (Poudel *et al.,* 2019), hence, knowing suitable climate for this agent is directly related to effective management of the weed.

1.3 Hypothesis

Present research is based on four main hypotheses:

- Climatically suitable areas of *Ageratina adenophora* expands in future with changing climate.
- Plant fitness of invasive alien plants of tropical origin decreases with increasing elevation in the mountain landscape.
- *Ageratina adenophora* reduces species richness, species diversity and alters soil chemical characteristics.
- Activity of stem galling insect (a biocontrol agent) peaks at a mid-elevation of distribution of *A. adenophora*.

1.4 Objectives of the study

A general objective of the present work was to analyse the distribution pattern, functional traits, impact and galling activity of the biocontrol agent of an invasive weed *A. adenophora*. The specific objectives are:

- To predict the current and potential future distribution of *A. adenophora* using ecological niche modelling in Chitwan-Annapurna Landscape, Nepal.

- To analyze the spatio-temporal distribution of *A. adenophora* using satellite imageries in Chitwan-Annapurna Landscape.
- To analyze the variation of plant functional traits of *A. adenophora* along the elevation gradient in central Nepal.
- To study the impact of *A. adenophora* on vegetation and soil characteristics.
- To assess the stem galling of *A. adenophora* by a biocontrol agent *Procecidochares utilis* along the elevation gradient.

1.5 Limitations

Following is the limitation of the present study:

- Impact data is based on single year sampling.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Biological invasions

Biological invasions have been identified as one of the major drivers of global environmental change with substantial global impact on biodiversity, ecosystem services, human health and livelihood (Vila & Hulme, 2017; IPBES, 2019; Rai & Singh, 2020). Adverse impact of biological invasion on biodiversity includes reduction of growth and fitness of native species, extinction of native and endemic species and change in community structure (Vila *et al.,* 2011; Bellard *et al.,* 2016; Pysek *et al.,* 2017). Different ecosystem services like supporting, regulating, provisioning and cultural services are also hampered by biological invasion (Vila & Hulme, 2017). Over the past few decades (1970-2017), the annual mean cost of biological invasion has reached to US $ 26.8 billion which is thought to be underestimated and is increasing threefold per decade (Diagne *et al.,* 2021). As human wellbeing is directly related to biodiversity and ecosystem services, varying impacts like psychological effects, discomfort, nuisance, phobias, skin irritations, allergies, poisoning, disease and even death are noticed due to biological invasion (CABI, 2018).

Currently, about one fifth of the earth's surface is at risk of biological invasion with probability of further increment in number and impact of invasive species by 2050 (IPBES, 2019; Seebens *et al.,* 2021). Increased trade and transport, climate change and socioeconomic change are predicted to significantly affect future impacts of invasive alien species on biodiversity (Essl *et al*. 2020). Climate change affects biological invasion mainly via three mechanisms- 1. Changing the nature of vectors and pathways, 2. Changing the abiotic environment and 3. Altering the biotic interactions in recipient communities (Robinson *et al.,* 2020). Number of studies from different countries have demonstrated that risks from plant invasion is going to increase due to climate change particularly by increasing climatically suitable areas for invasive plant species (Bradley *et al.,* 2010; Adhikari *et al.,* 2019; Shrestha *et al.,* 2019). In addition, parameters of climate change like increased temperature and CO_2 enrichment enhanced the performance of invasive species in comparison to native plant species (Liu *et al.,* 2017). Thus, predicting the distribution of invasive weeds under climate change scenarios and identifying the areas potentially at risk is an urgent need for effective management planning.

Biological traits, biogeographical origin and introduction history are the most important determinants of plant invasiveness (Ni *et al.,* 2021). Many studies have evaluated the relationship between functional traits of species and invasion success (van Kleunen *et al.,* 2010; Gallagher *et al.,* 2014). Invasive alien plant species had higher trait values for performance related traits in comparison to non-invasive species (van Kleunen *et al.,* 2010). A recent study conducted in China indicates that unintentionally introduced alien plant species with shorter life span and longer residence time are likely to become invasive and tend to invade more larger areas while taller plants introduced from Americas have higher chance of causing severe impact (Ni *et al.,* 2021).

2.2 Invasions by *Ageratina adenophora*

Till date, 938 invasive plant species have been reported globally (Turbelin *et al.,* 2017). Among them, *A. adenophora*, a native of Mexico, is one of the most noxious weeds that has extended its distribution range to 39 countries outside its native range (Poudel *et al.,* 2019). The earliest report of this weed outside of its native range was from United Kingdom where the weed was introduced as an ornamental plant in 1826 and then to other countries like USA, Australia and India (Poudel *et al*., 2019). Now, the weed has naturalized in several countries from tropical to temperate regions of the world (Asia, Oceania, Africa) (Muniappan *et al.,* 2009) and ecological niche modeling studies conducted in different regions (China, western Himalaya, South Africa, Nepal) all suggest the possibility of further expansion of the weed in these invaded regions in climate change scenarios (Wang *et al.,* 2017; Lamsal *et al*., 2018; Thapa *et al.,* 2018; Shrestha & Shrestha, 2019). In Nepal, the weed was first recorded in 1958 and is thought to have spread naturally from north-east India (Tiwari *et al.,* 2005). It is widespread in Nepal from eastern to western region including all physiographic regions (Shrestha & Shrestha, 2021). It forms monodominant stands in grasslands, wetlands, forest and roadsides.

Success of invasive species have been attributed to their differences in functional traits with native species (Pysek & Richardson, 2007; Mathakutha *et al.,* 2019). Studies conducted in China revealed that, compared to natives, *A. adenophora* had longer vegetative growth phase, higher biomass allocation, higher above/below-ground ratio, higher coverage, higher germination ratio and higher germination index (Gao *et al.,* 2013; Zhang *et al.,* 2014). Genetic differentiation (Gui *et al.,* 2008; Huang *et al.,* 2009) and phenotypic plasticity also have contributed to the invasion success of *A. adenophora* (Li & Feng, 2009; Zhao *et al*., 2012; Datta *et al.,* 2017). Several laboratory-based studies suggest that allelopathic interference also has helped the weed to establish monodominant stands in invaded communities, though there is lack of adequate field experiments to support these findings (Poudel *et al.,* 2019).

Figure 1: An open forest invaded by *A. adenophora* in central Nepal

2.3 Distribution mapping of invasive species

With the increasing number of invasive species, their profound negative impacts on biodiversity are also increasing. Climate change in addition is thought to exacerbate the impacts of invasive species with their further spread (Walther *et al.,* 2009). Therefore, to minimize the further spread and establishment of the invasive species, there is a need of early detection and rapid response at early stages of invasion which is the most cost-effective strategy of management (Westbrook, 2004; Simberloff, 2013). Knowledge on spatial distribution of invasive species is crucial for early detection and rapid response to invasive species (Westbrooks, 2004). In addition, distribution maps of invasive species also provide support to the scientists and resource managers for prioritizing management strategies and making decisions. Therefore, using different models for mapping the spatial distribution of the invasive species has been an emerging trend these days.

Ecological niche modelling of invasive weeds

Climate is the most important factor that determines the distribution of plant species including invasive weeds (Gaston, 2003; Mahgoub, 2019). And climate or habitat match between native and invaded range is one of the pre requisite for the invasion success of weeds (Thuiller *et al.,* 2005; Hayes & Barry, 2008). Though other factors other than climate like propagule pressure, dispersal ability, biotic interactions and human activities also determine the invasion success of alien species (Theoharides & Dukes, 2007) but climate matching is undoubtedly the most important one (Ficetola *et al.,* 2007). Based on this view Ecological Niche Models (ENMs) have been developed that identifies key climatic variables and determine the niche of a species. ENM is based on the assumption that species distribution is partly or fully determined by the aspects of climate (Araujo & Peterson, 2012). These models relate documented presence records of the focal species with environmental or spatial characteristics of the potential sites and are used to predict the current and future potential distribution of a species in a particular region of interest (Peterson, 2006; Elith & Leathwick, 2009; Franklin, 2009).

The niche concept is central to ENMs and is based on Hutchinson's concept of fundamental and realized niche (Araujo & Guisan, 2006). At an early stage of invasion, it is highly likely that an invasive species occupies only a portion of fundamental niche (i.e., realized niche) in its introduced range and there is always a risk of invasion in unoccupied part of the fundamental niche (Soberon & Nakamura, 2009). However, controversies persist over which facets of the niche are projected by ENMs (Araujo & Guisan, 2006; McInerny & Etienne, 2012).

Ecological niche models have been widely used in invasive species risks assessment (Qin *et al.,* 2016; Suarez-Mota *et al.,* 2016; Wan *et al.,* 2017; Shrestha *et al.,* 2018; Thapa *et al.,* 2018). These ENMs that consider future climate change scenarios are very helpful for managers to identify hotspots of invasion and monitor the spread of invasive species in future (Beaury *et al.,* 2020). Various modeling techniques have been applied for predicting suitability of invasive species. Among them, Maxent is one of the most popular modeling tools which uses presence only data and has been used globally in building habitat suitability maps of various invasive species under current climate and climate change scenarios (Phillips *et al.,* 2006; Merow *et al.,* 2013; West *et al.,* 2016; Lamsal *et al.,* 2018).

Climate change is one of the major drivers of invasive species distribution in future (Bellard *et al*., 2018). Invasive plant species are expected to gain climatically suitable areas under future climate change scenarios (Bradley *et al.,* 2010). Qin *et al.* (2016) predicted an increase in suitable habitat of *Ambrosia artimisifolia* and *Artimisia trifida* under projected future climate scenarios. Similarly, Thapa *et al.* (2018) also suggested an increase in suitable habitat of the invasive weeds like *Ageratina adenophora*, *Lantana camara* and *Chromolaena odorata.* A study carried out in China predicted a climatic niche shift between the native and invasive ranges of eight invasive weeds (Wan *et al.,* 2017). Studies conducted in different regions of the world till date have predicted gain in suitable areas of *A. adenophora* in future climate scenarios (Wang *et al.,* 2017; Thapa *et al.,* 2018; Lamsal *et al.,* 2018). In Nepal also*, A. adenophora* is expected to gain climatically suitable areas under future climate change (Shrestha & Shrestha, 2019). Though, a recent global study found shrinkage of invasion range of *A. adenophora* in future climate scenarios, the weed would gain climatically suitable areas in six biodiversity hotspot regions (Changjun *et al*., 2021).

Remote sensing approaches

Spatial bias in collection of occurrence points of the species strongly affect the ecological niche models (Phillips *et al.,* 2009). In addition, at regional and global scales, ground-based mapping is inefficient. Therefore, to improve these invasion risk assessment models and document the current extents of plant invasion, comprehensive mapping is required (Bradley, 2014). Remote sensing techniques have gained considerable interest since few years in the field of biological invasion as they are cost effective and provide fast and frequent mapping over large geographical extents (Underwood *et al.,* 2003; Mullerova *et al.,* 2013; Matongera *et al.,* 2017). In addition, remote sensing methods provide an opportunity for studying the temporal distribution of species across large areas (Turner *et al.,* 2003). Remote sensing approaches for mapping the distribution of individual species depends upon the biochemistry of the target species which includes leaf pigments, nutrient, water content and structural properties (Ustin *et al.,* 2004; He *et al.,* 2011; Feilhauer *et al.,* 2012). Each species has its own unique biogeochemistry which is responsible for difference in spectral signature between species. Use of remote sensing techniques in plant invasion dates back to 1970s when coloured infra-red (IR) photos were used to target herbicide application to water hyacinth infestations (Rouse *et al*., 1975). By then, remote sensing has flourished as a reliable approach for detection and mapping of invasive plant species spread (Bradley, 2014; Matongera *et al.,* 2017). Remote sensing has been successfully used in plant invasion studies (Huang & Asner, 2009), mostly for shrubs and trees (Fuller, 2005; Hamada *et al.,* 2007; Lawes & Wallace, 2008).

Remote sensing techniques include use of various sensors which differ in their spatial, spectral, temporal resolutions and detection algorithms. Among various sensors, multispectral remote sensors have also been applied to identify and predict distribution of invasive plant species on the basis of unique leaf colouration or cover (Fuller, 2005; Resasco *et al.,* 2007; Schneider & Fernando, 2010; Matongera *et al.,* 2017). Multispectral remote sensing records the intensity of reflected light at several different spectral wavelengths in the electromagnetic spectrum. Landsat is one of the examples of multispectral sensors that has moderate spatial resolution (30m) (Bradley, 2014). Landsat imageries are freely available and date back images till 1984 are also easily obtained so many studies have used Landsat images for detecting invasive species. Peterson (2005) used Landsat 7 ETM imagery of two different dates within a single year to distinguish invasive grass *Bromus tectorum* from other species. Bradley & Mustard (2006) used various Landsat Thematic Mapper images and spectral indices to detect grass invasion in grasslands. Similarly, Landsat TM and ETM + imagery was used to determine the spatial pattern of invasion by an invasive shrub *Lonicera maackii* in USA (Resasco *et al.,* 2007). Evangelista *et al.,* (2009) suggested that time series analysis of Landsat 7 ETM+ images were the best for mapping *Tamarix sp*. Similarly, Bishop *et al.* (2019) used Landsat 5 TM and Landsat 7 ETM to identify the spatial extent and temporal patterns of *Bromus tectorum* in USA.

With the advancement of new satellite images having more finer spatial resolution, the utility of commonly used Landsat images has been constrained. Multispectral sensors with high spatial resolution have also emerged as an efficient tool in detection and mapping of invasive plant species (Odidni *et al.,* 2014; Robinson *et al.,* 2016; Matongera *et al.,* 2017). Digital Globe sensor WorldView-2 is one of the high spatial resolution imageries that acquires 11-bit data in eight spectral bands. WV-2 imageries have been found to detect invasive plant species with high accuracy in comparison to Landsat imageries. Sankey *et al.* (2014) used WV-2 images to evaluate its potentiality to detect the presence, cover and biomass of *Brassica tournefortii* and reported overall accuracy of 88% but while using Landsat ETM images, the accuracy dropped by about 20%. Similarly, Stych *et al.* (2019) also reported higher accuracy of WV-2 images using Support vector machine in comparison to Landsat 8 images to classify forest infected by bark beetle outbreaks.

2.4 Plant functional traits along elevation gradients and invasion success

Any morphological or eco-physiological plant attributes that explain features of plant performance in the environment are known as plant functional traits (Violle *et al.*, 2007). Functional trait signifies ecological strategies of plants and their responses and influence on various ecosystem properties (Perez-Harguindeguy *et al.,* 2013). It also reflects adaptations varying with abiotic factors and tradeoffs among different functions within a plant (Lavorel *et al.,* 2007). Thus, functional trait-based approach is widely being used to 1) understand the functioning of organisms and its relation to the environment, 2) foster general rules in community ecology that governs the assembly of communities and 3) know how the functional traits of organisms affect ecosystem functioning (McGill *et al.,* 2006; Garnier & Navas, 2012; Funk *et al.,* 2017; Bu *et al.,* 2019).

As plant functional traits are related indirectly to plant fitness, they are good candidates to study causes of invasion success across wide environmental gradients (Violle *et al.,* 2007; Tecco *et al.,* 2010). Spread of invasive species over wide elevation gradients is attributed to the trait variability either due to phenotypic plasticity or local adaptation capability (Zhao *et al.,* 2012; Molina-Montenegro *et al.,* 2013; Colautti & Barett 2013; Hirano *et al.,* 2017). Phenotypic plasticity will allow short term responses to abiotic changes whereas genetic variation or local adaptation may permit evolutionary responses to abiotic changes (Bradshaw, 1965; Linhart & Grant, 1996). Intraspecific trait variability along elevation gradient has showed three trends 1. Trait values increased with increasing elevation 2. Trait values decreased with increasing elevation 3. Traits exhibited a unimodal pattern (Albert *et al.,* 2010).

However, the magnitude of trait variation along elevation gradient might be population specific within a species, therefore trait variation studies must be conducted in multiple locations (Pfennigwerth *et al.,* 2017).

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2.4.1 Specific leaf area (SLA)

Specific leaf area (SLA) is a growth and resource acquisition indicator and a good correlate of the potential growth rate of plant (Cornelissen *et al.,* 2003). It is directly related with relative growth rate and leaf nitrogen content (Leishman *et al.,* 2007; Perez-Harguindeguy *et al.,* 2013) and indirectly related with leaf thickness, carbon investment, stress tolerance etc. SLA is directly proportional to leaf nitrogen content and mass-based light saturated photosynthetic rate whereas indirectly proportional to leaf longevity and carbon investment in secondary compounds (Perez-Harguindeguy *et al.,* 2013). In resource-rich environments, species tend to have higher SLA whereas in environments with poor resources, species tend to have lower SLA (Cornelissen *et al.,* 2003). SLA is also positively correlated with higher water use efficiency (Hoffmann *et al.,* 2005). A high SLA ensures opportunistic capture of solar energy (Grottkop & Rejmanek, 2007).

High SLA is a trait that is associated with small seed mass, high relative growth rate and invasiveness (Grotkopp *et al.*, 2002; Hamilton *et al.*, 2005; Leishman &

Thomson, 2005). SLA is negatively correlated with leaf life span and assimilation rates and is generally lower in leaves of evergreen compared to deciduous species (Reich *et al.,* 1997). Lower SLA contributes to long leaf survival, nutrient retention and protection from desiccation (Mooney & Dunn, 1970). In context of invasive species, previous studies have shown that high SLA leading to higher relative growth rate is directly and positively related with invasiveness (Grotkopp & Rejmanek, 2007; Leishman *et al.,* 2007).

Along the elevation gradient, number of studies suggest the decreasing trend of SLA. In a study conducted in northern Spain, SLA of *Saxifraga trifurcata* decreased with increasing elevation (Milla *et al.,* 2009). Similarly, Ohdo & Takahashi (2020) also reported decreasing trend of SLA with increasing elevation. In a study conducted in Nepal Himalayas, *Rhododendron lepidotum* also exhibited similar decreasing trend of SLA along the elevation gradient (Pandey *et al.,* 2021). In a recent meta-analysis of 109 plant species of four continents, conducted by Midolo *et al.* (2019), similar decreasing trend of SLA with increasing difference in elevation was noticed. However, some studies have reported the highest SLA in mid-elevation ranges. In *Quercus aquifolioides*, SLA increased upto 2800 m asl and then decreased with the increasing elevation (Li *et al.,* 2006). Similar mid-elevation peak of SLA along the elevation gradient of 600 m was observed in *Lasiocephalus ovatus* (Almeida *et al.,* 2013). Mid elevation peak of SLA was noticed in bamboo *Pleioblastus amarus* also (Guo *et al.,* 2018). In a common garden and reciprocal transplant experiment conducted in India, no differences were found in SLA between plants of *A. adenophora* from different elevations indicating absence of clinal variation and local adaptation (Datta *et al.* 2017).

2.4.2 Leaf nitrogen content

Leaf nitrogen content (LNC) is the total amounts of nitrogen per unit of dry mass. It tends to be closely correlated with SLA and mass based maximum photosynthetic rate (Perez-Harguindeguy *et al.,* 2013). LNC is correlated with net photosynthetic rate which in turn affects stomatal conductance to water (Mezian & Shipley, 2001). Higher LNC associated with higher rates of maximum photosynthesis (Poorter & Evans, 1998). Both leaf nitrogen content and SLA decrease with leaf age and acclimate to leaf radiation microclimate (Reich *et al.,* 1997). LNC is also the link of carbon and respective nutrient cycle (Kattge *et al.,* 2011). High LNC means higher nutritional quality (Cornelissen *et al.,* 2003). With respect to invasive species, increased leaf nitrogen concentration enables them to allocate more nitrogen to photosynthesis which ultimately leads to higher relative growth rate (Grotkopp *et al.,* 2010). Thus, higher leaf nitrogen concentration is positively correlated with their invasiveness.

Declining foliar nitrogen with increasing elevation has been reported in treeline ecotones in Rolwaling of Nepal (Drollinger *et al.,* 2017). Along an elevation gradient of 1600 m asl in Ladakh in north western Himalaya, similar decreasing trend of foliar nitrogen was noticed (Macek *et al.,* 2012). Similarly, in a study conducted along an elevation gradient of 500-2300 m asl in north China, leaf N content decreased with the increasing elevation (Zhao *et al.,* 2016). A recent meta-analysis of 109 plant species from four continents reported decreasing nitrogen concentration per unit area with increasing difference in elevation (Midolo *et al.,* 2019). However, some studies have also noted the opposite trend of LNC along the elevation gradient. There was increase in LNC of *Acer pseudosieboldianum* with the increasing elevation along the elevation gradient of 600 m asl in a montane forest of Southern Korea (Nam & Lee, 2018). Shi *et al.* (2012) in a study of 291 plant species pooled together, found that leaf N kept on increasing until elevation of about 2200 m asl and then decreased with increasing elevation.

2.4.3 Reproductive traits

Reproductive traits indicate the resources invested in reproduction. These traits have an important influence upon the establishment and maintenance of population of a species in new areas and therefore upon its success as an invader (Pysek & Richardson, 2007; Correia *et al.,* 2014; Moravcova *et al.,* 2015). Thus, studies on reproductive traits of IAPS are necessary for the assessment of invasiveness of a species.

2.4.3.1 Number of floral heads per ramet

Number of floral heads per ramet determines the reproductive potential of the plant. Elevational change in reproductive traits is thought to be an adaptation to the short growing season at high elevations (Sakurai & Takahashi, 2016). Plants growing in high elevation have lower number of floral heads in comparison to plant growing in low elevation (Kelly, 1998; Alexander *et al.,* 2009). Similarly, number of floral heads per individual decreased with the increasing elevation in *Solidago virgaurea* in a study carried out in Japan (Hirano *et al.,* 2017). However, in *Solidago minuta*, the number of flower heads per plant decreased with the increasing elevation having its maximum at the centre of its investigated elevation gradient (Kieltyk, 2017). Some studies have also reported the decreasing flower number with the increasing elevation (Ngezahayo *et al.,* 2018)

2.4.3.2 Total seeds per floral head

Number of seeds per floral head is an indication of pollination success which ultimately determines the reproductive success of a plant (Primack, 1987; Chen *et al.,* 2018). Seed number per fruit decreased with the increasing elevation in *Pedicularis* species in eastern Tibetan plateau (Guo *et al.,* 2010). Similar observation was made in *Primula elatior* in Southern Poland (Olejnicjak *et al.,* 2018). Tradeoff is seen between seed size and seed number (Olejnicjak *et al.,* 2018). Similar inverse relationship is found between seed size and seed number (Jakobsson & Eriksson, 2000; Kuhner & Kleyer, 2008). However, seeds per fruit increased with increasing elevation in *Vaccinium myrtillus* along the elevation gradient (Pato & Obeso, 2012). Similar trend of increment in seed number with increasing elevation was reported in *Sinopodophyllum hexandrum* (Meng *et al.,* 2014). In a study conducted in China, *A. adenophora* exhibited unimodal curve of seed yield along the elevation gradient of 678-2356 m asl (Zhao *et al.,* 2012) indicating the increased reproduction at mid elevation environments.

2.4.3.3 Seed mass and seed size

Seed mass and seed size influences seed dispersal ability, seedling establishment success and population distribution pattern (Wang *et al.,* 2014). Seed mass has strong effects on processes that influence survivorship and fecundity and is often heritable (Westoby *et al.,* 2002). The mean seed size increased with increasing elevation in *Leucanthemum waldsteinii* and *Senecio subalpinus* (Olejniczak *et al.,* 2018). Similar increasing trend of seed size with increasing elevation has been suggested in *Centaurea solstitialis* in Anatolia and the Caucasus, Western Europe and Western US (Hierro *et al.,* 2019). However, some studies have also reported the mid elevation peak of seed size which decreased along the range margins (Vaupel & Matthies, 2012). In a study conducted in China, seed width as well as seed mass of *A. adenophora* increased with increasing elevation along the elevation gradient of 640-2430 m asl (Li & Feng, 2009).

Similar to the seed size, seed mass is also found to increase with the elevation. Pluess *et al.* (2005) reported a positive correlation between seed mass and elevation. Seed mass of *Solidago virgaurea* per individual at higher elevations was greater compared to that of lowest elevations in Mt. Norikura, Japan (Hirano *et al.,* 2017). However, negative correlation was reported between elevation and mean individual seed mass in *Pedicularis* species (Guo *et al.,* 2010). Further across *Picea* species, seed mass declined with the latitude (Miyazawa & Lechowicz, 2004).

2.5 Ecological impacts of plant invasion

Invasion by IAPS is one of the major drivers of current global change (IPBES, 2019). IAPS have caused significant negative impacts on biodiversity, ecosystem services, human health and socio-economy (Simberloff et al., 2013; Vila & Hulme, 2017; Rai & Singh, 2020). IAPS reduce the fitness and growth of native species which may even drive native species extinction (Vila *et al.,* 2011). Furthermore, they change the structure of plant community by decreasing the species abundance, species richness, species diversity and altering species composition (Hejda *et al.,* 2009; Vila *et al.,* 2011; Cordero *et al.,* 2016). Besides, intense competition between native plant species and invasive plant species for the resources might also create opportunity for other non-native species to invade leading to invasional meltdown (Simberloff & Von Holle, 1999; Braga *et al.,* 2018). Thus, IAPS may pose significant impacts at species, community and ecosystem level leading to biodiversity loss (Vila *et al.,* 2011).

Success of plant invaders is partly due to the invasion mediated changes in soil physio-chemical and biological environment (Gibbons *et al.,* 2017; Stefanowicz *et al.,* 2018; Lone *et al.,* 2019). Besides, changes in vegetation, IAPS also modify the properties of the soil after the invasion (Gibbons *et al.,* 2017; Lone *et al.,* 2019). Studies suggest that soil nutrients (carbon, nitrogen, phosphorus, potassium, calcium, magnesium) more often increase following invasion (Pysek *et al.,* 2012; Timsina *et al.,* 2011; Vila *et al.,* 2011; Gibbons *et al.,* 2017). However, this pattern is site specific. Soil nutrient concentration increase when the site is initially in low nutrient condition whereas opposite trend is noticed in the site initially with high nutrient

(Dassonville *et al.,* 2008). A global meta-analysis also reported that the differences in soil nutrient concentration between native and invasive species decreased with the increase in nutrient resources of the environment (Sardans *et al.,* 2017). Furthermore, some studies also report the negative or little effect of invasive weeds on soil nutrient concentration (Vanderhoeven *et al.,* 2006; Martin *et al.,* 2009).

Though, there is no doubt that invasive species cause significant negative impacts on biodiversity, impacts of invasion are context dependent and vary with both magnitude and direction (Hulme *et al.,* 2013). Different parameters like characteristics of invading species, its abundance, time since introduction, composition of the recipient community, habitat condition, abiotic changes, other stressors, spatial scale, ecological complexity and interactions among these parameters all effect the magnitude and direction of invasion impacts (Kumshick *et al.,* 2015; Kunzi *et al.,* 2015). The decrease in species richness in invaded plot in comparison to uninvaded plots is largely driven by the identity of invading species (Hejda *et al.,* 2009) whereas the major determinants in the decrease of Shannon's diversity and evenness are the cover and height of invading species. Though a number of studies have examined the impacts of invasive plants on plant diversity and community composition, only handful of researches have tested whether these impacts have arisen due to the allelopathic interference, competition, alteration of ecosystem variables or other processes (Levine *et al.,* 2003). Thus, for a comprehensive understanding of the success of invasive plant management, quantitative impact assessment studies comparing invaded, non-invaded and removal sites and also the observation of the resulting native species assemblage after its removal is necessary (Andreu & Vila, 2011).

Ageratina adenophora has already naturalized in 40 countries with significant ecological and economic impacts. The weed has invaded forests, grasslands and wetlands forming monodominant stands and has reduced the cover, richness and diversity of native plant species (Ding *et al.,* 2007; Gao *et al.,* 2013; Baral *et al.,* 2017). In China as well as in India, *A. adenophora* invasion changed the species composition as well as species diversity of understory plant community (Kumar *et al.,* 2020; Fu *et al.,* 2018). About 29-40% reduction in species number was reported in the site invaded by *A. adenophora* (Kumar *et al.,* 2020). In addition to reduction in species diversity, *A. adenophora* also altered the functional diversity of the understory community in South west China (Wu *et al.,* 2020). The weed has caused serious threats to forest occupying vacant places of teak, rubber and other forest plantations

(Dogra *et al.,* 2010). Furthermore, plant communities at lower montane vegetation in central Nepal are vulnerable to *A. adenophora* invasion (Thapa *et al.,* 2016). It also suppresses the growth of native tree seedlings so because of this ability, *A. adenophora* is known as “Banmara” or “forest killer” in Nepal (Tiwari *et al.,* 2005; Fu *et al.,* 2018; Thapa *et al.,* 2020). *A. adenophora* has also caused decline in agricultural productivity and forage production. Its invasion resulted in 18% yield loss of corn, 9% yield loss of rice and 5% loss of economic forest per year in Guanxi of China. Similarly, in Nepal, reduction in cereal crops and grasses was reported in Kaski district of Nepal (Bhushal, 2009). Furthermore, dispalcement of native species like *Artimisia indica*, *Solanum surattense*, *Urtica dioica* by *A. adenophora* invasion were also reported in Panchase of Kaski district (Baral *et al.,* 2017). Species with lower LNC were lost first with the increasing invasion intensity of *A. adenophora* in *Pinus yunnanensis* in China (Fu *et al.,* 2018).

Apart from impact on vegetation, *A. adenophora* also causes change in the bio-chemical characteristics of the soil. Soil invaded by *A. adenophora* had higher amount of nitrogen, available phosphorus and potassium in comparison to non-invaded soil (Niu *et al.,* 2007; Li *et al.,* 2009; Kumar *et al.,* 2020; Wu *et al.,* 2020). However, its impact on soil organic matter, soil nitrogen, soil phosphorus and soil potassium are related to seasons, exposure and degree of invasiveness (Yu *et al.,* 2014). In addition, soil biota also changed after the invasion of *A. adenophora*, vesicular-arbuscular mycorrhiza being the most abundant one and fungi/bacteria ratio also increased creating a hospitable environment for itself (Niu *et al.,* 2007; Li *et al.,* 2009).

2.6 Biological control by stem galling insect

Various methods like mechanical, chemical and biological control have been known for the management of IAPS. However, biological control methods are gaining popularity because they are environmentally friendly, cost effective and sustainable as well. The main principle lying behind the biological control of the weed is that the IAPS have escaped from their natural enemies that limit their multiplication and spread in their native range (Ani *et al.,* 2018). Therefore, biological control involves using specific natural enemies that can reduce the development and reproduction of their host plant and prevent their spread.

Use of biocontrol agents for the control of IAPS is a growing approach worldwide (Schwarzlander *et al.,* 2018). Among different biocontrol agents known so far, herbivorous arthropods especially gall inducers have been widely used as biocontrol agent against invasive weeds worldwide and have been found to retard the vegetative growth and reproduction of these weeds (Erasmus *et al.,* 1992; Florentine *et al.,* 2005; Bitume *et al.,* 2019). Gall inducers are host specific and have low probability of harming non-target plants; therefore, they are considered as good biocontrol agents (Harris & Shorthouse, 1996). An estimated 132,930 species of gall inducing insects have been reported globally (Espirito-Santo & Fernandes, 2007). Among them, *Procecidochares utilis* is a gall forming fly that was introduced as a biocontrol agent against *A. adenophora* which is now established in eight countries outside of its native range, Mexico (Poudel *et al.,* 2019). In these different regions, the effectiveness of the fly in controlling the weed has been highly variable (Poudel *et al.,* 2019). In Hawaii, the gall fly had significant negative impact on the weed but in high rainfall areas, the gall fly was not successful in control of the weed (Bess & Haramato, 1972). Partial success in the control of the weed was observed in NewZealand (Fowler *et al.,* 2000). In Australia as well as South Africa, the gally fly reduced the vegetative growth and reproductive vigour of *A. adenophora* (Erasmus *et al.,* 1992; Page & Lacey 2006; Winston *et al.,* 2014). However, a recent study conducted in south west China viewed that in response to compensation for galling, *A. adenophora* produced more branches and also increased seed production (Yuan *et al.,* 2020). In context of Nepal, the gall fly is reported to be naturally dispersed from India and was first observed at Kanyam, in Illam district of Nepal (Sharma & Chhettri, 1977). Though the first study by Sharma & Chhettri (1977) indicated the probability of the gall fly to control the weed in Nepal, the effectiveness of the gall fly in Nepal seems to be low currently. Therefore, there is a need of additional research on the ecology of gall fly, its abundance and its impact in Nepal as well.

Distribution and abundance of galling insects along the elevation gradient is mediated by two effects, i.e., direct effect by the variation of abiotic factors like temperature and precipitation and indirect effect by the variation in biotic factors like suitability of host plant and plant insect interaction (Hodkinson, 2005; Mooney *et al.,* 2016; Moreira *et al.,* 2018). Infestation by galling insects result in the formation of galls and gall size is determined by the tritrophic interaction between insect genotype, plant genotype and environmental factors (Weis & Abrahamson, 1986; Weis & Gorman, 1990). Evidences suggest that gall size play a strong role in the performance of gall inducing insects as biocontrol agent (Sopow & Quiring, 2001; Egan *et al.,* 2011).

Generally, small sized galls are more susceptible to parasitism by parasitoids than the large sized galls (Weis *et al.,* 1985; Marini-Filho & Fernandes, 2012). Also, the number of galling insects is positively correlated with the gall size (Sopow & Quiring, 2001; Tabuchi & Amano, 2004). Most of the studies related to abundance of gall inducers are restricted to evaluating abundance with respect to seasonality, host plant abundance, age, architecture, and soil nutrients (Cuevas-Reyes *et al.,* 2011; Stokes & Stiling, 2013; Silva *et al.,* 2015; Boaventura *et al.,* 2018). In general, species are the most abundant towards the centre of their geographic range as well as climatic space and become progressively rarer and patchier in occurrence near the edges of their distribution (Hengeveld & Hack, 1982; Brown, 1984). A greater abundance of galling insects has been reported in xeric habitats than in mesic habitats (Fernandes & Price, 1992). Though, some studies have reported that gall abundance decreased with the increasing elevation (Lawton *et al.,* 1987; Fuentes-Contreras *et al.,* 1999), knowledge on the role of elevation on gall size and gall abundance is still poor. Studies suggest that heavy and persistent galling is necessary for the long-term detrimental effect on *A. adenophora* (Bess & Haramato, 1959; van Staden & Bennett, 1991; Page & Lacey, 2006); therefore, knowledge on abiotic factors (elevation) affecting stem galling abundance is central to the successful management of the weed.

Galls formed by the infestation by gall-inducing insects on host plant provide good nutrition, protection, and shelter to the residing insects (Shorthouse *et al.,* 2005; Raman, 2007). To explain the adaptive nature of gall induction, three hypotheses have been proposed which are related to gall morphology (Stone & Schonrogge, 2003). They are: The nutrition hypothesis, the microenvironment hypothesis, and the enemy hypothesis (Price *et al.,* 1986). The nutrition hypothesis states that plant galls are adaptations that provide insects with higher nutritional food sources in comparison to ungalled plant tissue (Price *et al.,* 1986; Price *et al.,* 1987; Stone & Schonrogge, 2003). The ability of gall inducers to act as a biocontrol agent also depends upon the efficiency of galls to act as nutrient sinks (Harris & Shorthouse, 1996). Establishment of sinks in galls deprives the host plant of resources that could be otherwise utilized by the plant in growth and reproduction exerting negative impact on host plant (McCrea *et al.,* 1985; Abrahamson & Weis, 1997; Marini-Filho & Fernandes, 2012). Bagatto and Shorthouse (1991) reported higher levels of Cu, Zn, Fe, Mn and Ni in galls formed by *Hemadas nubilipennis* in the host plant *Vaccinium angustifolium* in comparison to normal tissues. Similarly, galls induced by *Epiblemma strenuana* on *Parthenium hysterophorus* increased concentration of chloride and magnesium in comparison to others (Florentine *et al.,* 2005). Higher concentration of P, Ca and Mg was also reported in galls induced by *Rhopalomyia* sp. on *Diplusodon orbicularis* in comparison to ungalled tissues (Marini-Filho & Fernandes, 2012). However, ability of stem galls formed on *A. adenophora* to act as nutrient sink has not been explored till date. Therefore, exploring this ability of stem gall formed by *P. utilis* would also increase our understanding on its efficacy to act as a biocontrol agent.

2.7 Research gaps

Ageratina adenophora has already spread in vast areas of Nepal occupying wide elevation range of 130-3280 m asl (Shrestha & Shrestha, 2021). Ecological niche modeling studies suggest the probability of further expansion of the weed in Nepal with climate change in future. In CHAL, reduction of forage supply, displacement of native species causing loss of biodiversity and prevention of forest regeneration by *A. adenophora* has already been reported (WWF, 2013; Shrestha *et al.,* 2019). Therefore, there is an urgent need of risk assessment studies that could predict potential areas of distribution of *A. adenophora* in CHAL under current and future climate scenarios that would provide valuable information to managers and policy makers to take necessary action in time for preventing its further spread. Phenotypic plasticity is one of the factors that has helped *A. adenophora* to occupy wide range of climatic habitats (Poudel *et al.,* 2019). Though study regarding variation in some functional traits like plant height, biomass, seed yield and seed germination rate of *A. adenophora* along an elevation gradient of 678-2356 m asl has been conducted in China (Zhao *et al.,* 2012), Nepal lacks such study. As, the weed is found along wide elevation gradient in Nepal, it is important to understand its invasion mechanism facilitated by its functional traits that has helped the weed to spread rapidly. It has been generally stated that *A. adenophora* negatively impacts biodiversity by reducing species diversity, species richness and cover of native species (Ding *et al.,* 2007; Gao *et al.,* 2013; Baral *et al.,* 2017). However, there is mostly lack of empirical data to support such assertion. Therefore, knowing the impacts of *A. adenophora* would be helpful to prioritise the management actions. As a biological control agent of *A. adenophora*, the gall forming insect, *P. utilis* remains active in Nepal for more than four decades with limited impacts on the weed (Sharma & Chhettri, 1977; Poudel *et al.,* 2019). Due to a wide elevation range of distribution of the weed, the activities of the gall forming insect is also expected to vary along the elevation gradient. However, no such study has been undertaken in Nepal Himalaya.

CHAPTER 3

3. MATERIAL AND METHODS

3.1 Study area

This study was conducted in different locations of central Nepal (83º E to 86º 30' E). Central Nepal is a region which is considered as a transition zone of eastern and western Himalaya. It comprises five physiographic regions namely Terai, Siwalik, Middle Mountain, High Mountain and High Himalaya. Extending from subtropical climate in lowlands to Alpine in the High Himalayas and a cold dry climate in the trans Himalayan region, central Nepal exhibits a high climatic variability. This geographic and climatic heterogeneity makes this region a home of diverse flora and fauna. The overall climatic condition of physiographic regions of central Nepal is presented in Table 1.

For the distribution modeling studies using Maxent and satellite imageries, an important landscape of central Nepal i.e., Chitwan-Annapurna Landscape (CHAL) was selected (Figure 2). This landscape covers 19 districts with an area of 32,057 km^2 and has a wide elevation gradient ranging from 200 to 8091 masl (WWF, 2013). It spans four physiographic regions namely, Siwalik, Middle Mountain, High Mountain and High Himalaya with diverse climatic conditions. This geographic and climatic variation has led to the CHAL's rich biodiversity. Forests and grasslands are the main natural ecosystems, occupying 35.5% and 8.6% of the landscape, respectively, whereas 21.1% of the area is under agriculture (WWF, 2013). The region has a population of 4.5 million people (CBS, 2013). The average minimum and maximum temperatures are 5ºC and 40ºC, and the average annual rainfall ranges from 165 to 5244 mm (MFSC 2016). The combination of diverse natural environment along with anthropogenic disturbances has made the region vulnerable to biological invasions (WWF, 2013).

Plant functional trait study was carried out along the elevation gradients at three sites of central Nepal i.e., 1. Eklefaant-Simbhanjyang (hereafter called Simbhanjyang) 2. Khairenitaar-Panchase (hereafter called Panchase) and 3. Chandragiri (Figure 2, Table 2). In addition, two additional sites (Chitwan and Rasuwa districts) were also included for the study which represented the lowest and highest elevational range of *A. adenophora* distribution in central Nepal.

Simbhanjyang belt (Ekleafaant-Simbhanjyang)

This site represented the longer elevation gradient of 2000 m asl (500-2500 m asl). The transect for the study expanded in two districts i.e., Dhading and Makwanpur (Table 2). Eklefaant and Archaley were the sites representing the 500 m asl and 1000 m asl elevation respectively, which lied in Dhading district. Other three sites Damechour, Nayagaun and Simbhanjyang lied in Makwanpur district which represented the elevation of 1500 m, 2000 m and 2500 m asl respectively. Eklefaant represented the tropical climate, Archaley and Damechour represented the subtropical climate whereas Nayagaun and Simbhanjyang represented the temperate climate.

Panchase belt Khairenitaar-Panchase

This site also represented the longer elevation gradient of 2000 m asl (500-2500 m asl). The transect for the study expanded in three districts, i.e., Tanahun, Kaski and Parbat (Table 2). Khairenitaar was selected as the lowermost elevational distribution site (500 m asl) for *A. adenophora* which lies in upper tropical climatic zone of Tanahun district. Maadibaang and Sidane represents the site having elevation of 1000 m and 1500 m asl, respectively which lies in subtropical climate zone of Kaski district. Bhanjyang and Panchase which represent the site having elevation of 2000 m and 2500 m asl, respectively lies in temperate climatic zone of Kaski district.

Chandragiri belt

This short elevation gradient of 1030 m asl (1500-2530 m asl) is located in Kathmandu district (Table 2). Chandragiri municipality was selected for the study that lies in south western part of the Kathmandu valley of Bagmati province. This municipality covers an area of 43.92 sq km with a total population of 87,533. Chandragiri lies in 27°43’ 36.486” N in North, 27°32’ 45.029” N in South, 85°16’ 39.509” E in East, 85°11’8.685” E in West. Elevation ranges from 1310 m asl to 2551 m asl in Chandragiri (GoN). It lies in subtropical climate zone (1000 to 2000 meters) and deciduous monsoon forest zone (elevation range of 1,200–2,100 meters). During the month of May and June highest temperature is recorded whereas lowest temperature is recorded in December and January. Balkhu khola, Daudali khola and Ghatte khola are the three main rivers that flow in Chandragiri.

Lowest and highest elevation site

Jutpaani was selected as the site representing the lowest elevational distribution limit of *A. adenophora* in central Nepal. It lies in Chitwan district and has the elevation of 240 m asl (Table 2). It harbors monsoon humid subtropical climate.

For the representation of the highest elevational distribution limit of *A. adenophora* in central Nepal, Dhimsaa was selected at an elevation of 2963 m asl in Rasuwa district (Table 2). Dhimsa lies in Gosainkunda rural municipality and has temperate climate. It lies in High Mountain region.

For assessment of the impact of *Ageratina adenophora*, two sites were selected i.e., Simbhanjyang and Chandragiri. In Simbhanjyang, sampling was carried out in two elevation belts (1000 and 2000 m asl) whereas in Chandragiri, three elevation belts (1500, 2100 and 2400 m asl) were taken into account.

For assessing the stem galling of the weed by a biocontrol agent *Procecidochares utilis* along the elevation gradient, two sites (Simbhanjyang and Chandragiri) were taken into consideration. Two additional sites were also selected that represented the lowest (Jutpani, Chitwan district) and highest (Dhimsa, Rasuwa district) elevational range of distribution of *A. adenophora* in central Nepal. These sites represent the same site that were considered for functional trait study.

Table 1: Climatic condition in different physiographic regions of central Nepal as proposed by Stearn (1960) (Source: DHM, 2017)

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Terai	$T_{Max.}$	20.03	24.81	30.77	35.79	36.07	35.48	33.59	33.54	33.17	31.61	28.36	22.60
	$T_{Min.}$	8.89	11.00	15.14	20.19	23.50	25.70	25.89	25.63	24.78	20.90	14.80	10.56
	Rain	7.49	18.93	13.55	31.00	114.44	228.77	423.43	366.99	216.80	56.37	2.52	1.54
Siwalik	$T_{Max.}$	21.55	25.44	31.24	35.80	35.70	34.85	33.22	33.28	33.07	31.41	27.92	23.40
	$T_{Min.}$	8.93	11.08	15.29	19.79	22.52	24.50	24.90	24.83	23.94	20.13	14.66	10.54
	Rain	10.00	20.06	18.52	48.45	175.82	353.82	597.67	557.49	312.08	63.08	3.88	1.76
Middle Mountain	$T_{Max.}$	18.89	21.39	25.73	29.09	29.63	29.84	28.89	28.99	28.45	26.66	23.19	19.85
	$T_{Min.}$	5.44	7.52	11.14	14.64	17.19	19.93	20.84	20.58	19.27	15.44	10.57	6.76
	Rain	9.67	34.85	34.55	61.81	150.79	318.59	525.00	457.70	297.44	69.04	4.97	3.52
High Mountain	$T_{Max.}$	15.34	17.13	21.48	24.67	25.81	26.34	25.37	25.25	24.83	22.96	19.45	16.64
	$T_{Min.}$	3.47	4.81	8.36	11.59	13.86	16.56	17.35	16.71	15.80	12.08	8.01	4.97
	Rain	20.79	44.25	49.37	68.82	147.45	419.01	738.32	666.02	425.35	91.01	7.74	5.55
High Himalaya	$T_{Max.}$	10.31	11.84	15.30	18.23	20.23	21.74	21.33	21.36	20.73	17.72	15.15	12.67
	$T_{Min.}$	-3.80	-2.30	0.83	3.59	6.48	10.39	11.84	11.49	9.56	4.24	0.44	-2.32
	Rain	21.51	30.97	32.18	29.98	30.56	57.55	103.91	85.12	52.16	23.04	2.96	4.83

Note: $T_{Max.}$– Maximum temperature (°C), $T_{Min.}$– Minimum temperature (°C), Rain– Total rainfall (mm)

Table 2: Details of sample collection site

Gradients	Sites	District	Province	Elevation (m asl)	Climatic zone	Land use
Eklefaant-Simbhanjyang	Eklefaant	Dhading	Bagmati	500	tropical	Fallowland
	Archaley	Dhading	Bagmati	1000	Subtropical	Fallowland
	Damechour	Makwanpur	Bagmati	1500	Subtropical	Roadside
	Nayagaun	Makwanpur	Bagmati	2000	Temperate	Roadside
	Simbhanjyang	Makwanpur	Bagmati	2500	Temperate	Roadside
Khairenitaar-Panchase	Khairenitaar	Tanahun	Gandaki	500	Upper tropical	Roadside
	Maadibaang	Kaski	Gandaki	1000	Subtropical	Fallow land
	Sidane	Kaski	Gandaki	1500	Subtropical	Roadside
	Bhanjyang	Kaski	Gandaki	2000	Temperate	Open forest
	Panchase	Kaski, Parbat, Syangja	Gandaki	2500	Temperate	Forest
Chandragiri		Kathmandu	Bagmati	1500	Subtropical	Open forest
		Kathmandu	Bagmati	1600	Subtropical	Open forest
		Kathmandu	Bagmati	1750	Subtropical	Open forest
		Kathmandu	Bagmati	1950	Subtropical	Open forest
		Kathmandu	Bagmati	2150	Temperate	Open forest
		Kathmandu	Bagmati	2350	Temperate	Open forest
		Kathmandu	Bagmati	2480	Temperate	Open forest
		Kathmandu	Bagmati	2530	Temperate	Open forest
Dhimsaa		Rasuwa	Bagmati	2932	Temperate	Forest
		Rasuwa	Bagmati	2944	Temperate	Forest
		Rasuwa	Bagmati	2965	Temperate	Forest
Jutpaani		Chitwan	Bagmati	240	Lower Tropical	Roadside
		Chitwan	Bagmati	240	Lower Tropical	Agricultural land
		Chitwan	Bagmati	240	Lower Tropical	Agricultural land

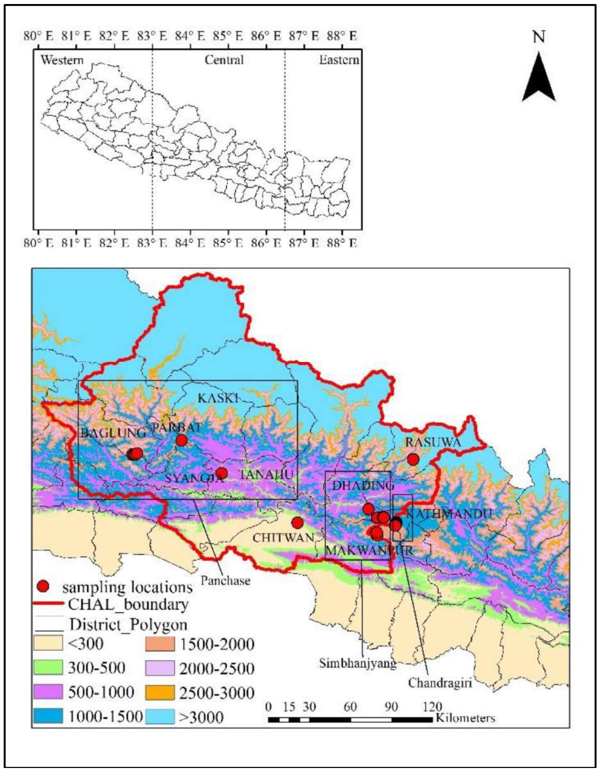


Figure 2: Location of study area and sampling sites in Nepal

3.2 Ecological niche modeling

Ecological niche models are used to predict the climatically suitable regions of species across a landscape by relating documented presence records of focal species with the environmental or spatial characteristics of the potential sites. Maxent model was used to predict the climatically suitable area of the weed in Chitwan-Annapurna Landscape (CHAL).

3.2.1 Species occurrence data

A total of 686 occurrence points were collected from different sources, 245 occurrence points from secondary sources (Shrestha & Shrestha, 2019; Siwakoti *et al.,* 2016) whereas remaining 441 occurrence points were collected during field visit from 2016-2019. Occurrence points were mainly collected through field surveys along road networks and trekking routes as road networks are the major conduit of dispersal for *A. adenophora* (Dong *et al.,* 2008). This sampling bias was addressed by spatial filtering of the data. Duplicate records of these occurrence points were deleted and spatially thinned using spThin package (Aiello-Lammens *et al.,* 2015) in R software (version 3.4.4) (R core team 2017) so that only single location occurred in each grid cell (1 km*1 km). Spatial filtering allows reducing overfitting to sampling bias in ecological niche models (Boria *et al.,* 2014). Thus, a total of 403 occurrence records were used for constructing the model.

3.2.2 Environmental variables

Nineteen grid-based bioclimatic variables representing annual trends, seasonality and extreme environmental conditions were downloaded on December 2018 from the worldclim database (version 1.4) (www.worldclim.org; Hijmans *et al.,* 2005) at 30 arc sec spatial resolution (~1 km^2) (Appendix 1). Elevation was obtained from Shuttle Radar Topographic Mission (SRTM) at 90 m spatial resolution; this was then resampled into 30 arc sec spatial resolution by using the nearest neighbor resampling technique in Arc GIS (version 10.4). Slope and aspect rasters of the study area were derived from the elevation data. All these downloaded datasets were extracted to study area (CHAL) by using Spatial Analyst Tool and Extraction tool in Arc GIS version 10.3. These datasets which were in raster format were then changed into ASCII files in Arc GIS. The same procedure was repeated to prepare the predicted distribution maps in two future climate scenarios (2050 and 2070).

For predicting the suitable areas in future climate scenarios, projections from Community Climate System Model (CCSM4) under b1 emission scenarios was used which is based on the fifth phase of the Coupled Model Intercomparison Project 5 (CMIP5). Three greenhouse gas (GHGs) concentrations pathways were selected, also known as Representative Carbon Pathways (RCP 2.6, RCP 4.5 and 8.5) for two different time periods (2050 and 2070) as adopted by IPCC in its fifth assessment report (AR5) (IPCC, 2013). The RCP 2.6, RCP 4.5 and RCP 8.5 represents the lowest, middle and highest emission scenario which accounts for 1°C, 1.4-1.8°C and 2-3.7°C projected change in global mean surface temperature, respectively (IPCC, 2013; van Vuuren *et al.,* 2011).

3.2.3 Model building

Maxent (version 3.3.3) software was used to build the ecological niche models (Phillips & Dudik, 2008). Maxent is one of the most popularly used habitat suitability modeling techniques that uses presence only data and is widely used for invasive species (Phillips *et al.,* 2006; Shrestha *et al.,* 2018; Maharjan *et al.,* 2019). Maxent works well with incomplete or limited data and gaps so can provide robust estimates of habitat suitability for invasive species at small spatial extent (Jarnevich *et al.,* 2006; Jarnevich & Reynolds, 2011; West *et al.,* 2016).

A multicollinearity test was performed for 19 bioclimatic and three topographic variables using Pearson's correlation coefficient. Variables with a Pearson correlation ≥ 0.80 or less than -0.80 were dropped to eliminate multicollinearity among predictor variables and overfitting of the Ecological Niche Models (Merow *et al.,* 2013) (Appendix 2). The decision to drop or select the predictor variables was done on the basis of knowledge of species' ecological and biological characteristics. The remaining seven bioclimatic variables: Isothermality (bio 3), Minimum temperature of the coldest month (bio 6), Temperature Annual Range (bio 7), Precipitation of driest month (bio 14), Precipitation seasonality (bio 15), Precipitation of warmest quarter (bio 18), Precipitation of coldest quarter (bio 19) and two topographic variables: aspect and slope were used as predictors to build the habitat suitability model.

The Maxent model used 75% of the data for training and remaining 25% for testing. Logistic format was used as it improves model calibration by estimating species probability of presence conditioned on environmental variables (Phillips & Dudik, 2008). The convergence threshold was set to 10^{-5}, a maximum of 5000 iterations, 15 replications with the replicated run type subsample and 10,000 random background points. As a threshold rule, tenth percentile training presence was chosen on the basis of highest values of Area Under Curve (AUC) and True Skill Statistics (TSS). Furthermore, tenth percentile training presence is considered as a highly conservative estimate of a species tolerance with respect to each climatic variable (Svenning *et al.,* 2008). Other values were kept as default.

The Maxent output is continuous data with values ranging from 0 (lowest) to 1 (highest) probability of distribution. The Maxent output data was imported in Arc GIS and converted into raster format for further analysis. The map, thus, obtained was classified with the help of "reclassify" tool into two classes – suitable habitat and unsuitable habitat on the basis of 10th percentile training presence logistic threshold. Thus, a binary habitat suitability map was prepared for current and all future climate scenarios. Also, the suitable area at present and future climate scenarios as well as changes in the suitable areas in terms of gain, loss and stable areas in future in all the scenarios was calculated in Arc GIS. The data of physiographic regions and protected areas were further clipped to suitable habitat maps of current and future climate scenarios to see the current and future suitable areas of this weed in these physiographic regions and protected areas. Changes in the upper and lower elevational distribution range in future climate scenarios in comparison to current was quantified using extraction tool and DEM raster for all maps (current and future) in Arc GIS.

3.2.4 Model evaluation

Model performance and prediction accuracy was evaluated by threshold independent (Area under ROC curve (AUC)) and threshold dependent ((True Skill Statistics (TSS)) measures (Fielding & Bell, 1997; Allouche *et al.,* 2006; Franklin, 2009). AUC values range from 0-1. Values 0.5-0.7 were considered low, 0.7-0.9 as moderate and >0.9 as high (Swets, 1988; Manel *et al.,* 2001). TSS value ranges from -1 to +1, +1 value indicating the perfect agreement and values equal to zero or less indicates a performance no better than random (Allouche *et al.,* 2006). Marginal response curves were used to visually investigate the relationship between predictor variables and predicted index of habitat suitability of *A. adenophora.* The relative contribution of different predictor variables to the Maxent model was assessed by the variable percentage contribution and Jacknife procedures (Elith *et al.,* 2011). Two jackknife tests were taken into consideration, i.e., jackknife test of regularized training gain and jackknife test of AUC. Jacknife test of regularized training gain shows the training of variable when used in isolation, training gain of variable when omitted and compares it with the training gain of all variables. Similarly, jackknife test of AUC shows the predictive performance of the variable when used in isolation, when omitted and compares it with the AUC value when all the variables are used (Phillips, 2017).

3.3 Spatio-temporal distribution

3.3.1 Data collection

Field visits was conducted from the year 2017 to 2019 in 16 districts of CHAL to collect the occurrence point of *A. adenophora* using the Global Positioning System (GPS). With the help of clinometer, slope and aspect of the place of occurrence were measured. Along with this, other information like land use, vegetation types were also noted down. The patch size of *A. adenophora* was noted down for every occurrence point recorded. A patch size of $2\,\mathrm{m} \times 2\mathrm{m}$, $5\,\mathrm{m} \times 5\,\mathrm{m}$ and $10\,\mathrm{m} \times 10\,\mathrm{m}$ were considered as low, medium and large intensity for the weed. A total of 435 occurrence points of *A. adenophora* were collected and used for further analysis.

Two different multispectral satellite imageries (Landsat TM/ETM and Worldview-2) were used to study the distribution of *A. adenophora* in CHAL. Landsat TM/ETM Imageries for four different years (2018, 2009, 1999 and 1992) were downloaded from the Archives of United States Geological Survey (USGS) Earth Explorer (https://earthexplorer.usgs.gov). While downloading the images, preference was given to the images with least cloud cover. Landsat 8 OLI/TIRS data were used for the year 2018 whereas for the years 2009, 1999 and 1992, Landsat 5 TM data was used. The spatial resolution of all the Landsat data was $30\,\mathrm{m} \times 30\,\mathrm{m}$. Detailed information about Landsat data are presented in (Appendix 3) and the detailed information of data sources are in Annex. ASTER Dem data were also freely downloaded from Archives. Worldview-2 imageries of spatial resolution 2 m × 2 m of multispectral band in different parts of CHAL (Dhading, Tanahun and Kaski) were purchased from the authentic sources (Appendix 4). Climatic data (minimum temperature, maximum temperature and average rainfall) were obtained from Department of Hydrology and Meteorology, Government of Nepal (Appendix 5).

3.3.2 Image processing

The multispectral images were then mosaiced by using Data management tool and mosaic tool in Arc GIS version 10.3. After mosaicing the dataset, histogram equalization was done for image enhancement. Now, image classification was carried out. Unsupervised classification was carried out for only near infra-red band assigning 100 classes for clustering in Arc GIS version 10.3. Supervised classification was carried out in ERDAS Imagine 2014 for creating land cover map. Representative samples for each cover class were created using polygon and assigned each cover class a definite land use type. Then these training samples were applied to the entire image to get a land cover map.

Ultimately, seven different raster datasets (Elevation, landuse, NDVI, reflectance value, minimum temperature, maximum temperature and rainfall) were used for knowledge-based classification.

Normalized Difference Vegetation Index (NDVI)

Normalized Difference Vegetation Index (NDVI) is based on the difference of reflectance in the near-infrared and red bands. To develop NDVI map following pre-processing of the images were performed:

i) The reflectance value of both ETM and TM data was calculated for vegetation analysis. For ETM/OLI sensor data of 2018 band 5 and band 4 was used and for TM sensor data band 4 (NIR) and band 3 (Red) was used in 2009, 1999 and 1992.

ii) The raw images of NIR band, Red band was converted to radiance (Landsat 7 Data Users Handbook – USGS). The formula used in this process is as follows:

$$L_\lambda = \frac{\mathrm{LMAX}_\lambda - \mathrm{LMIN}_\lambda}{\mathrm{QCALMAX} - \mathrm{QCALMIN}} \times (\mathrm{QCAL} - \mathrm{QCALMIN}) + \mathrm{LMIN}_\lambda$$

Where,

L_λ is the cell value as radiance

QCAL = digital number

LMIN_λ = spectral radiance scales to QCALMIN

LMAX_λ = spectral radiance scales to QCALMAX

QCALMIN = the minimum quantized calibrated pixel value (typically = 1)

QCALMAX = the maximum quantized calibrated pixel value (typically = 255)

iii) After converting DN to radiance the output file was, thus, converted into reflectance by using following equation

$$\rho\lambda = \frac{\pi * \mathrm{L}_{\lambda} * d^2}{\mathrm{ESUN}_{\lambda} * \cos\theta_s}$$

Where,

ρλ = Unitless plantary reflectance

Lλ = spectral radiance (from earlier step)

d = Earth-Sun distance in astronmoical units

ESUNλ = mean solar exoatmospheric irradiances

θ_s = solar zenith angle

iv) Reflective band DN's can be converted to TOA reflectance using the rescaling coefficients in the MTL file. The conversion was performed using parameters provided with the metadata file of the Landsat 8 satellite images and the following formula set

$\rho\lambda' = M\rho Qcal + A\rho$

Where,

ρλ′ = ToA planetary spectral reflectance without correction for the solar angle (unitless)

Mρ = Reflectance multiplicative scaling factor for the band

Aρ = Reflectance additive scaling factor for the band

Qcal = L1 pixel value in the DN

This process does not include correction for the solar elevation angle. The following additional formula was used to obtain the true ToA reflectance:

$\rho\lambda = \rho\lambda'/\sin(\theta SE)$

Where,

ρλ = ToA Planetary Reflectance (unitless)

θ SE = Solar Elevation Angle

After above pre-processing of the images, NDVI was calculated by using following formula (Weier & Herring, 2000):

$$\mathrm{NDVI} = \frac{(\mathrm{NIR} - \mathrm{Red})}{(\mathrm{NIR} + \mathrm{Red})}$$

Where NIR and RED are the spectral reflectance in the sensor's near-infrared and red bands, respectively.

High NDVI values result from the combination of a high reflectance in the near infrared and lower reflectance in the red band. Non-vegetated areas, including bare soil, open water, snow/ice, and most construction materials, have much lower NDVI values. NDVI was calculated and the suitable value ranges for the species was 0.2-0.4. Along with NDVI various other variables were incorporated to knowledge-based classification (Table 3).

3.3.3 Reclassification of variables

All seven variables to be used for knowledge-based classification were reclassified using reclassify tool in Arc GIS software. These raster datasets were classified in two classes, i.e., suitable (value 1 and unsuitable (value 0). For all the years, criteria for suitable class were kept same (Table 3).

Table 3: Variables and their criteria used for knowledge-based classification of *A. adenophora*

Rules	Calculation	Suitable criteria	Source
Elevation	Reclassifying DEM file	240-2965 m	Field observation
Temperature (max)	DHM data	<33.45°C	Station based
Temperature (min)	DHM data	8>°C	Station based
Precipitation	DHM	550-5000 mm	Station based
NDVI	(NIR - Red)/(NIR + Red)	0.21-0.38	Weier & Herring 2000, From GPS coordinates compiled from the field survey
Digital number value	Unsupervised classification	Extracted from occurrence points	From GPS coordinates taken during field survey
Land uses	Supervised classification	Open area, degraded forest/disturbed forest/Thin forest, mid hill slopy forest	Field observation

3.3.4 Model building by knowledge-based classification

Knowledge based classification include use of decision trees incorporating spatial knowledge that has been used to encode additional information on existing land use types. The knowledge-based classifier performs multistage classifications by using a series of binary decisions to place pixels into classes. Each decision divides the pixels in a set of images into two classes based on an expression. Each new class was divided into two more classes based on another expression to define as many decision nodes as needed. The results of the decisions are classes. Then data from many different sources and files together were used to make a single decision tree classifier.

3.3.5 Change detection

Change detection procedures involved data acquired by the same (or similar) sensor and be recorded using the same spatial resolution, viewing geometry, spectral bands, radiometric resolution, and time of day. For detecting changes at time scales greater than one year, anniversary dates were preferred to minimize sun angle and seasonal differences.

Supervised and knowledge-based classification was used to classify the combined image into temporal-categorical “classes”; some of these classes were stable (i.e., the same class on both dates), while other classes represented individual types of change. Further, for any given pixel or group of pixels a “temporal signature” was extracted, showing the seasonal evolution of NDVI within the pixel(s). The seasonal cycle in NDVI was distinct. With the help of NDVI, DNs value and other ancillary data temporal scale was analyzed. With temporal image differentiation change detection was observed.

3.3.6 Accuracy assessment

Accuracy assessment was performed through field validation. Accuracy assessment was carried out by using the principles proposed by Congalton and Green (2009). Training data were collected using an “ad-hoc” sampling design that is neither systematic nor random, instead emphasizing the opportunistic acquisition of ground truth at sites that were readily accessible on the ground, readily interpretable in higher-resolution imagery. Sample size must also weigh heavily in the development and interpretation of classification accuracy figures. As a broad guideline, it has been suggested that a minimum of 50 samples of each land cover category be included in the error matrix (Congalton & Green, 2009). Error matrices compare, on a class-by-class basis, the relationship between known reference data (ground truth) and the corresponding results of the classification procedure. All nondiagonal elements of the matrix represent errors of omission or commission. Omission errors correspond to nondiagonal column elements Commission errors are represented by nondiagonal row elements. The accuracies of individual in each category by either the total number of pixels in the corresponding row or column. While evaluating error matrices overall accuracy as well as individual accuracies were also calculated. Overall accuracy was computed by dividing the total number of correctly classified pixels (i.e., the sum of the elements along the major diagonal) by the total number of reference pixels. It indicates the quality of the map classification. It can be calculated by dividing the total number of correctly classified pixels by the total number of reference pixels.

Evaluating classification error matrices

The Kappa Coefficient is generated from a statistical test to evaluate the accuracy of a classification (Bishop *et al.*, 1975). Kappa essentially evaluates how well the classification performed as compared to just randomly assigning values, i.e., did the classification do better than random. The Kappa Coefficient can range from -1 to 1 (Table 4). A value of 0 indicated that the classification is no better than a random classification. A negative number indicates the classification is significantly worse than random. A value close to 1 indicates that the classification is significantly better than random.

The k ^ (“kappa” or “KHAT”) statistic was calculated by using following formula:

$$\text{Khat} = \frac{(\text{n} * \text{SUM Xii}) - \text{SUM (Xi} + * \text{X} + \text{i)}}{\text{n2} - \text{SUM (Xi} + * \text{X} + \text{i)}}$$

Where,

SUM = sum across all rows in matrix

Xii = diagonal

Xi + = marginal row total (row i)

X+I = marginal column total (column i)

n = number of observations

Table 4: Value range of kappa coefficient and its interpretation

Values	**Interpretation**
Smaller than 0.00	Poor agreement
0.00 to 0.20	Slight agreement
0.21 to 0.40	Fair agreement
0.41 to 0.60	Moderate agreement
0.61 to 0.80	Substantial agreement
0.81 to 1.00	Almost perfect agreement

3.4 Plant functional traits

3.4.1 Field data and sample collection

Three elevation gradients were taken into account *viz*. Eklefaant to Simbhanjyang (hereafter referred as Simbhanjyang), Khairenitaar to Panchase (hereafter referred as Panchase) and Chandragiri hill. Sampling was done in five elevation belts of Simbhanjyang and Panchase (500, 1000, 1500, 2000 and 2500 m asl) whereas for Chandragiri, eight elevation belts (1500, 1600, 1750, 1950, 2150, 2350, 2480, 2530 m asl) were taken into consideration. Additional data was collected from Jutpaani of Chitwan district (240 m asl) and Dhimsaa in Langtang National Park of Rasuwa district (2965 m asl) which represented the lowest and highest distributional limit of *A. adenophora* in the study area (Table 2).

For field sampling, in each elevation belt, three quadrats (10 m × 10 m) at 50 m apart from each other were sampled. A total of 15 quadrats in Simbhanjyang, 15 quadrats in Panchase, 24 quadrats in Chandragiri, 3 quadrats in Chitwan and 3 quadrats in Rasuwa were sampled for the vegetative and reproductive traits. Leaf samples from Simbhanjyang and Panchase were collected during December, 2016 whereas from Chandragiri and other two sites (Chitwan and Rasuwa), leaf samples were collected during December, 2017. From each quadrat 15 healthy individuals were selected and from each individual, 3 fully exposed and healthy leaves were plucked and kept in a plastic ziplock bag. Altogether 675 leaves (3 quadrats × 5 elevation belts × 15 individuals × 3 leaves) from Simbhanjyang, 675 leaves from Panchase (3 quadrats × 5 elevation belts × 15 individuals × 3 leaves), 1080 leaves (3 quadrats × 8 elevation belts × 15 individuals × 3 leaves) from Chandragiri, 135 leaves (3 quadrats × 15 individuals × 3 leaves) from Chitwan and 135 leaves from Rasuwa were sampled.

The oven dried leaves, after the Specific Leaf Area (SLA) calculation (refer to laboratory analysis mentioned below), were further processed for estimation of Leaf Nitrogen Content (LNC) following micro Kjeldahl method (Horneck & Miller, 1998; Appendix 6). Then 45 leaves from each single quadrat were crushed to fine particles and mixed so that each quadrat had one sample. For each mixed sample, three subsamples were prepared for analyzing LNC. LNC was estimated for altogether 45 samples (3 quadrats × 5 elevation belts × 3 subsamples) from Simbhanjyang, 45 from Panchase (3 quadrats × 5 elevation belts × 3 subsamples), 72 from Chandragiri (3 quadrats × 8 elevation belts × 3 subsamples), 9 from Chitwan (3 quadrats × 3 subsamples) and 9 samples from Rasuwa (3 quadrats × 3 subsamples).

For reproductive traits, sampling at the lower elevation belts (240-1000 m asl) were done during the month of April, for mid elevation belts (1500-1950 m asl) during the month of May and for higher elevation belts (2000-2500 m asl), sampling was conducted during June. Data were collected in 2017 at Simbhanjyang and Panchase, and in 2018 at other sites. The number of flower heads per ramet was counted in 10 healthy individuals from each quadrat. Then 100 floral heads (10 floral heads from each individual) from each quadrat were collected and wrapped in paper bag individually. Total number of mature seeds (black coloured seeds) were counted in 30 flower heads picked up randomly from 100 stored floral heads from each quadrat.

Seed length and seed breadth were measured for 30 seed samples from each quadrat. For this, photographs of each seed to be measured was captured by an inbuilt digital camera of the microscope (Excelis-Unitron1505206) and stored in the computer. The digital camera was first calibrated by using a stage micrometer, and then seed size was measured by using the software CaptaVision version 3.6.9.1. Seed mass per 100 seeds was measured for thirty samples using a digital balance (0.0001 g) (KERN ALS220-4N) after oven drying the seeds at 80°C for 48 hours (Perez-Harguindeguy *et al.,* 2013).

3.4.2 Laboratory analysis

Photographs of each leaf collected during the field visits were taken twice to reduce error on the same day of collection using digital camera (Canon 700D). After that, the leaves collected were tagged, kept in paper bags and brought to laboratory. The leaves were then oven dried at 60°C for 72 hours (Perez-Harguindeguy *et al.,* 2013) and dry mass of leaves was determined using digital weighing balance (0.001 g). SLA of each leaf were calculated using following formulae (Zobel *et al.,* 1987):

$$\mathrm{SLA} = \frac{\text{one sided area of fresh leaf (cm)}}{\text{dry mass (g)}}$$

The oven dried leaves after the SLA measurement were used for the estimation of LNC. The LNC of those dried leaves was analyzed using micro-Kjeldahl method (Horneck & Miller, 1998).

3.4.3 Statistical analysis

Values of SLA, LNC, number of heads per ramet, total number of seeds per floral head (mature seeds), seed length, seed breadth and seed mass were evaluated for normality using Shapiro-Wilk normality test and Bartlett test for homogeneity of variances. Differences in these traits along the elevation gradients in all the three sites were compared using analysis of variance (ANOVA) followed by posthoc Tukey test for normally distributed data or Kruskal-Wallis test with Bonferroni's correction for non-normal data. Pearson's correlation coefficient was used to see the relationship between SLA and LNC. All the statistical analyses were carried out in R version 3.4.4 (R core team, 2019).

3.5 Impact assessment

3.5.1 Field data and sample collection

Impact assessment was carried out in two elevation belts (1000 m and 2000 m asl) at Simbhanjyang and three elevation belts (1500 m, 2100 m and 2400 m asl) at Chandragiri. In each elevation belts, three sites, 50 m apart from each other were selected in such a way that both invaded and non-invaded patches were at ca. 5 m apart. Thus, at each site, 5 quadrats (2 m × 2 m) were sampled in the invaded patch (*A. adenophora's* cover ≥ 80%) and 5 quadrats in the non-invaded patch. In each quadrat, enumeration of all the vascular plants was done and cover of each species was estimated visually following Daubenmire's cover class method (Daubenmire, 1959). In the invaded plots, the average height and number of ramets of *A. adenophora* was also recorded. Each individual plant species was collected, tagged and pressed between newspapers using herbarium press. Along with the collection, field notes were taken which included habitat, flower colour, odour, location, collection date of all the species observed inside the quadrat. Photographs of the species were also taken.

Field data were used to calculate species richness, species diversity and similarity index. Species richness was calculated as the total number of species rooted in a quadrat (Magurran, 2004). Shannon and Simpson diversity indices were calculated as a measure of species diversity as follows:

Shannon diversity index (H') = $-\sum \text{Pi} \ln \text{Pi}$ (Shannon & Weaver, 1963)

Where,

H' = Shannon diversity index

P_i = Proportion of individuals found in the i^{th} species for a sampling community

It was calculated as:

$Pi = n_i/N$ where n_i = Average coverage of each individual species and

N = Total average coverage of all species

Simpson diversity index (1–D) = $1-\frac{\Sigma ni\ (ni-1)}{N\ (N-1)}$ (Simpson, 1949)

Where,

n_i = the coverage of individual in i^{th} species

N = total average coverage of all species

n_i = Relative coverage = $\frac{\text{Average coverage of each individual species}}{\text{Total coverage of all species}} \times 100$

Sorensen similarity index between invaded and non-invaded quadrats was calculated using presence/absence data and expressed in percentage as follows:

Sorensen similarity index (Qs)= $\frac{2j}{a+b}$ (Sorensen, 1948)

Where,

j = Species common to both invaded and non-invaded plots

a = Total species of invaded plots

b = Total species of non-invaded plots

Soil samples were also collected from each quadrat. The soil sub-samples were collected from 0-10 cm depth using soil borer from four corners and centre of the quadrat (Niu *et al.,* 2007). Single sample per quadrat was obtained by mixing all sub-samples to reduce the microsite variation. Thus, altogether 150 soil samples were collected from two sites i.e., Chandragiri (30 quadrats × 3 elevation belts) and Simbhanjyang (30 quadrats × 2 elevation belts).

3.5.2 Laboratory analysis

When the plant specimens completely dried up, they were mounted on the herbarium sheet and labeled. Preliminary identification of species was done by the help of relevant taxonomic literatures such as A handbook of flowering plants of Nepal (Vol. 1) (Rajbhandari & Rai, 2017), Vegetation and Flora of South-West Kathmandu Valley (Chandragiri Range) (Baniya & Shakya, 1999), Flowering plants of Makawanpur (Chapagain *et al.,* 2016). Specimens were verified from voucher specimens deposited in National Herbarium and Plant Laboratories (KATH) and Tribhuvan University Central Herbarium (TUCH). Experts' help was also taken for the identification of doubtful specimens. From the checklist of identified species, those included in the "Global Register of Introduced and Invasive species (GRIIS)-Nepal" were considered as 'naturalized species' and the remaining as 'native species. Those naturalized species that were considered as 'invasive' in the list reported by Shrestha (2016) were considered as invasive and the remaining as 'non-invasive naturalized' species.

The collected soil samples were kept in refrigerator at 4°C until further analysis. Then soil organic carbon was estimated by following Walkley and Black method (Gupta, 2000; Appendix 7) whereas soil nitrogen concentration was analyzed by following micro-Kjeldahl method (Horneck & Miller, 1998). Available soil phosphorus was estimated using the Olsen bicarbonate method (Appendix 8) and exchangeable soil potassium was determined by using flame photometer method (Trivedy & Goel, 1986; Appendix 9).

3.5.3 Statistical analysis

Species richness were compared between invaded and non-invaded quadrats by using paired sample t test. Shannon diversity and Simpson diversity indices of invaded and non-invaded quadrats were compared using paired sample t test. Similarly, soil nutrients (C, N, P and K) between invaded and non-invaded quadrats were also compared using paired sample t test. Species composition difference between invaded and non-invaded quadrats was analyzed by ordination, quadrat type being the only environmental variable. Detrended correspondence analysis was done in order to know the nature of species response curve. The axis length of DCA 1 determined the further analysis. DCA analysis revealed that the axis length of first axis was greater than 2.5 s. d. unit (Appendix 13; Appendix 14) therefore, Canonical Correspondence analysis (CCA) was done to see the difference in species composition between invaded and non-invaded quadrats (Timsina *et al.,* 2011). All the statistical analyses were carried out in R version 3.4.4 (R core team, 2019).

3.6 Biological control by stem galling insect

3.6.1 Field sampling

Distribution survey of galls were carried out during November-December 2018 along the elevation gradients at two sites: Simbhanjyang and Chandragiri (Figure 2). Two additional sites were also surveyed that represented the highest and lowest elevational range of *A. adenophora* distribution in Nepal; they were Dhimsa in Langtang National Park (Rasuwa district) and Jutpani in Chitwan district, respectively. Five different elevation belts (500, 1000, 1500, 2000 and 2400 m asl) in Simbhanjyang, eight elevation belts (1500, 1650, 1750, 1950, 2150, 2350, 2480 and 2530 m asl) in Chandragiri, two elevation belts (2630 and 2965 m asl) in Dhimsha and at single location in Jutpani (240 m asl), distribution survey of galls was carried out.

For the data and sample collection, three quadrats of size 10 m × 10 m, each quadrat being 50 m apart from each other, were sampled at each elevation belt. One hundred ramets were counted in each quadrat sampled and the gall abundance was recorded as the number of ramets having gall (Smith *et al.,* 2011). From each quadrat, 20 ramets having fresh galls without the emergence hole were selected. The length and breadth of each gall was measured using digital vernier calipers (WT 4171). The diameter of stem with gall was measured 1 cm below the gall (Marini-Filho & Fernandes, 2012). The ramet of the same bunch (i.e., genet) without gall was considered to be normal stem (i.e., control). The diameter of the normal stem was also measured at the same height as that of galled stem. Galls and a stem section of 5 cm in length (just 1 cm below the gall) was cut from the galled stem. Similar stem section (5 cm) was also cut out from the normal stem at the same height as that of galled stem (Tooker *et al.,* 2008). In this way, from one genet, three samples were collected, namely gall samples, stem below gall (galled stem), and normal stem (ungalled stem). The samples were kept in a paper bag and allowed to dry in shade until taken to laboratory. From each elevation, altogether 60 (20 ramets × 3 sites) samples of gall, stem below gall, and normal stem were collected.

3.6.2 Laboratory analysis

From each gall sample, the gall flies in different stages were counted and removed carefully. Then the gall, stem below gall and normal stem samples were oven dried at 80°C for 24 hours (Florentine *et al.,* 2005). The dried samples were ground into powder with electric grinder. To have adequate amount of plant materials for nutrient analysis, the samples from three different quadrats of the same elevation belt were mixed. Then they were kept in the fridge (4°C) until further analysis. The samples were analyzed for three essential nutrients such as nitrogen (N), phosphorus (P) and potassium (K). Nitrogen was analysed using Micro-Kjeldahl method (Horneck & Miller, 1998). Wet method using sulphuric acid and hydrogen peroxide was used for digestion of plant samples for determination of phosphorus and potassium concentration (Campbell & Plank, 1998). Phosphorus was estimated calorimetrically using vanado-molybdate method (Juo, 1978) and potassium by using flame photometer (Horneck & Hanson, 1998). Nitrogen estimation was done in Regional Soil Testing Laboratory of Hetauda (Ministry of Agriculture Development) whereas phosphorus and potassium were estimated in the Agricultural Technology Centre, Lalitpur in Kathmandu.

3.6.3 Statistical analysis

Values of gall abundance (number of galls per 100 ramets), nutrient concentrations, gall diameter, diameter of stem with and without galls were evaluated using Shapiro-wilk normality test and Bartlett test for homogeneity of variances. To examine the variation in gall abundance (response variable) with elevation (predictor variable), a generalized linear model with linear as well as quadratic terms were fitted using the Poisson distribution with logit function in R version 3.4.4 (R core team, 2019). We used Akaike Information Criteria (AIC) to select the best model. For this, the data of gall abundance of all the sites were used to get a full distributional range of the fly (n=48). The Pearson's correlation coefficient and a simple linear regression were used to analyse the relationship between number of larvae per gall and gall diameter.

Similar analyses were used to examine the relationship between gall diameter and diameter of the stem with gall. Differences in gall diameter among different elevations were compared using analysis of variance (ANOVA) followed by post hoc tukey test. To assess the impact of galling on the stem diameter of the weed, Kruskal Wallis test with Bonferroni's correction was used. Nutrient data of samples from Chandragiri were compared using Kruskal Wallis test whereas of Simbhanjyang and Rasuwa were compared using one way analysis of variance (ANOVA) followed by Posthoc Tukey test.

CHAPTER 4

4. RESULTS

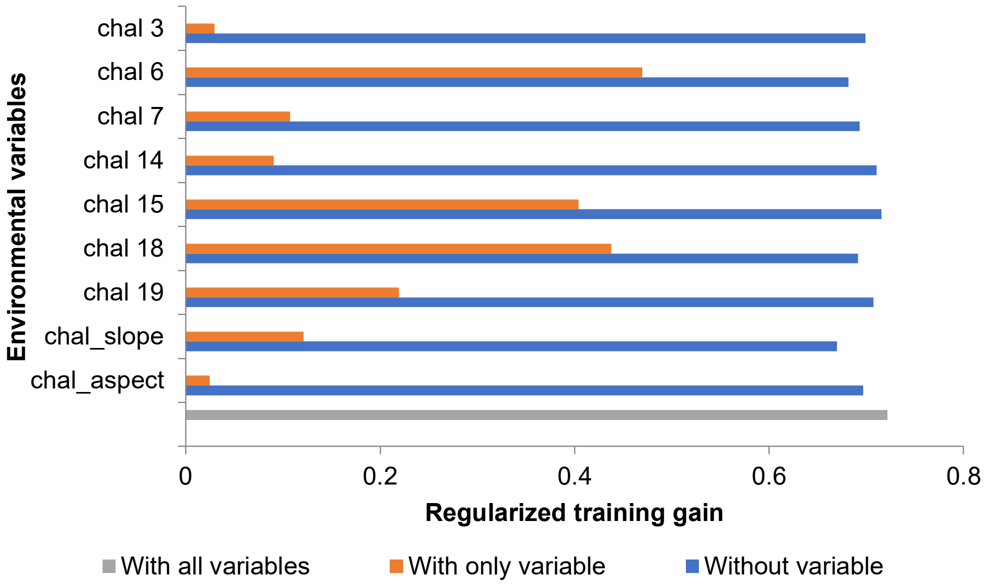
4.1 Climatically suitable areas of *Ageratina adenophora* in Chitwan-Annapurna Landscape

4.1.1 Model performance and variable contribution

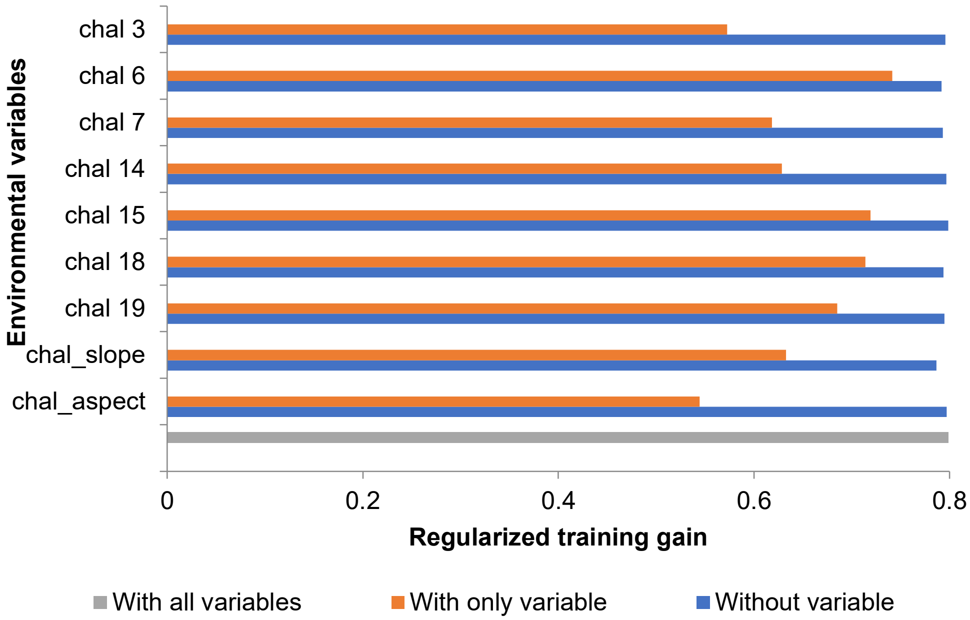
The current model built for *A. adenophora* performed better than random, with a mean training AUC value 0.85, mean test AUC value 0.80 and TSS value 0.52 (Appendix 10). Responses of each predictor variables are shown in response curve (Appendix 11). Out of 9 predictor variables used for model building, minimum temperature of the coldest month (bio 6) had the highest contribution (48.7%) followed by precipitation of the warmest quarter (bio 18) whereas aspect had the lowest contribution (2.9%) (Table 5). Maxent's jackknife test of variable importance also showed that minimum temperature of the coldest month (bio 6) had the highest training gain and AUC followed by precipitation of the warmest quarter (bio 18) when used in isolation (Figure 3). The response curve of the variable minimum temperature of the coldest month (bio 6) shows that the probability of occurrence of the weed below 1°C was the least and it increases with the increasing minimum temperature (Appendix 11). In addition, the minimum temperature of the coldest month of about 96% of the occurrence points used in model building was above 0°C (Appendix 12). Similarly, the response curve of precipitation of warmest quarter (bio 18) indicates that the probability of presence of the weed increases with the increase in precipitation of the warmest quarter above 500 mm (Appendix 11).

Table 5: Relative contribution of the environmental variables to the Maxent model built for current climatic condition

Predictor variables	% contribution	Permutation importance
bio 6	48.7	50.1
bio 18	19.4	8.1
slope	6.6	9.5
bio 7	5.9	8
bio 15	5.7	3.4
bio 19	3.8	5
bio 3	3.5	8.8
bio 14	3.4	2.7
aspect	2.9	4.3



Jacknife of regularized training gain for *Ageratina adenophora*



Jacknife of AUC for *Ageratina adenophora*

Figure 3: Results of Jacknife test of relative importance of predictor variables for *A. adenophora* for the current distribution

4.1.2 Current climatically suitable areas

Currently, 38% (12,215 km²) of total area of CHAL is found to be suitable for *A. adenophora* (Table 6, Figure 4). All districts of CHAL within elevational range of 119-2824 m asl had climatically suitable areas for this weed. The most suitable areas for *A. adenophora* mainly lie in the Middle Mountain (75%) followed by Siwaliks (37%), High Mountains (29%) and High Himalaya (0.1%) (Table 7). High Himalaya is less suitable for the weed with the fewest suitable areas found in the districts of Manang and Mustang. Nonetheless, all districts were found to have climatically suitable areas for this weed. Because the Middle Mountain and High Mountain regions had the most suitable areas for the weed, we tried to observe the change in climatically suitable areas in three protected areas situated in these physiographic regions. Among three protected areas (Annapurna Conservation area, Langtang National Park and Manaslu Conservation area), Langtang National Park was predicted to have the highest percentage of suitable areas for the weed (Figure 5).

4.1.3 Future invasion risk and change in climatic suitability

The predicted climatically suitable areas for *A. adenophora* would increase under RCP 2.6 for the year 2070 and RCP 4.5 for both the years 2050 and 2070 (Table 6, Figure 4). The highest increase (2%) in area of suitable habitat was predicted for the year 2070 under RCP 2.6 and 4.5. This gain in suitable areas is more prominent in districts like Lamjung, Gorkha, Dhading, Makwanpur, Chitwan and Tanahun. However, the increase in radiative force (from RCP 4.5 to 8.5) would decrease climatically suitable areas for the weed in both the years 2050 and 2070 (Table 6, Figure 4).

Though the suitable area is predicted to decrease in extreme climate scenarios (RCP 8.5), the upper elevational distribution limit would expand by 31 m and 42 m for the year 2050 and 2070, respectively (Figure 6). Though a small decrease (24 m) in upper elevation limit is predicted under RCP 2.6 in 2050, the highest increase of 48 m is expected under RCP 2.6 for the year 2070 in comparison to current climatic condition. However, under middle emission scenarios (RCP 4.5) for both the years 2050 and 2070, contraction in the upper elevational limit is predicted. In contrary, the lower elevation limit of *A. adenophora* would either remain stable or contract in future climate scenarios (Figure 6).

With climate change, all physiographic regions except Middle Mountain, were expected to gain climatically suitable areas. Though, a minimal loss in suitable areas in Middle Mountain was expected, this region will contain the most suitable areas for the weed followed by High Mountains, Siwaliks and High Himalaya in all the future climate scenarios (Table 7). In four of the future climate scenarios, i.e., RCP 2.6 (2050 and 2070) and RCP 8.5 (2050 and 2070), Siwaliks will gain the climatically suitable areas. Except under RCP 8.5 for the year 2050, High Mountains will also gain more suitable areas in all the future climate scenarios. Furthermore, under RCP 4.5 and 8.5 for the year 2070, climatically suitable area is predicted to increase in High Himalayas as well. Among all physiographic regions, percentage gain in suitable area is highest (2.4%) in Siwaliks under RCP 8.5 for the year 2050 (Table 7).

Like under current climatic condition, in future climate scenarios as well, Langtang National Park will have more suitable areas than other two protected areas (Figure 5). Under RCP 2.6 and 4.5 for both the years 2050 and 2070, climatically suitable areas are predicted to increase in Annapurna Conservation Area while under extreme climate scenarios, it seems to lose some suitable areas. For Langtang National Park, except RCP 4.5 (2050 and 2070), the climatically suitable areas will increase in RCP 2.6 and 8.5 for both the years. For, Manaslu Conservation Area, a remarkable gain in suitable areas is predicted only under RCP 8.5 for the year 2070.

Table 6: Predicted climatically suitable area of *A. adenophora* under current and future climate scenarios in Chitwan-Annapurna Landscape, Nepal

Scenarios	Suitable area (km²)	Suitable area (%)	Change in suitable area (%)
Current	12215	38	
RCP 2.6, 2050	12113	38	0
RCP 2.6, 2070	12758	40	2
RCP 4.5, 2050	12385	39	1
RCP 4.5, 2070	12889	40	2
RCP 8.5, 2050	11836	37	-1
RCP 8.5, 2070	11823	37	-1

Table 7: Predicted suitable area of *A. adenophora* in different physiographic regions of Chitwan-Annapurna Landscape, Nepal

Suitable area (%)				
Scenarios	**Siwalik**	**Middle mountain**	**High mountain**	**High Himalaya**
Current	36.56	75.09	29.25	0.09
RCP 2.6, 2050	36.33	73.83	30.14	0.07
RCP 2.6, 2070	37.70	77.90	31.81	0.09
RCP 4.5, 2050	34.06	76.00	31.63	0.10
RCP 4.5, 2070	36.29	78.65	33.38	0.10
RCP 8.5, 2050	43.28	71.13	26.77	0.07
RCP 8.5, 2070	40.15	70.53	29.65	0.15

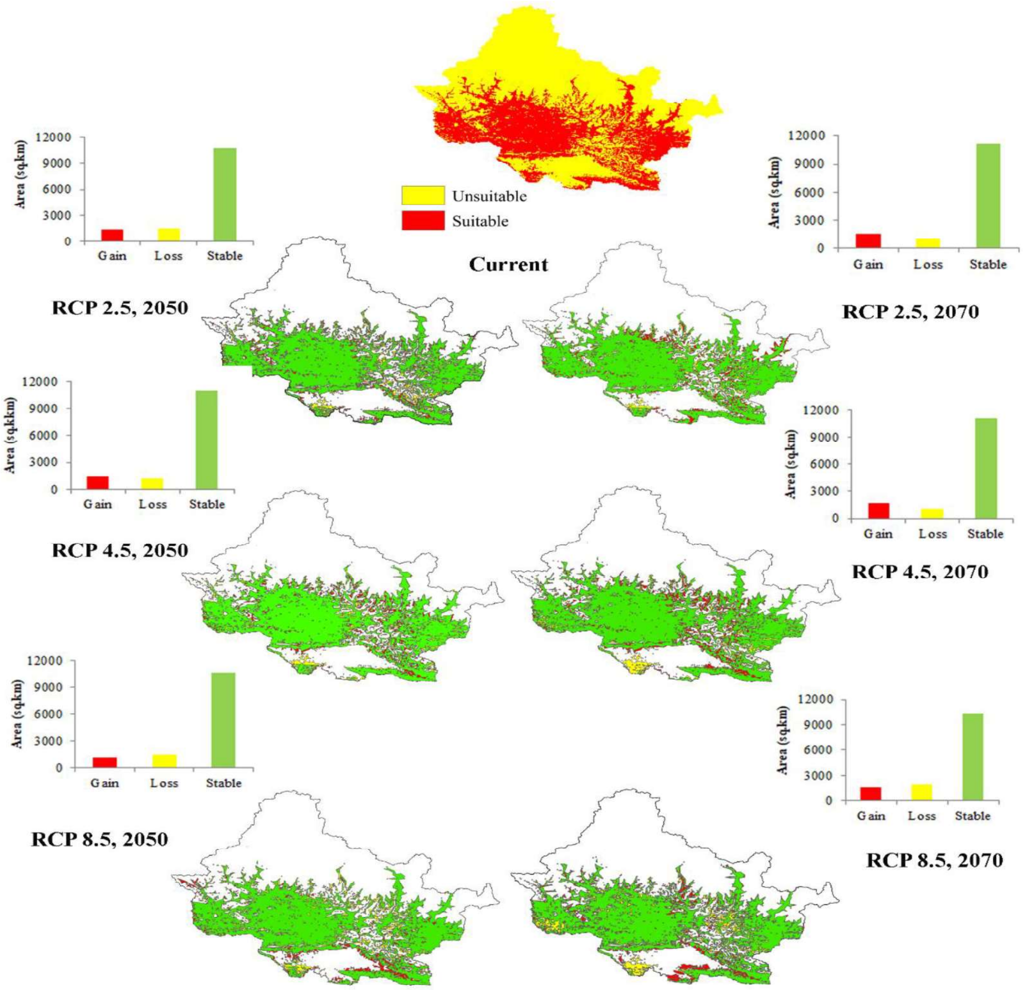


Figure 4: Projected climatically suitable areas of *A. adenophora* in CHAL showing likely stable, gain and loss areas under RCP 2.5, 4.5 and 8.5 in the year 2050 and 2070 with respect to the current suitable areas

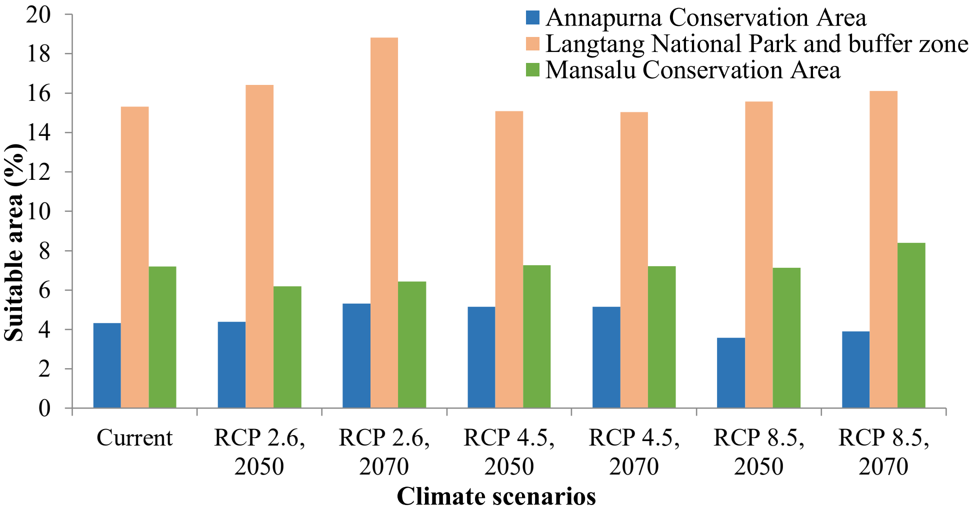
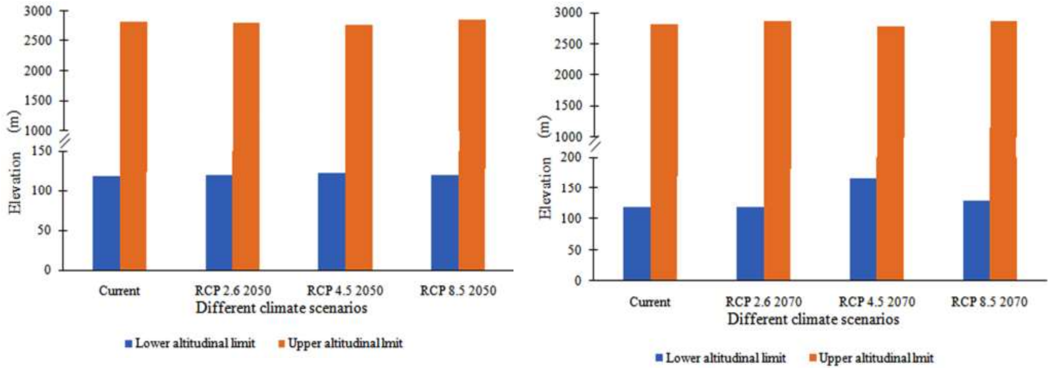


Figure 5: Change in suitable areas of *A. adenophora* in different protected areas of Chitwan-Annapurna Landscape, Nepal under current and future climate scenarios



(a) (b)

Figure 6: Change in upper and lower elevation range of *A. adenophora* in future climate scenarios in comparison to current (a) Year 2050 (b) Year 2070

4.2 Spatiotemporal distribution of *A. adenophora* in Chitwan-Annapurna Landscape predicted from satellite imageries

4.2.1 Change in distribution between 1990-2018

An increase in areas invaded by *A. adenophora* was observed during the period between 1990 and 2018 (Figures 7-11). In 1990, the distribution of *A. adenophora* in CHAL was 69 km^2 occupying 0.22% of the total area of CHAL (Figure 7). The area of distribution reached 1076 km^2 in the year 2000 occupying 3.35% of the total area of CHAL (Figure 8) and by 2010, the area of distribution reached to 1140 km^2 covering 3.55% of CHAL (Figure 9). Ultimately in 2018, area invaded by *A. adenophora* has increased to 1459 km^2 accounting 4.55% of CHAL (Figure 10).

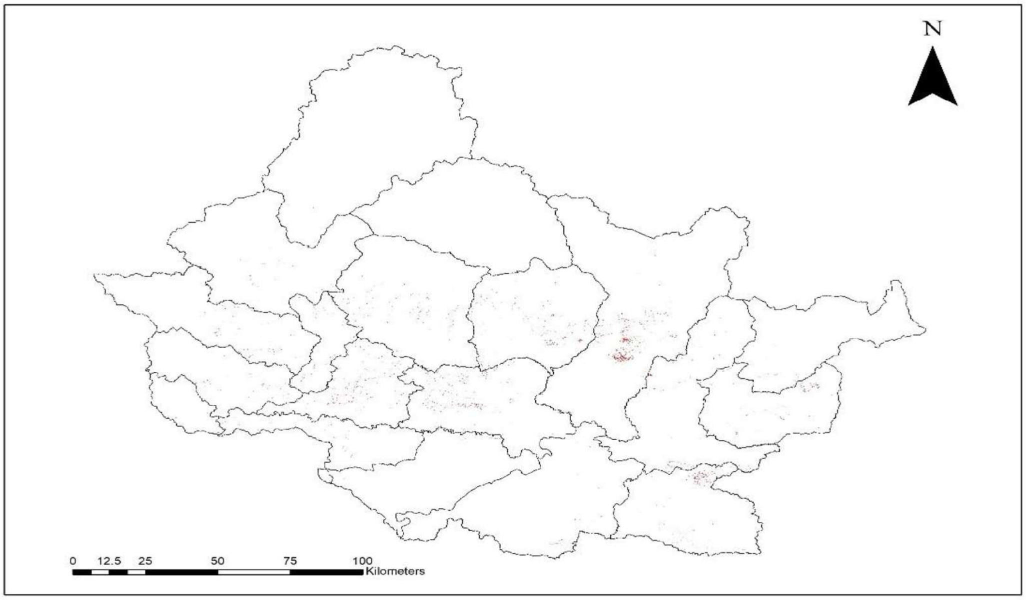


Figure 7: Distribution of *Ageratina adenophora* in CHAL in 1990

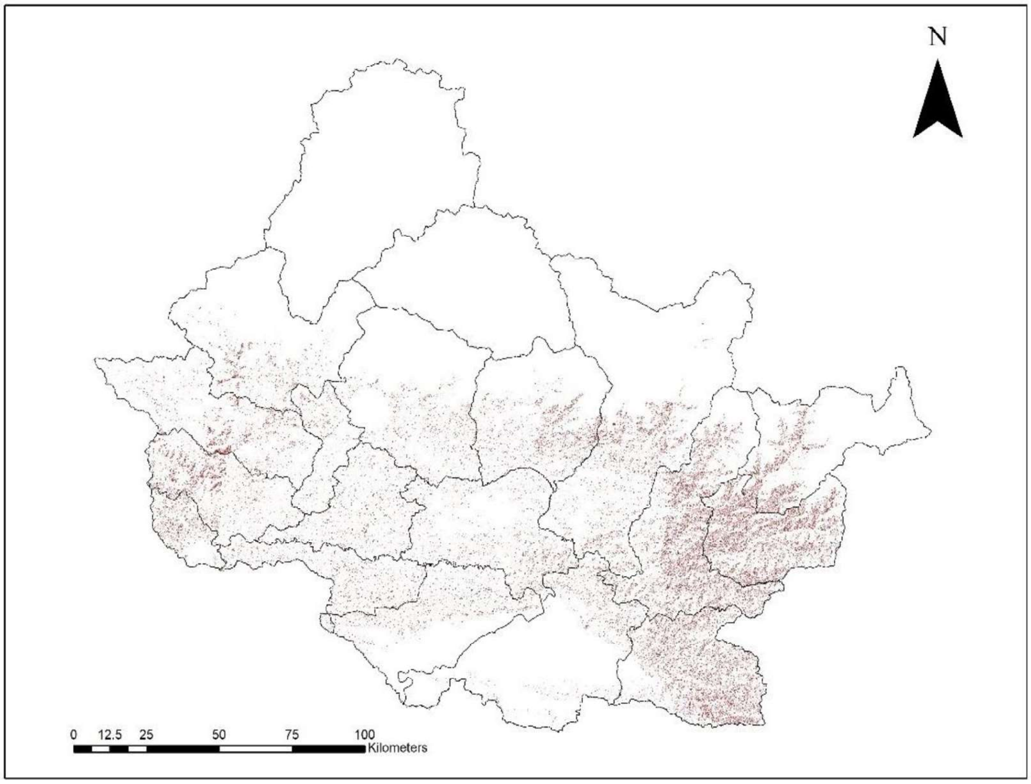


Figure 8: Distribution of *A. adenophora* in CHAL in 2000

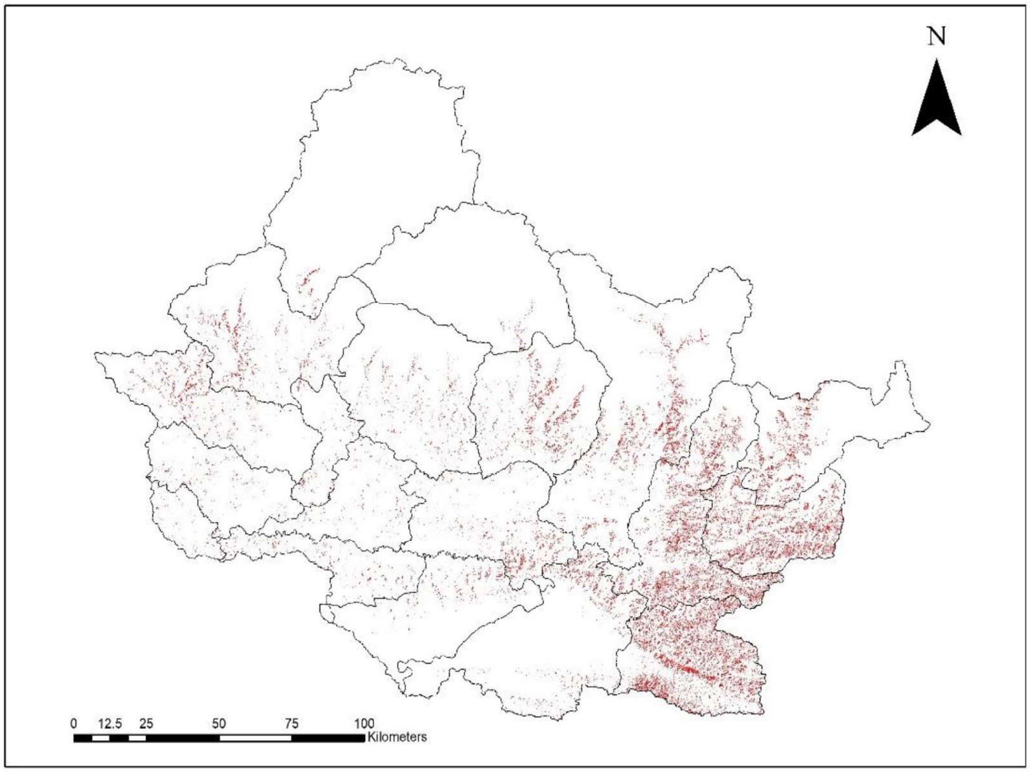


Figure 9: Distribution of *A. adenophora* in CHAL in 2010

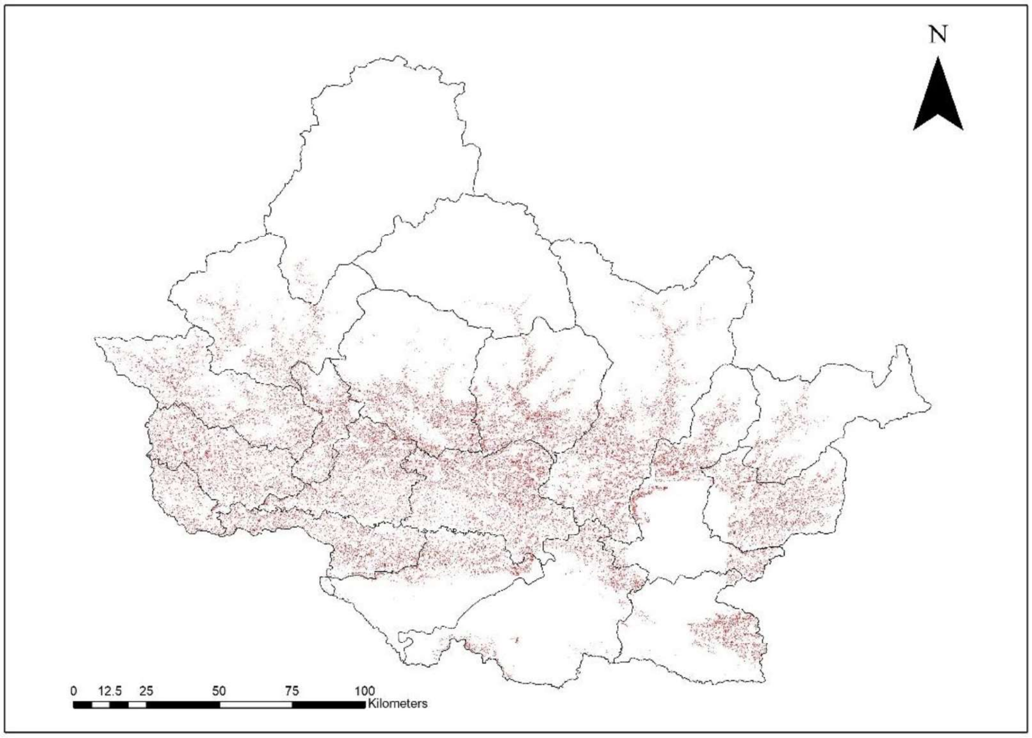


Figure 10: Distribution of *A. adenophora* in CHAL in 2018

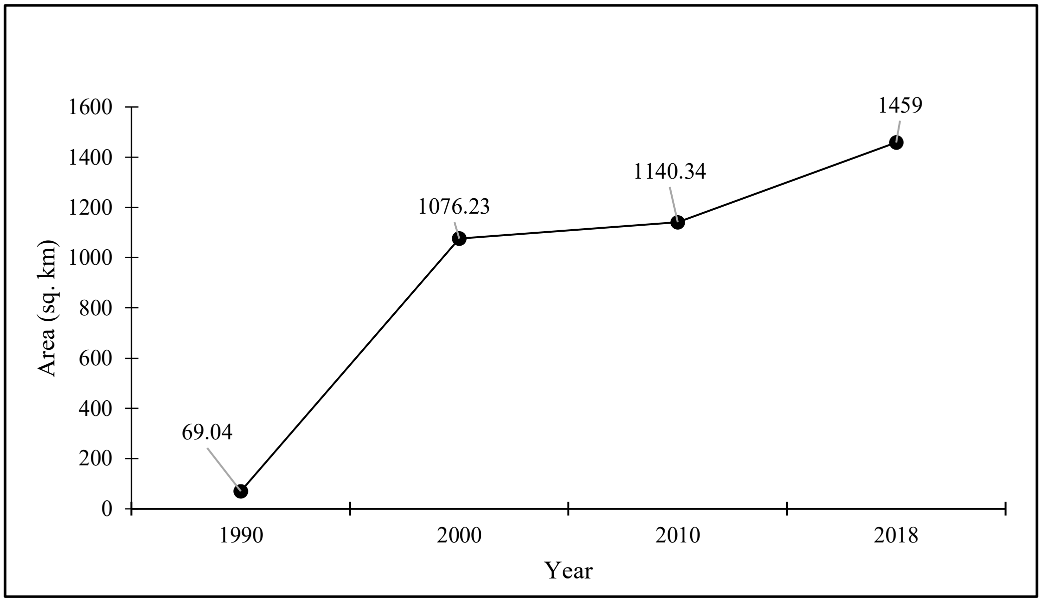


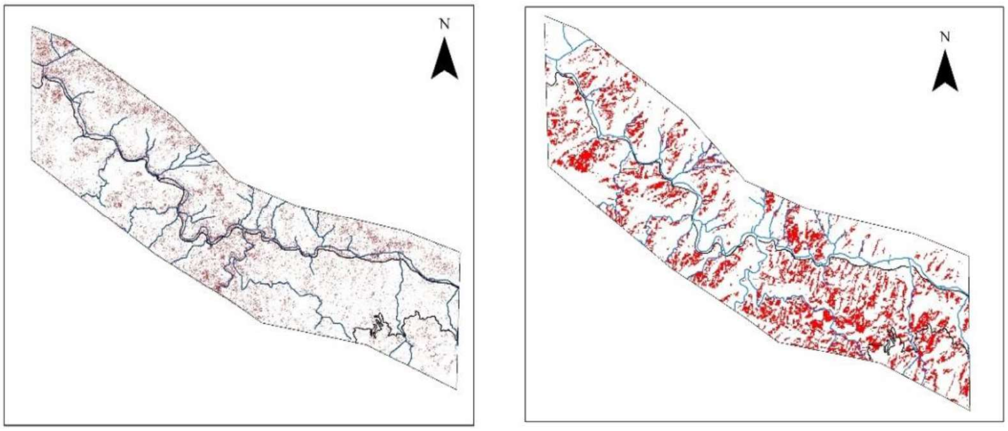
Figure 11: Trendline showing the distribution of *Ageratina adenophora* from 1990-2018 in CHAL

4.2.2 Invaded areas predicted using Worldview-2 (WV-2) and Landsat 8 imageries of 2008 and 2018

The area invaded by *A. adenophora* in Landsat imageries increased from 2008 to 2018 in two of the smaller area of interest i.e., Sarangkot and Ghashikuwa (Table 8).

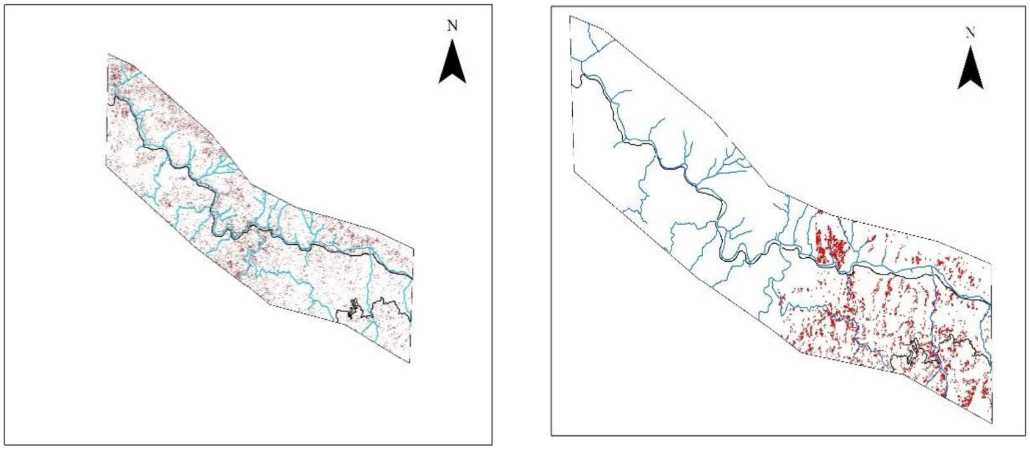
The highest increment of 16.56 % in invaded area of *A. adenophora* was observed in Sarangkot followed by Ghasikuwa which reported an increase of 14.38% in a span of ten years (Figure 14 & 15, Table 8). However, in Mahadevbesi, the invaded area decreased from 2008 to 2018 by 12.8% (Table 8). Similar trend was observed in Worldview-2 images as well. The percentage invaded area increased by 5.43% in Ghasikuwa from 2008 to 2018 whereas in Mahadevbesi, the area invaded by *A. adenophora* decreased by 4.23% (Table 8).

Though, similar trend was observed in WV-2 and Landsat imageries in a span of ten years (2008-2018), some differences was noticed in the estimated invaded area between the two imageries. In the year 2008, Mahadevbesi had the highest percentage of invaded area in comparison to Ghasikuwa in both WV-2 and Landsat imageries (Table 8). However, a difference of 6% in the estimated invaded area was noticed between WV-2 and Landsat images (Figure 12, Table 8). Similarly in 2018, *A. adenophora* invaded highest percentage of area (10.02%) in Sarangkot in both WV-2 and Landsat imageries but a difference of 7.29% was observed in the estimated invaded area (Figure 15, Table 8). Furthermore, in all the imageries except Ghasikuwa for the year 2008 (Figure 16) and Mahadevbesi for the year 2018 (Figure 13), invaded area was found to be more in Landsat imageries in comparison to WV-2 images (Table 8). The highest difference of 7.41% was observed in Ghasikuwa in 2018 between the two imageries (Figure 17, Table 8) whereas the lowest difference of 1.54% was also observed in Ghasikuwa in 2008 between the two images (Figure 16, Table 8).



(a) (b)

Figure 12: Invaded areas of *A. adenophora* in Dhading (Mahadevbesi) in a) Worldview-2 imageries and b) Landsat imageries in the year 2008



(a) (b)

Figure 13: Invaded areas of *A. adenophora* in Dhading (Mahadevbesi) in a) Worldview 2 imageries and b) Landsat imageries in the year 2018

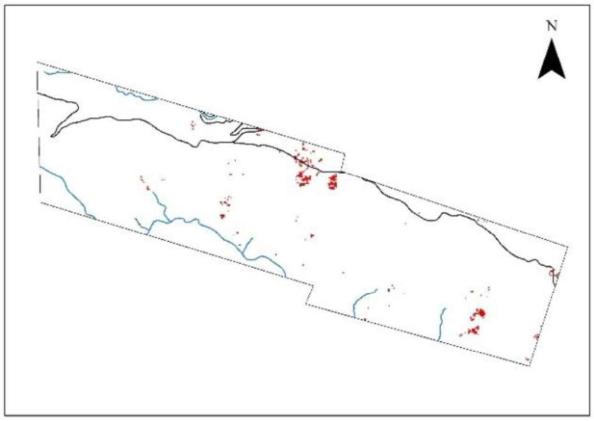
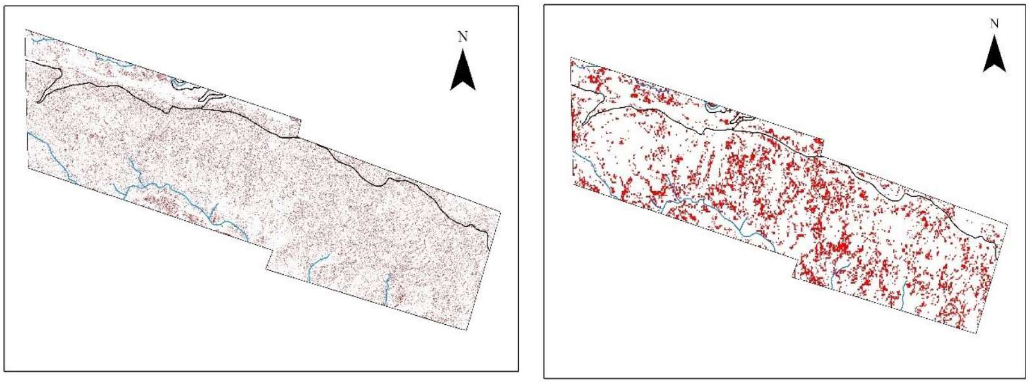
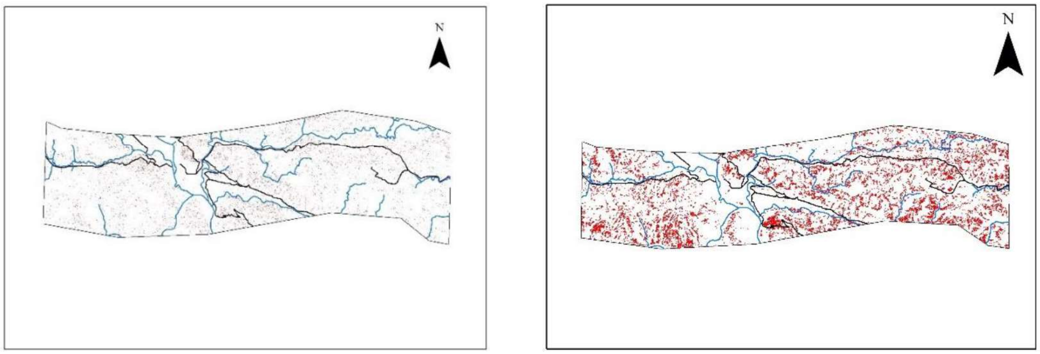


Figure 14: Invaded areas of *A. adenophora* in Kaski (Sarangkot) in Landsat imageries in the year 2008



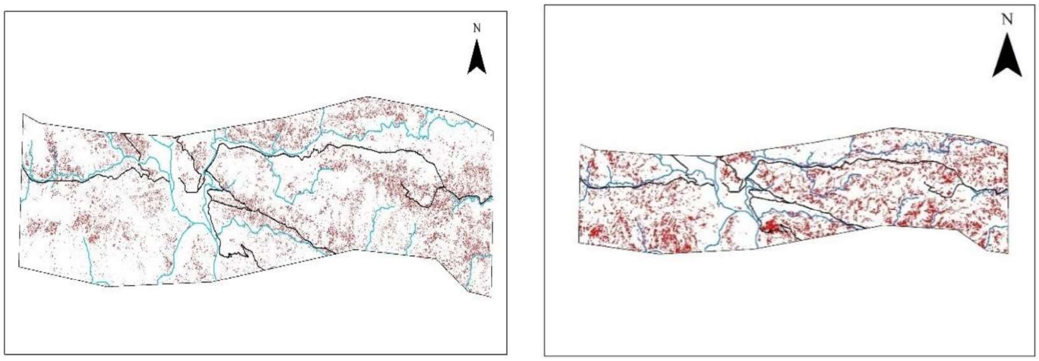
(a) (b)

Figure 15: Invaded areas of *A. adenophora* in Kaski (Sarangkot) in (a) Worldview-2 imageries and b) Landsat imageries in the year 2018



(a) (b)

Figure 16: Invaded areas of *A. adenophora* in Tanahun (Ghasikuwa) in a) Worldview imageries and b) Landsat imageries in the year 2008



(a) (b)

Figure 17: Invaded areas of *A. adenophora* in Tanahun (Ghasikuwa) in a) Worldview imageries and b) Landsat imageries in the year 2018

Table 8: Change in area invaded by *A. adenophora* from 2008 to 2018 in different AOI of Worldview-2 and Landsat images

Area of interest (AOI)	Year	Total area of interest (km^2)	Landsat		Worldview-2	
			Area (km^2)	% Invaded area	Area (km^2)	% Invaded area
Dhading (Mahadevbesi)	2008	60.18	10.8	17.95	7.2	11.96
	2018	60.18	3.1	5.15	4.6	7.64
Kaski (Sarangkot)	2008	32.93	0.25	0.75	not available	not available
	2018	32.93	5.7	17.31	3.3	10.02
Tanahun (Ghasikuwa)	2008	67.37	0.41	0.61	1.45	2.15
	2018	67.37	10.1	14.99	5.11	7.58

4.2.3 Accuracy assessment of distribution of *A. adenophora* in Worldview-2 and Landsat imageries

The overall accuracy obtained in CHAL area was 62% with 0.35 kappa value (Table 9). In Worldview-2 image of Tanahu (Ghasikuwa), 72.4% of overall accuracy was obtained with a kappa value of 0.50 whereas for the same area of interest, in Landsat image, overall accuracy of 38.8% was obtained. The kappa value obtained for this image was 0.40 (Table 10).

Table 9: Accuracy assessment for the distribution of *A. adenophora* in Landsat 8 image of CHAL in 2018

Location	**Overall accuracy (%)**	**Kappa**
CHAl	62	0.35

Table 10: Comparison of accuracy assessment for the distribution of *A. adenophora* between Landsat and Worldview-2 imageries

District	**AOI**	**World view 2 imageries**		**Landsat images**		**P-value of overall accuracy**
		Overall Accuracy (%)	**Kappa**	**Overall Accuracy (%)**	**Kappa**	
Tanahun	Ghasikuwa	72.4	0.50	38.8	0.40	<0.05

4.3 Plant functional traits along the elevation gradients

Among eight functional traits studied, except Specific Leaf Area (SLA) and Leaf Area (LA), the mean values of other functional traits (Leaf Nitrogen Content (LNC), Number of floral heads per ramet, Total seeds per floral head, Seed length, Seed breadth and Seed mass) was higher in Chandragiri in comparison to other two sites i.e., Simbhanjyang and Panchase (Table11).

Table 11: Mean values of the functional traits of *Ageratina adenophora* with minimum and maximum value and sample size

Traits	**Mean values of the functional traits of *Ageratina adenophora***						**Range (min-max)**		
	Sample size			**Mean ± S.D**					
	Simbhanjyang	**Panchase**	**Chandragiri**	**Simbhanjyang**	**Panchase**	**Chandragiri**	**Simbhanjyang**	**Panchase**	**Chandragiri**
SLA (cm^2/ gm)	675	675	1080	332 ± 105	337 ± 86	217 ± 67	117 - 902	176 - 661	94 - 508
LA (cm^2)	675	675	1080	37 ± 10	36 ± 6	28 ± 6	19 - 58	19 - 62	14 - 58
LNC (%)	45	45	72	1.62 ± 0.26	1.91 ± 0.30	3.24 ± 0.48	1.18 - 2.24	1.46 - 2.63	2.49 - 4.28
No. of floral heads/ ramet	150	150	240	97 ± 26	98 ± 28	279 ± 71	78 - 103	30 - 180	89 - 470
Total seeds (mature seeds)/floral head	450	450	540	36 ± 10	28 ± 10	38 ± 8	5 to 73	1 to 62	13 to 64
Seed length (mm)	450	450	720	1569 ± 175	1591 ± 170	1699 ± 144	1128 - 2337	1103 - 2305	1212 - 2163
Seed breadth (mm)	450	450	720	324 ± 39	342 ± 38	350 ± 38	232 - 455	226 - 461	242 - 572
Seed mass (per100 seeds) g	150	150	240	0.0041 ± 0.0009	0.0035 ± 0.0007	0.0055 ± 0.0006	0.0023 - 0.0066	0.0019 - 0.0051	0.0045 - 0.0082

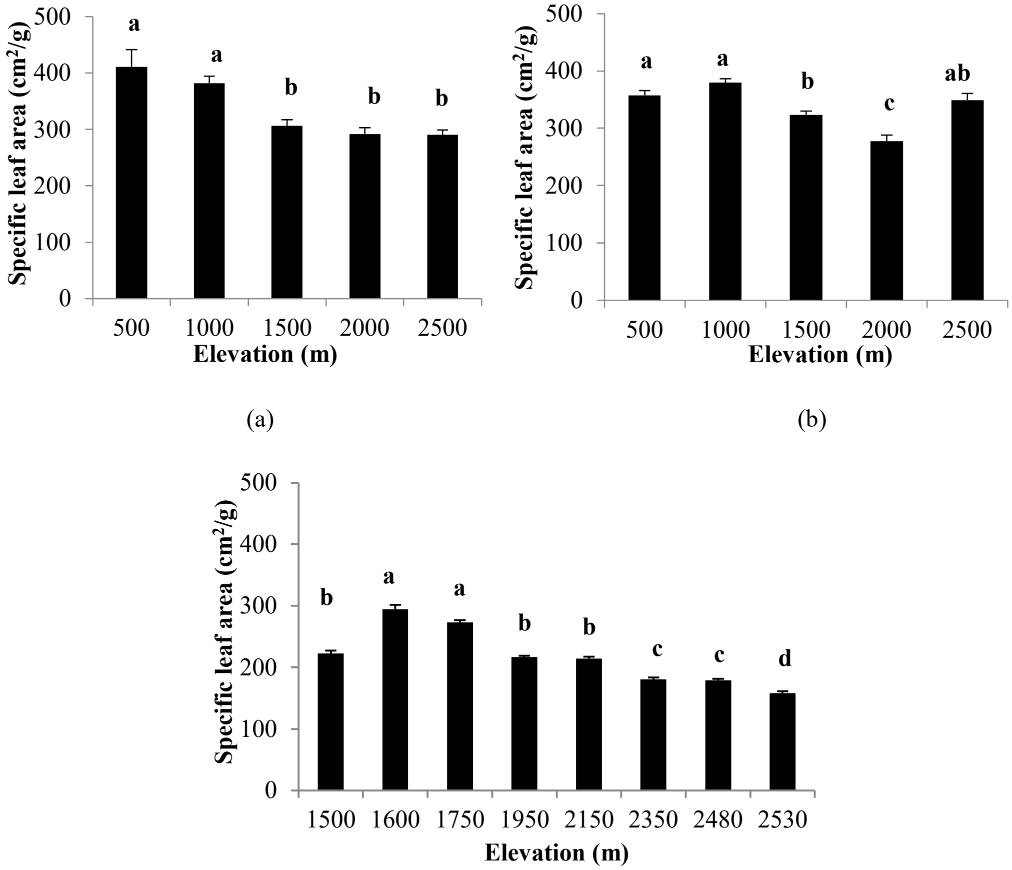
Table 12 : Mean values of the functional traits of *A. adenophora* with minimum and maximum value and sample size at highest and lowest elevation sites in central Nepal

	Sample size		**Mean ± S.D**		**Range (min-max)**	
Traits	Chitwan	Rasuwa	Chitwan	Rasuwa	Chitwan	Rasuwa
SLA (cm^2/gm)	135	135	422±165	275±62	216-847	150-451
LNC (%)	9	9	3.68±0.03	2.93±0.09	3.64-3.7	2.9-3.08
No. of floral heads/ramet	30	30	143±29	*	94-215	*
Total seeds (mature seeds/ floral head	30	30	34±8	29±9	26-47	20-48
Seed length (mm)	90	90	1451±98	2109±166	1254-1648	1640-2565
Seed breadth (mm)	90	90	301±29	376±43	219-362	287-489
Seed mass (g)	90	90	0.0021±0.0002	0.0059±0.0004	0.0018-0.0024	0.0052-0.0066

Note: * represents the unavailability of data

4.3.1 Specific leaf area (SLA)

Specific leaf area (SLA) of a fully expanded leaf of *A. adenophora* ranged from 94-902 cm^2/g along the elevation gradients at three sites (Table 11). SLA of *A. adenophora* at the lowest elevational site (Chitwan) was about 1.5 times higher than that of the highest elevational site i.e., Rasuwa (Table 12). SLA did not exhibit same pattern in all three sites. SLA along elevation gradients in Simbhanjyang decreased with the increasing elevation (χ^2 = 132, d.f = 4, P<0.001) (Figure 18a) whereas in Panchase, SLA first decreased with the increasing elevation upto 2000 m asl and then increased at 2500 m asl (χ^2 = 126, d.f = 4, P<0.001) (Figure 18b). However, in Chandragiri, SLA increased upto 1750 m with respect to 1500 m asl and then decreased with the increasing elevation (χ^2 = 524, d.f = 7, P<0.001) (Figure 18c).



(a)

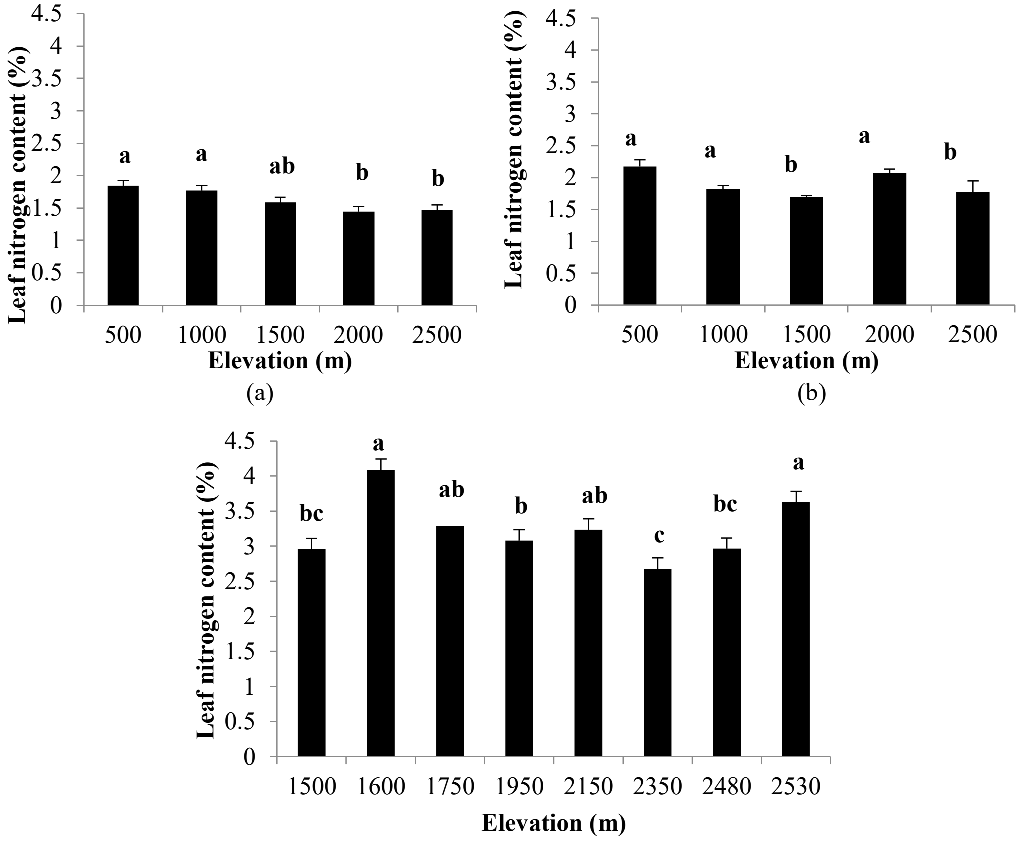
(b)

(c)

Figure 18: Variation in SLA along elevation gradients in study site. a. Simbhanjyang, b. Panchase c. Chandragiri. Different letters above bars indicate statistically significant differences between elevations at P<0.001 according to Kruskal Wallis test followed by bonferroni's correction

4.3.2 Leaf nitrogen content (LNC)

Leaf nitrogen content (LNC) of *A. adenophora* ranged from 1.18 to 4.28% along the elevation gradients of all three sites. LNC at the lowest elevational site (Chitwan) was about 1.3 times higher than that of the highest elevational site (Rasuwa) (Table 12). LNC showed a diverse trend across three sites. In Simbhanjyang, LNC decreased with the increasing elevation along the elevation gradient (F = 22, d.f = 43, P<0.001) (Figure 19a). Though LNC decreased with the increasing elevation upto 1500 m asl in Panchase, it again increased at 2000 m asl and then decreased at 2500 m asl (Panchase; $\chi2$ = 15, d.f = 4, P<0.001) (Figure 19b). However, in Chandragiri, LNC first increased at 1600 m and then decreased being constant upto the elevation 2480 m asl. At 2530 m, LNC again increased being similar to that at 1600 m asl ($\chi2$ = 51, d.f = 7, P<0.001) (Figure 19c). LNC in Chandragiri was nearly 2 times higher than the other two sites.



(a) (b)

(c)

Figure 19: Variation of LNC along the elevation gradients in three sites. a. Simbhanjyang, b. Panchase and c. Chandragiri. Different letters above bars indicate statistically significant differences between elevations at $P<0.001$ according to one way ANOVA followed by post hoc Tukey test for the site Simbhanjyang whereas Kruskal Wallis test followed by bonferroni's correction for the sites Panchase and Chandragiri

4.3.3 Relationship between SLA and LNC

A negative relationship was observed between SLA and LNC (Figure 20). As SLA increased, LNC content decreased.

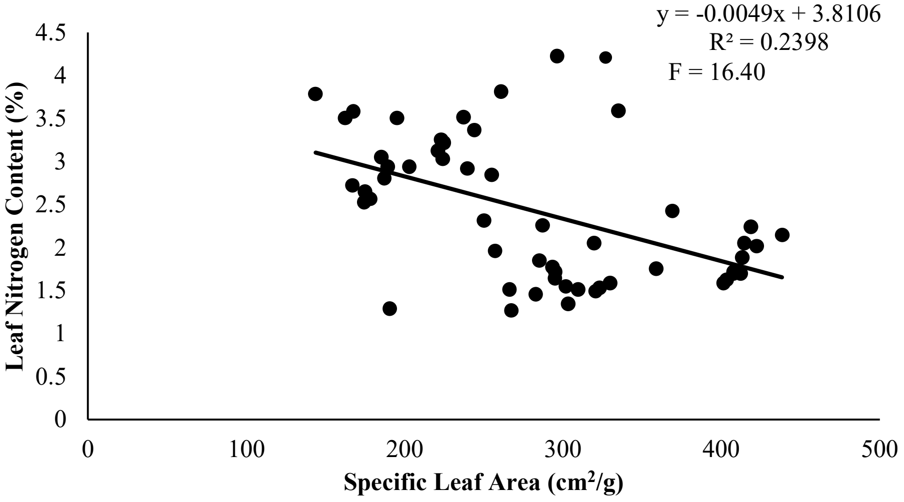
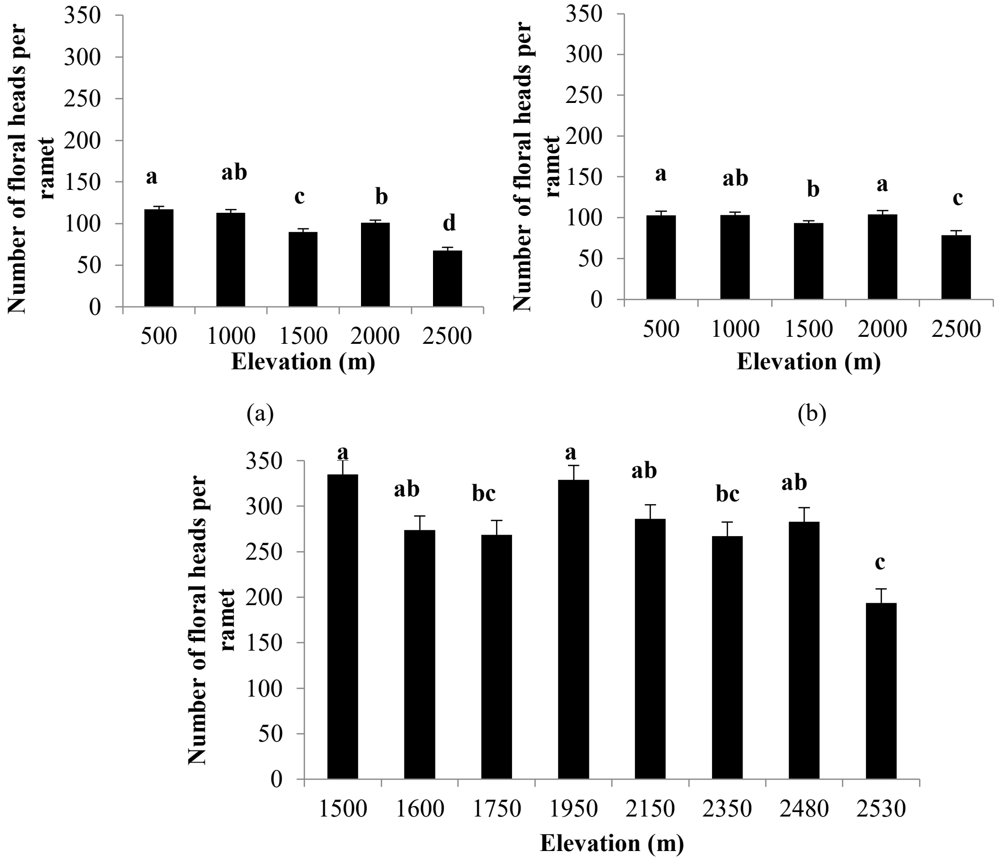


Figure 20: Relationship between Specific Leaf Area (SLA) and Leaf Nitrogen Content (LNC) of *A. adenophora*

4.3.4 Number of floral heads per ramet

Along the elevation gradient at three sites, the number of floral heads ranged from 30-470 per ramet (Table 11). In all three sites, the number of floral heads per ramet was the lowest at the uppermost elevational limit. Though, the number of floral heads per ramet decreased with the increasing elevation in all the three sites, at elevation 1950-2000 m asl, the number first increased and then decreased (Simbhanjyang: χ^2 = 70, d.f = 4, P<0.001; Panchase: χ^2 = 29, d.f = 4, P<0.001; Chandragiri: χ^2 = 62, d.f = 7, p<0.001) (Figure 21a, 21b & 21c).



(a) (b)

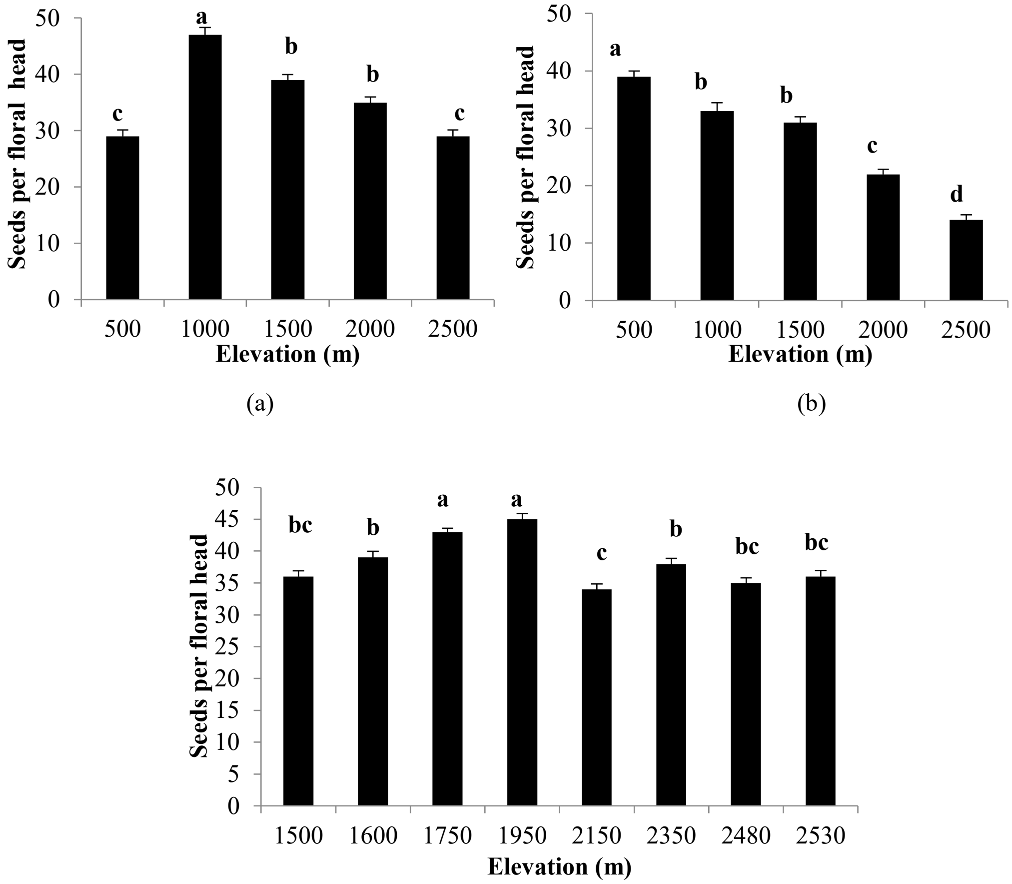
(c)

Figure 21: Number of floral heads per ramet along the elevation gradient in a. Simbhanjyang, b. Panchase and c. Chandragiri. Different letters above bars indicate statistically significant differences between elevations at P<0.001 according to Kruskal Wallis test followed by bonferroni's correction

4.3.5 Seeds per floral head

A floral head of *A. adenophora* produced 1-73 seeds along the elevation gradients at three sites (Table 11). In Simbhanjyang, number of seeds per floral head first increased upto 1000 m asl and then decreased after that ($\chi2$ = 117, d.f = 4, P<0.001; Figure 22a). Similarly, in Chandragiri, total seeds per head increased upto 1750 m asl and then decreased after 2150 m asl ($\chi2$ = 125, d.f = 7, P<0.001; Figure 22b).

However, in Panchase, mature seeds per head decreased with the increasing elevation (χ2 = 206, d.f = 7, P<0.001; Figure 22c).



(a)

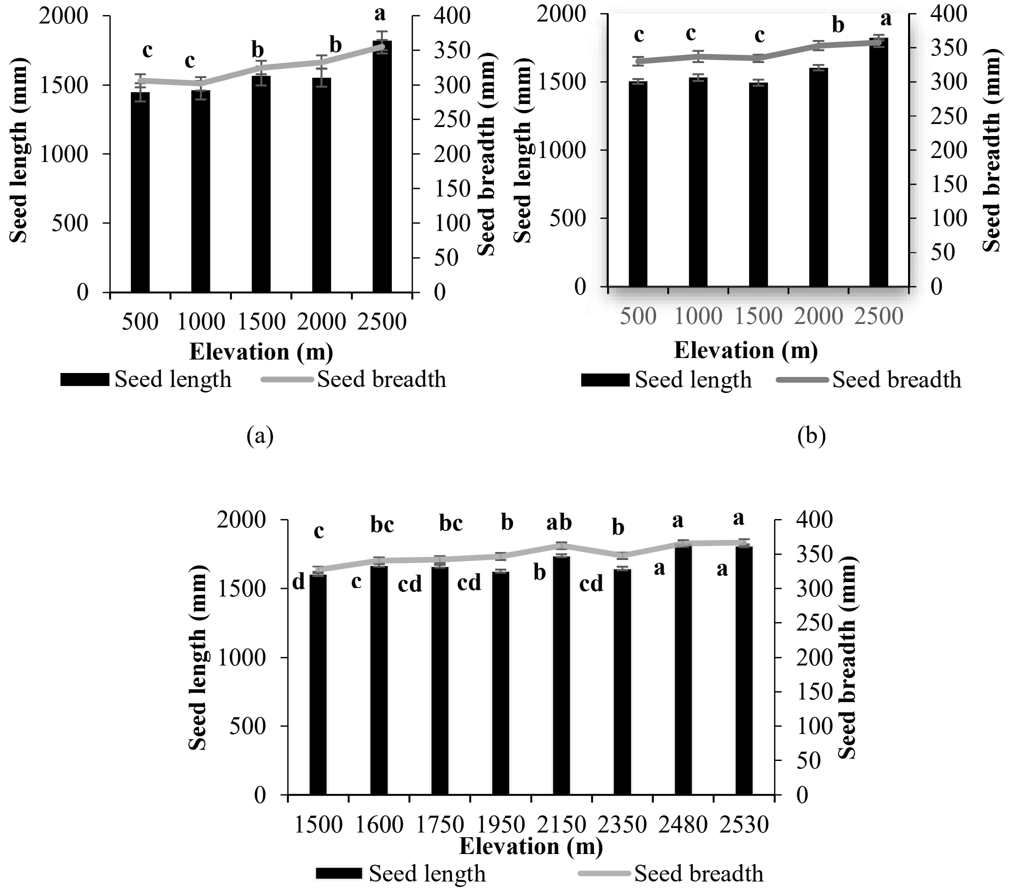
(b)

(c)

Figure 22: Variation of seeds per floral head along the elevation gradients in three sites. a. Simbhanjyang, b. Panchase and c. Chandragiri. Different letters above bars indicate statistically significant differences between elevations at P<0.001 according to Kruskal Wallis test followed by bonferroni's correction

4.3.6 Seed size

The seed length and seed breadth of *A. adenophora* ranged from 1103 to 2337 mm and 226-572 mm along the elevation gradients in three sites (Table 11). However, the individual with the largest seed length (2565 mm) was observed at the highest elevational site i.e., Rasuwa (Table 12). The seed length of *A. adenophora* at the highest elevation site (Rasuwa) was about 1.5 times larger than that of the lowest elevation site (Chitwan) (Table 12). In all three sites, seed length as well as breadth increased with the increasing elevation along the elevation gradient (χ2 = 243, d.f = 4, P<0.001, Simbhanjyang, Figure 23a; χ2 = 210, d.f = 4, P<0.001, Panchase, Figure 23b; χ2 = 224, d.f = 7, P<0.001, Chandragiri, Figure 23c).



(a)

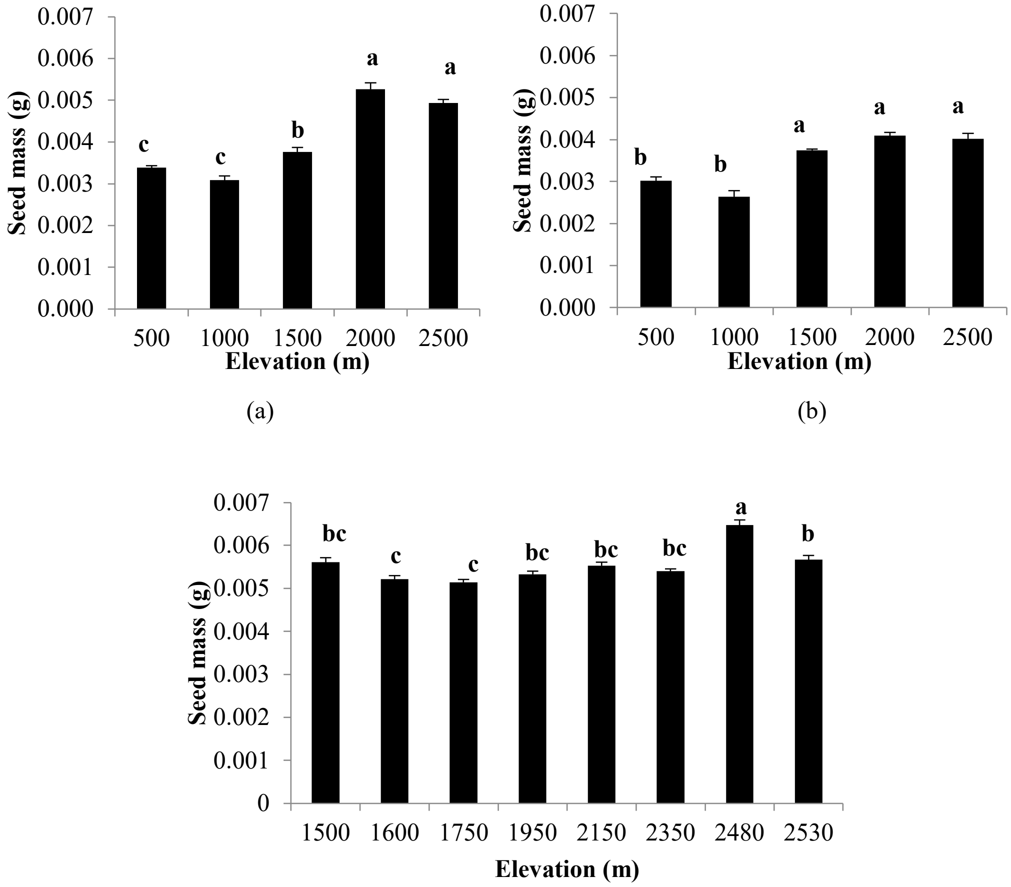
(b)

(c)

Figure 23: Variation in seed length and seed breadth along the elevation gradient in a. Simbhanjyang, b. Panchase and c. Chandragiri. Different letters above bars indicate statistically significant differences between elevations at P<0.001 according to Kruskal Wallis test followed by bonferroni's correction

4.3.7 Seed mass

The mass of seeds produced by *A. adenophora* ranged from 0.0019 to 0.0082 g (per 100 seeds) along the elevation gradient in three sites (Table 11). Individuals with lowest seed mass was recorded from Chitwan (Table 12). In Simbhanjyang as well as Panchase, seed mass increased with the increasing elevation along the elevation gradient (χ2 = 119, d.f = 4, P<0.001, Simbhanjyang, Figure 24a; χ2 = 70, d.f = 4, P<0.001, Panchase, Figure 24b)). However, in Chandragiri, seed mass remained constant upto elevation 2350 m asl and then increased at elevation 2480 m asl (χ2 = 75, d.f = 7, P<0.001, Figure 24c).



(a) (b)

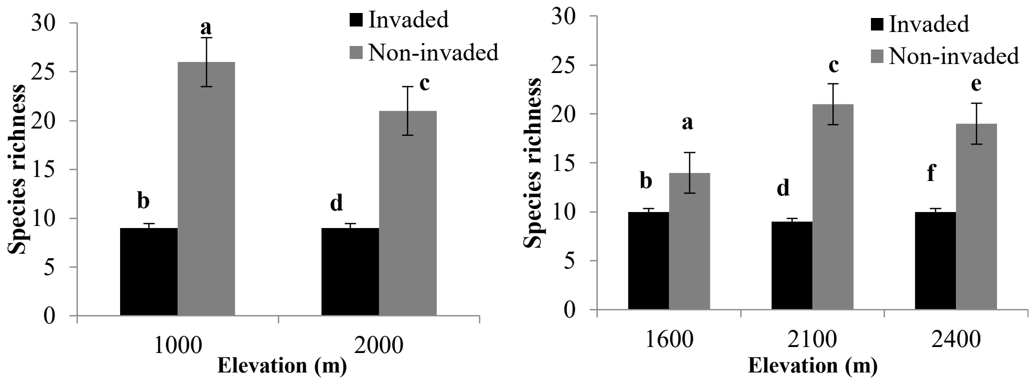
(c)

Figure 24: Variation in seed mass along the elevation gradient in a. Simbhanjyang, b. Panchase and c. Chandragiri. Different letters above bar indicate statistically significant differences between elevations at P<0.001 according to Kruskal Wallis test followed by bonferroni's correction

4.4 Impact of *Ageratina adenophora* on vegetation and soil characteristics

4.4.1 Species richness

There was significant difference (P<0.001) in species richness between invaded and non-invaded plots (Figure 25). Species richness of invaded plots in both sites was nearly 1.5 times less than species richness of non-invaded plots. In Simbhanjyang, the highest number of species (87) was recorded at an elevation of 1000 m asl in non-invaded plots whereas in Chandragiri the highest number of species (100) was observed at an elevation of 2100 m asl in non-invaded plots (Table 13; Figure 25). The average highest number of species recorded in a plot of non-invaded sites was 29.



(a) (b)

Figure 25: Mean species richness (per plot) at different elevations in a. Simbhanjyang and b. Chandragiri. Different letters above bars indicate statistically significant difference between invaded and non-invaded sites at P<0.001 according to Independent sample t test

Table 13: Total number of species recorded in invaded and non-invaded plots at different elevation bands of Simbhanjyang and Chandragiri

Sites	**Elevation (m asl)**	**Non-invaded plots**		**Invaded plots**	
		Number of species recorded	Number of families recorded	Number of species recorded	Number of families recorded
Simbhanjyang	1000	87	38	44	24
	2000	73	25	47	22
Chandragiri	1500	62	24	45	23
	2100	100	38	58	31
	2400	89	42	51	33

4.4.2 Species diversity

The Shannon's diversity index was found to be higher in non-invaded plots in comparison to invaded plots in both the study sites (Table 14). Similarly, Simpson's diversity index was also higher in non-invaded plots in comparison to invaded plots in all the elevation ranges in both the sites (Table 14). Shannon's diversity index in the invaded plots ranged from 1.18 to 1.39 whereas in non-invaded plots ranged from 1.84 to 2.63 (Table 14). Similarly, Simpson's index in invaded plots ranged from 0.5 to 0.62 whereas in non-invaded plots ranged from 0.77 to 0.9 (Table 14).

Table 14: Diversity index of invaded and non-invaded plots of different sites at different elevation ranges

	Shannon's diversity index (H)					**Simpson's diversity index (1-D)**				
Sites	**Daman**		**Chandragiri**			**Daman**		**Chandragiri**		
Elevation	1000	2000	1500	2100	2400	1000	2000	1500	2100	2400
Invaded	1.39a	1.31a	1.18a	1.21a	1.32a	0.62a	0.54a	0.5a	0.53a	0.56a
Non-invaded	2.63b	2.17b	1.84b	2.4b	2.43b	0.9b	0.81b	0.77b	0.86b	0.87b

*Different letters represent significant difference between the values

4.4.3 Species composition variation

Plot type were the only variables that showed the effect on species composition while doing ordination on paired plots. In Simbhanjyang, there were 55 species common to both plot types whereas 25 species were found only in invaded plots and 71 species were found only in non-invaded plots (Appendix 15). In invaded plots of - Simbhanjyang, 3 IAPS and 4 naturalised noninvasive species were recorded whereas in noninvaded plots, 4 IAPS and 7 naturalised noninvasive species were recorded (Appendix 15). In Chandragiri, there were 88 species common to both plot types whereas 18 species were found only in invaded plots and 91 species were found only in non-invaded plots (Appendix 16). Among these species, one IAPS and 6 noninvasive naturalized species were observed in invaded plots whereas in non-invaded plots, one IAPS and 9 noninvasive naturalized species were documented (Appendix 16). The Sorenson's similarity index between invaded and non-invaded plots at Simbhanjyang and Chandragiri was 52% and 51%, respectively (Table 15). Low similarity index was also revealed by canonical correspondence analysis (CCA); many species present in non- invaded plots were absent in invaded plots (Figure 26 & 27). Species of genus *Cyanotis*, *Desmodium*, and grasses like *Cyperus* were absent in the invaded plots of Simbhanjyang. Similarly, in Chandragiri, species of genus like *Cyanotis*, *Desmodium*, *Indigofera* and grasses like *Pycreus* were absent in the invaded plots. Pteridophyte like *Adiantum caudatum* were absent in the invaded plots of both the sites.

Table 15. Similarity index between invaded and non-invaded plots of the sites at different elevation

	Elevation	Sorenson's similarity index	Sorenson's similarity index (mean)
Simbhanjyang	1000	0.54	0.52
	2000	0.5	
Chandragiri	1500	0.46	
	2100	0.53	0.51
	2400	0.53	

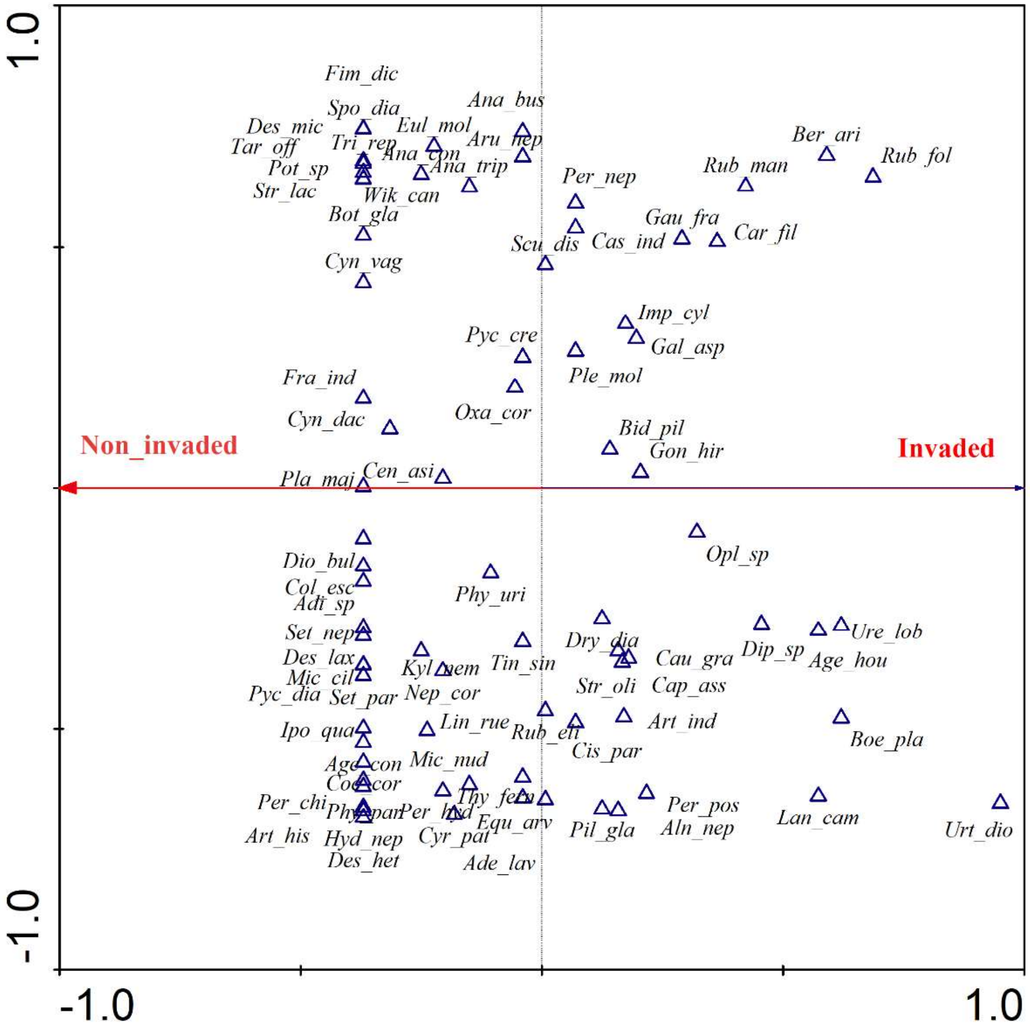


Figure 26: Results of canonical correspondence analysis (CCA) showing species composition variation between invaded and non-invaded plots of Simbhanjyang. Full name of each species is given in Appendix 15.

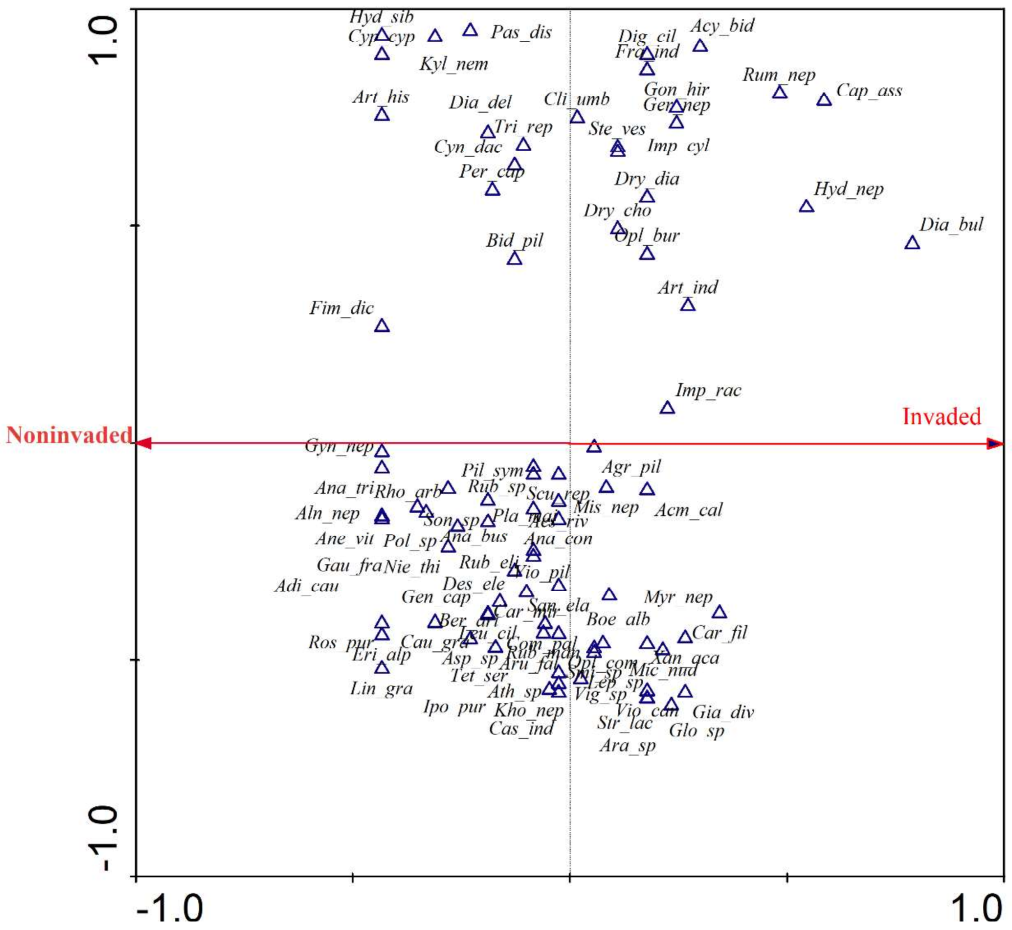
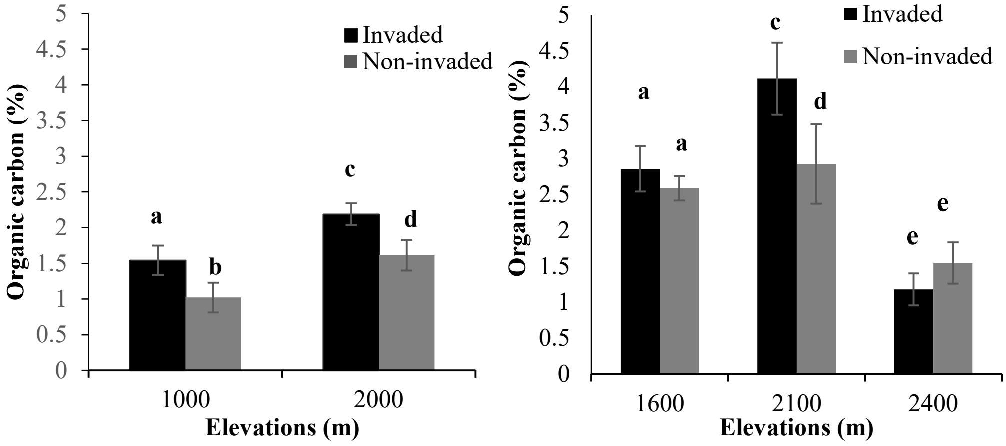


Figure 27: Results of canonical correspondence analysis (CCA) showing species composition variation between invaded and non-invaded plots in Chandragiri. Full name of species is given in Appendix 16.

4.4.4 Soil chemical properties

4.4.4.1 Soil carbon

In Simbhanjyang, at both elevations, i.e., 1000 m and 2000 m asl, soil organic carbon was higher in invaded sites in comparison to non-invaded sites (Figure 28a). However, in Chandragiri, soil organic carbon did not differ between invaded and non-invaded sites at two elevations i.e., 1600 m and 2400 m asl (Figure 28b).

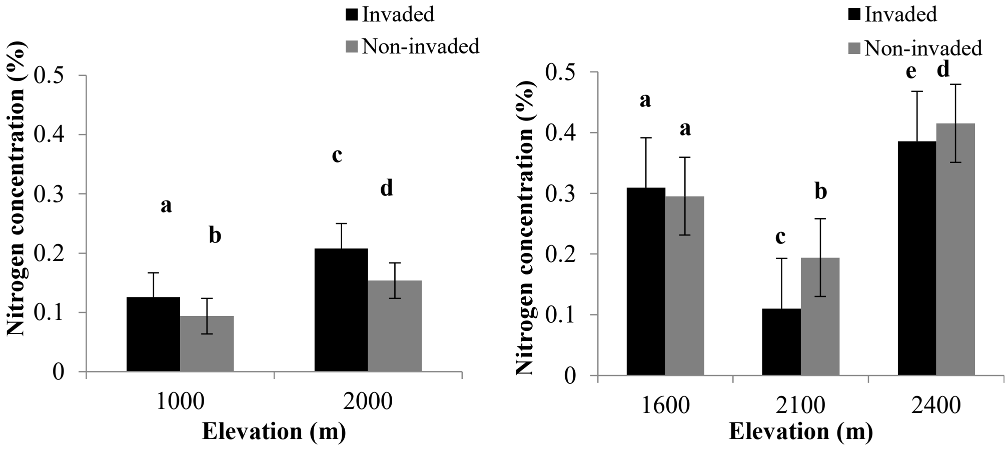


(a) (b)

Figure 28: Mean carbon concentration of invaded and non-invaded sites at different elevations in a. Simbhajyang and b. Chandragiri. Different letters above bars indicate statistically significant difference between invaded and non-invaded sites at P<0.005 according to paired t test.

4.4.4.2 Soil nitrogen

In Simbhanjyang, at both elevations, i.e., 1000 m and 2000 m asl, nitrogen concentration was higher in invaded site in comparison to non-invaded site (Figure 29a). Except the sites at the lowest elevation (1600 m asl), nitrogen concentration differed between the invaded and non-invaded sites in Chandragiri (Figure 29b).

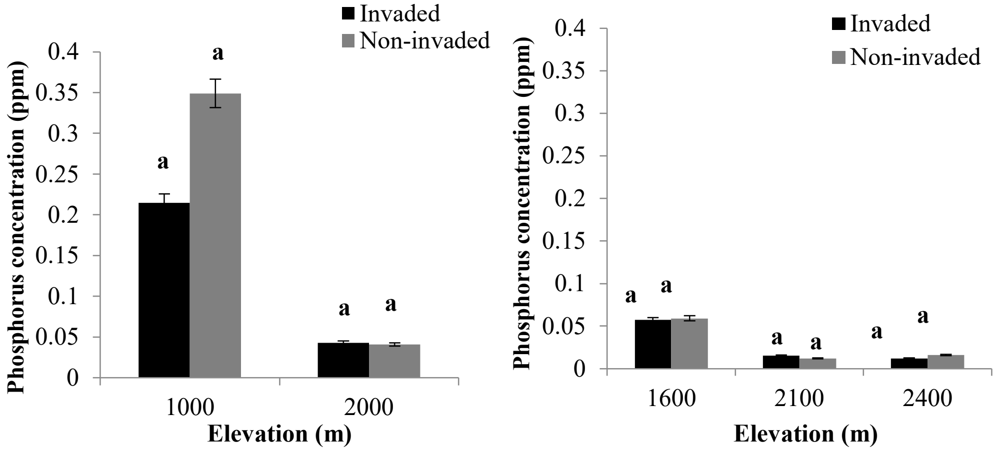


(a) (b)

Figure 29: Mean nitrogen concentration of invaded and non-invaded sites at different elevations in a. Simbhajyang and b. Chandragiri. Different letters above bars indicate statistically significant difference between invaded and non-invaded sites at P<0.001 according to paired t test.

4.4.4.3 Soil phosphorus

In Simbhanjyang as well as Chandragiri, the soil phosphorus did not differ between the invaded and non-invaded sites in both the elevations (Figure 30a & 30b).

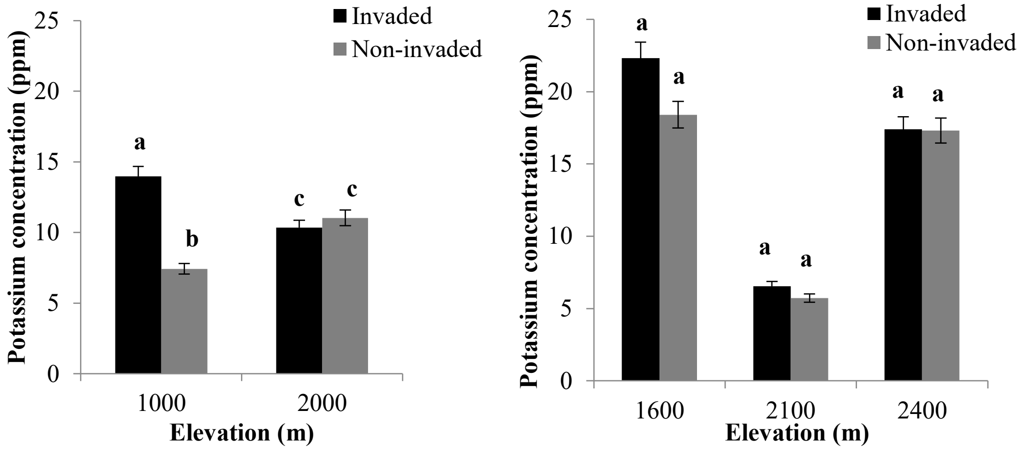


(a) (b)

Figure 30: Mean phosphorus concentration at different elevations in invaded and non-invaded sites in a. Simbhanjyang b. Chandragiri. Different letters above bars indicate statistically significant difference between invaded and non-invaded sites at P<0.005 according to paired t test.

4.4.4.4 Soil potassium

In Simbhanjyang, soil potassium was higher in invaded site in comparison to non-invaded site at elevation 1000 m asl whereas was the same for elevation 2000 m asl (Figure 31a). However, in Chandragiri, soil potassium did not differ between invaded and non-invaded sites in all the elevation ranges (Figure 31b).



(a) (b)

Figure 31: Mean potassium concentration in invaded and non-invaded sites of different elevation range in a. Simbhanjyang and b. Chandragiri. Different letters above bars indicate statistically significant difference between invaded and non-invaded sites at P<0.005 according to paired t test

4.5 Stem galling of *A. adenophora* by *P. utilis* along an elevation gradient in central Nepal

4.5.1 Elevation effects on *P. utilis* gall abundance

The relationship between elevation and gall abundance (n = 48) was unimodal (R^2 = 0.78, P < 0.001; Figure 32). Elevation explained about 78% of the variance in frequency of galls. The frequency of galls per 100 ramets were the highest in plots between 1940–2000 m asl, which is close to the mid-elevation of the entire elevation range of the distribution of *A. adenophora* (240-2965 m asl) in Nepal.

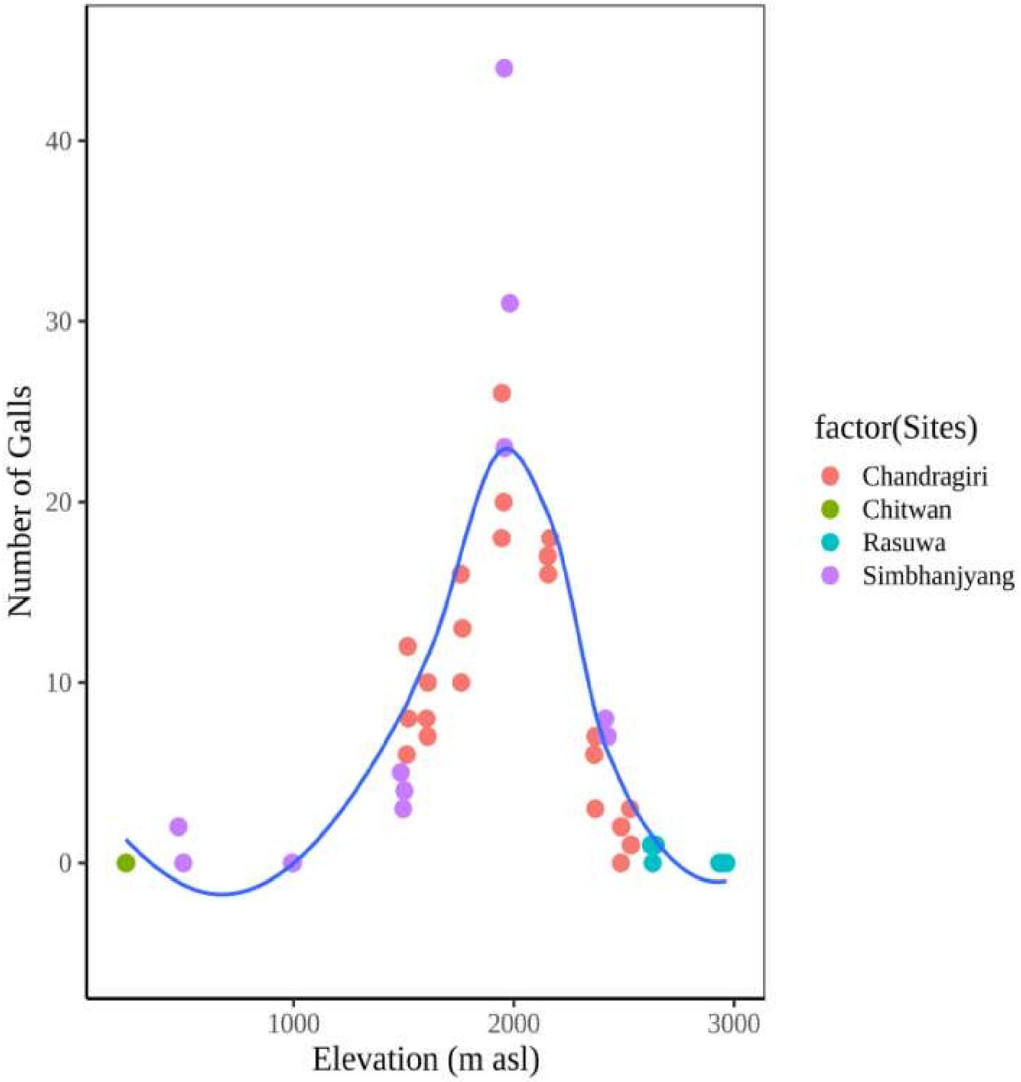
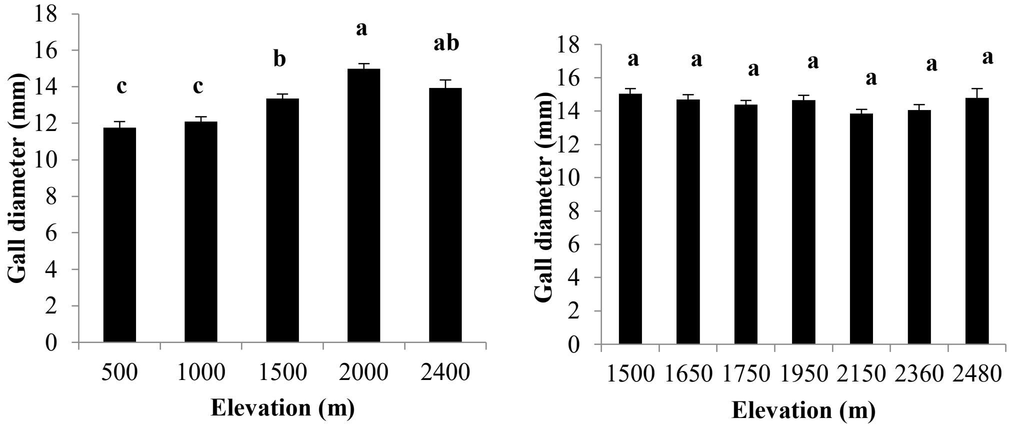


Figure 32: Gall abundance along the elevation gradient in central Nepal

4.5.2 Elevation effects on *P. utilis* gall size

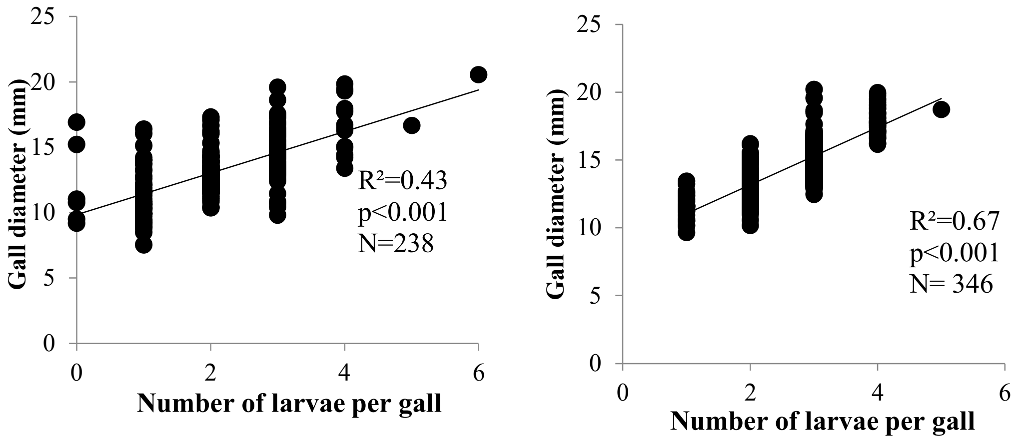
The mean gall diameter in Simbhanjyang was 13.33±2.38 mm whereas it was 14.50±2.14 mm in Chandragiri. Change in gall diameter with elevation was different between the two sites with wide (i.e., Simbhanjyang) and narrow (i.e., Chandragiri) elevation gradients. The gall diameter increased with the increasing elevation up to 2000 m asl and then decreased after that in Simbhanjyang ($F = 57.31$, $P < 0.001$), where the elevation difference between the lowest and the highest sites was 1920 m asl (Figure 33a). However, in Chandragiri with narrow elevation difference (980 m asl), the gall diameter did not vary significantly along the elevation gradient ($F = 5.134$, $P = 0.07$; Figure 33b).



(a) (b)

Figure 33: Mean gall diameter along the elevation gradient in two sites in central Nepal a. Simbhanjyang and b. Panchase. Different letters above the bar denotes the significant differences among the samples from different elevation belts ($P<0.001$, Tukey HSD test)

The number of larvae per gall in the study area ranged from one to six. There was a significant positive relationship between number of larvae per gall and gall diameter ($R^2 = 0.43$, $P < 0.001$ (Simbhanjyang); $R^2 = 0.67$, $P < 0.001$ (Chandragiri); Figure 34a & 34b). Also, gall diameter was significantly positively correlated with stem diameter ($r = 0.33$, $P < 0.001$ (Simbhanjyang); $r = 0.32$, $P < 0.001$ (Chandragiri); Appendix 18). At both sites, gall diameter increased with the increasing number of larvae per gall (Figure 34a & 34b).

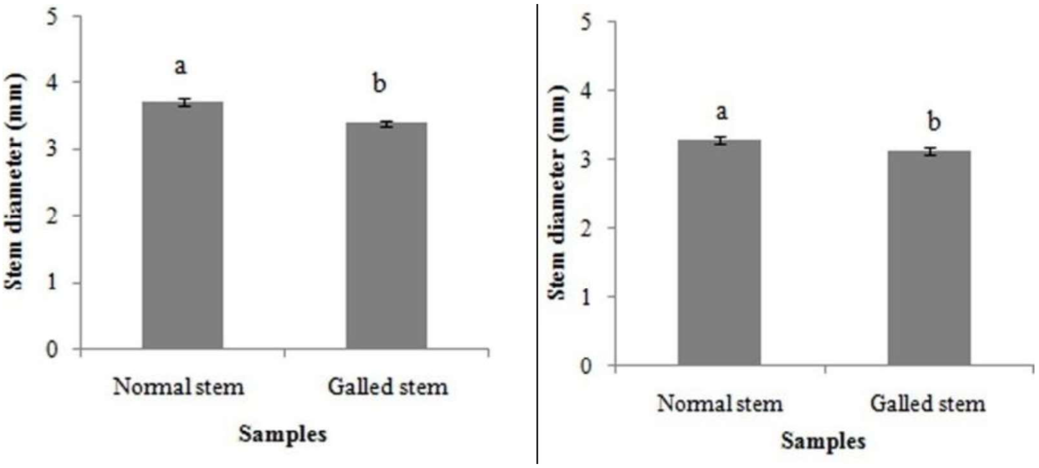


(a) (b)

Figure 34: Relationship between number of larvae per gall and gall diameter in two sites in central Nepal a. Simbhanjyang and b. Chandragiri

4.5.3 Effect of gall on stem diameter

The mean diameter of galled stems was significantly lower than stems without galls (χ^2 = 7.4, d.f. = 1, P<0.05 (Simbhanjyang); χ^2 = 24.2, d.f. = 1, P<0.001 (Chandragiri); Figure 35a & 35b) in both the sampling sites.

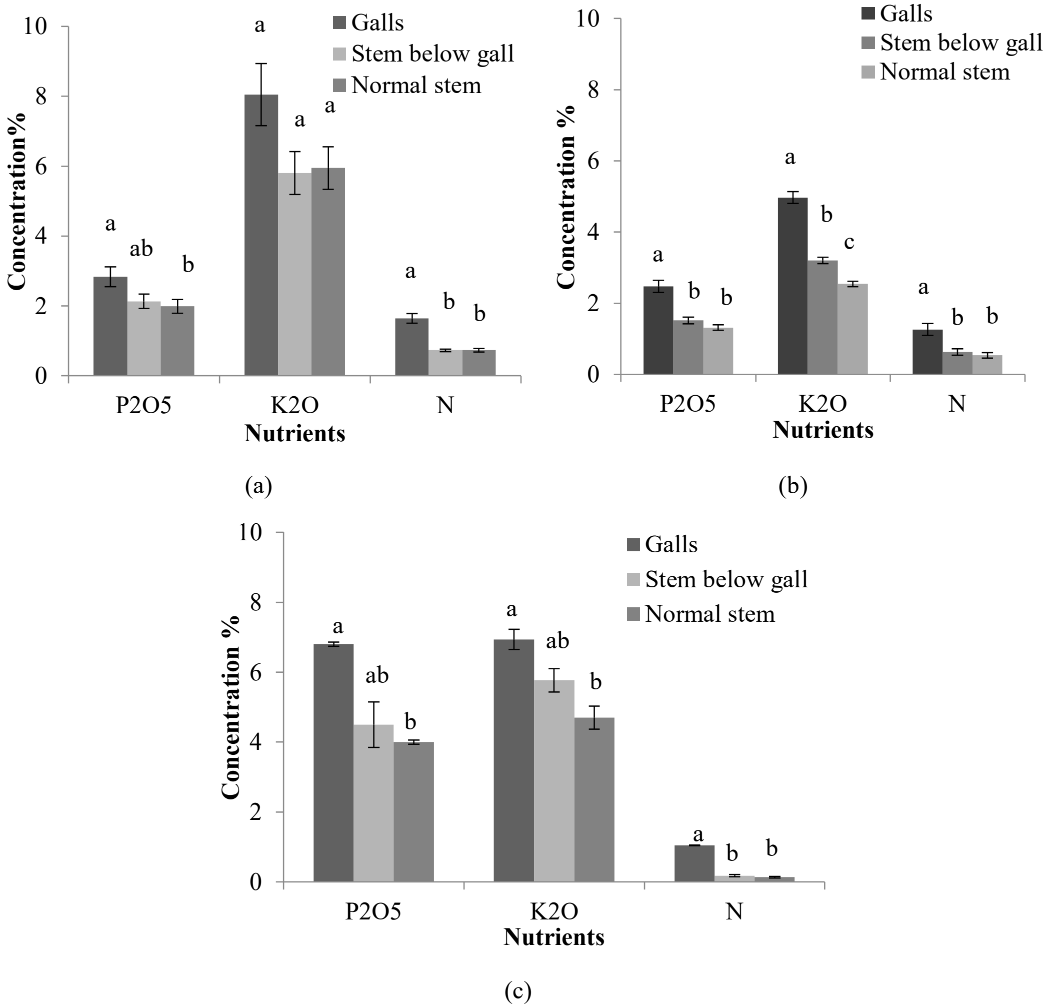


(a) (b)

Figure 35: Diameter (mean ± s.e) of normal stem and galled stem in two sites of central Nepal a. Simbhanjyang and b. Chandragiri

4.5.4 Nutrient concentrations

In Simbhanjyang, nitrogen concentration was higher in gall samples than in stems below galls and normal stems without galls ($F_{2,42}$ = 61.69, P<0.001) whereas phosphorus concentration was significantly higher in gall samples than in normal stems without galls ($F_{2,42} = 3.958$, $P<0.001$). However, potassium concentration did not differ among the samples ($F_{2,42} = 2.597$, $P = 0.0864$; Figure 36a). In contrast, all three nutrients (N, P_2O_5 and K_2O) were significantly higher in galls than in stem below galls and normal stem without galls in Chandragiri ($\chi^2 = 40.913$, d.f = 2, $P<0.001$ (N): $\chi^2 = 33.811$, d.f = 2, $P<0.001$ (P_2O_5); $\chi2 = 37.375$, d.f = 2, $P<0.001$(K_2O); Figure 36b). In Rasuwa (the highest elevation), though all the three nutrients were significantly higher in galls in comparison to normal stem ($F_{2,6} = 483.7$, $P<0.001$ (N); $F_{2,6} = 15.56$, $P<0.001$ (P_2O_5); $F_{2,6} = 12.42$, $P<0.001$ (K_2O); Figure 36c), the potassium concentration in gall samples did not differ with that of stem below gall but the difference was significant for nitrogen and phosphorus. All three nutrients, i.e., nitrogen, phosphorus and potassium were significantly positively correlated with each other in both sites (Table 16).



(a) (b)

(c)

Figure 36: Mean nutrient concentrations in galls, stem below galls and normal stem in a. Simbhanjyang, b. Chandragiri and c. Rasuwa

Table 16: Correlation between mineral nutrients

Gradients		Nutrients	
Chandragiri		P_2O_5	K_2O
	K_2O	0.54***	
	N	0.6***	0.64***
Simbhanjyang		P_2O_5	K_2O
	K_2O	0.73***	
	N	0.44***	0.41***

Note: * denotes significant difference at $P<0.05$

CHAPTER 5

5. DISCUSSION

5.1 Ecological niche modeling

This study predicted the current and future suitable habitat of *A. adenophora* in Chitwan-Annapurna Landscape, Nepal using Maxent software. The model evaluation parameters obtained for the models, i.e., AUC and TSS both lied within the range that confirms the robustness of these built models (Appendix 10). AUC values above 0.8, TSS values closer to one are considered to be acceptable for the models to be considered robust (Swets, 1988; Manel *et al.*, 2001; Allouche *et al.,* 2006). Though this study did not include a model transferability assessment, this would provide a valuable information for model validation as well as model selection (Wenger & Olden, 2012).

Climatic factors like temperature and precipitation play pivotal role in determining the pathways and success of plant invasion (Kathiresan & Gualbert, 2016; Wang *et al.,* 2017). The predictions of this study suggest that the minimum temperature in the coldest month is the most important climatic factor in determining the distribution of *A. adenophora*. This concurs with the finding of other researchers (Wang & Wang, 2006; Zhu *et al.,* 2007; Wang *et al.,* 2017; Lamsal *et al.,* 2018; Thapa *et al.,* 2018; Datta *et al.,* 2019). According to the model of this study, the minimum temperature in the coldest month alone accounts for about 49% contribution to the habitat suitability model (Table 5) and the probability of occurrence of the weed decreased almost to zero beyond the minimum winter temperature of 1°C (Appendix 11). This indicates that the distribution of the weed is constrained by the lower minimum winter temperature which is similar to that of the observations in Indian Himalaya where the low temperature in winter limits the uppermost distribution range of *A. adenophora* (Datta *et al.,* 2017).

Ecological niche models built in this study was based on the occurrence data of invaded regions only which indicates that the potential niche which is estimated is a part of the fundamental niche of the species (Elith & Leathwick, 2009; Soberon & Nakamura, 2009). Moreover, factors such as biotic interactions and dispersal limitations also restrict the species from occupying its full potential niche (Soberon & Nakamura, 2009). Thus, use of occurrence data from both native and invaded ranges would provide a more accurate estimate of the potential niche of the species (Jimenez-Valverde *et al.,* 2011; Kumar *et al.,* 2015).

In the current climatic condition as well as in future climate scenarios, Middle Mountain is found to be climatically more suitable for *A. adenophora* in comparison to other physiographic regions. The elevation of Middle Mountain ranges from 1000 m to 2500 m asl (DHM, 2017) which lies within the suitable range for the distribution of this weed (Wang & Wang, 2006; Zhu *et al.,* 2007). A recent study which modeled the distribution of 24 invasive alien plants in Nepal, also identified the Middle Mountain region as having greater area of invasion hotspots, with suitable areas for the largest number of species studied (Shrestha & Shrestha, 2019). Displacement of native species such as *Artemisia indica* and *Urtica dioica*, and reduction of the ground vegetation layer of *Digitaria* sp., *Eragostris* sp., and *Imperata cylindrica* by *A. adenophora* have already been observed in the Middle Mountain region (Tiwari *et al.,* 2005; Baral *et al.,* 2017). Therefore, this region requires an urgent attention of policy makers and land resource managers to implement effective management plans to prevent further spread of this weed. In contrast, High Himalaya is unsuitable for the weed because this region has a low minimum winter temperature (−10°C to 5°C) and low annual precipitation (400-1000 mm) (DHM, 2017) which limits the distribution of the weed (Datta *et al.,* 2017). Most of the current areas predicted to be suitable for *A. adenophora* were found along road verges and river networks, which is in accordance with the study conducted in China (Wang & Wang, 2006). These river and road networks enhance the rapid spread of *A. adenophora* (Dong *et al.,* 2008; Sang *et al.,* 2010). Therefore, control and monitoring efforts for the management of this weed should be focused on areas near roads and rivers.

There is increasing evidence that climate change is likely to increase risk of plant invasions creating more suitable areas in future (Bradley *et al.,* 2010). The projections of this study also showed increase in climatically suitable area of *A. adenophora* in future. The weed is going to gain suitable areas in two future climate scenarios, i.e., RCP 2.6 (2050) and RCP 4.5 (2050 and 2070). A recent study also views that climatically suitable niches of *A. adenophora* would expand by 5.3% in future climatic condition (RCP 6.0) in Nepal (Shrestha & Shrestha, 2019). Studies in other parts of the world also have predicted the expansion of climatically suitable areas of *A. adenophora* in future climate scenarios (Wang & Wang, 2006; Zhu *et al.,* 2007; Wang *et al.,* 2017; Lamsal *et al.,* 2018; Thapa *et al.,* 2018). However, a study conducted in Bhutan predicted a contraction of suitable areas of *A. adenophora* by 0.22% in 2050 (Thiney *et al.,* 2019). Similarly, a recent global study also predicted that the potential suitable areas of *A. adenphora* would shrink globally under climate change scenarios but would increase in six biodiversity hotspot regions (Changjun *et al.,* 2021). An experimental warming study conducted in China stated that warming (2°C rise) increased biomass allocation and canopy cover of the weed making it more stress tolerant (He *et al.,* 2012). Similarly, CO_2 enrichment also increased the relative growth rate and biomass allocation of the weed (Lei *et al.,* 2012). Thus, increase in suitable habitats in future climate scenarios with increased temperature and CO_2 concentration can be attributed to the innate and evolutionary increased ecophysiological tolerances of *A. adenophora* favouring its growth (Blossey & Notzold, 1995; He *et al.,* 2012; Lei *et al.,* 2012). Though, under extreme climate scenarios (RCP 8.5), a loss in total climatically suitable areas is predicted, the weed will still thrive to gain suitable areas in protected areas and physiographic regions. Districts like Lamjung, Gorkha, Dhading, Makwanpur, Chitwan and Tanahun are predicted to be vulnerable to further invasion by the weed due to climate change, so it is crucial to implement scientifically informed site-specific management policies, with the participation of local communities.

Despite, being an apomicitc plant having evolutionary constraints, niche expansion has been noticed in *A. adenophora* (Datta *et al.,* 2019). In addition, the weed is found to exhibit phenotypic plasticity that assist the weed to occupy broader climatic niche (Zhao *et al.,* 2012). The future climate models also demonstrate the expansion of upper elevational distribution limit of the weed. This result of this study also coincides with that of the findings in Western Himalaya where the weed was predicted to expand by 981 m in upper elevation compared to that of current climatic condition (Thapa *et al.,* 2018). This indicates that the weed will spread towards cooler and drier regions in future. Similar trend was noticed in the study carried out to observe spatio-temporal patterns in China (Zhu *et al.,* 2007). Increased cold tolerance due to epigenetic modifications might help the weed in future to gain more suitable habitats in cooler and drier places at high elevation (Xie *et al.,* 2015). With the climate change, all three protected areas will gain areas of potential suitable habitat. Though the weed has already been identified as the most problematic weed in Annapurna Conservation Area impacting the native diversity and livelihood of people (Thapa & Maharjan, 2014), no such studies has been carried out in Langtang National Park and Manaslu Conservation Area. Upward movement and colonization of *A. adenophora* due to recent climate changes have already been observed in Langtang National Park (Lamsal *et al.,* 2017). The ecological niche models also predicted that Langtang National Park has highest proportion of climatically suitable areas in current climatic condition and continues to remain same in future climate scenarios. A range shift of *A. adenophora* might threaten the habitat of two endangered animals, *Aliurus fulgens* (red panda) and *Moschus chrysogaster* (Himalayan musk deer), in Langtang National Park (Lamsal *et al.,* 2017). Thus, this information will act as a prompt for land managers, scientific community, conservationists as well as policy makers to adapt precautionary measures and formulate effective policies for preventing the further spread of this weed in the novel regions (Poudel *et al.,* 2020a).

Taking climatic and topographic factors into consideration, niche model predicted the elevational range expansion, as well as an increase in suitable areas in future climate scenarios. However, other factors, such as biotic interactions, dispersal ability, demography, evolution, adaptation, and land use change, also play key roles in determining the species range shift with climate change (Sinclair *et al.,* 2010; Urban *et al.,* 2016). Furthermore, long term temporal predictions in climate change scenarios area associated with two other main errors. The first is the extrapolation of data beyond training range to non-analogue environmental conditions (climate change scenarios), which might make predictions less reliable (Fitzpatrick & Hargrove 2009). Another risk is that as the relationship between climatic variables could change with time, the correlation structure of current and future climate variables might also change, thus reducing the certainty of models (Dormann *et al.,* 2013). To overcome these uncertainties and achieve more realistic predictions of species distribution, all factors, abiotic and biotic, that shape the distribution of invasive species should be incorporated in the species distribution models (Gonzalez-Salazar *et al.,* 2013; Leach *et al.,* 2016). Nevertheless, ENMs provide predictive information on species distribution required by vegetation managers and conservation practitioners for developing effective strategies to prevent further invasion (Peterson, 2003). Thus, this study provides useful information about the current distribution of *A. adenophora* and identify areas that may be at risk in the future on a local scale, demonstrating the urgent need for formulating effective management strategies to mitigate the impact of the weed.

5.2 Spatio-temporal distribution of *Ageratina adenophora* in CHAL

In initial phases of invasion (1990), *Ageratina adenophora* population was found to be in small patches mostly confined towards eastern region of CHAL. This was expected because the invasion of this weed began from eastern Nepal from where it spread towards western Nepal (Sharma & Chhettri, 1977; Tiwari *et al.,* 2005). Within a time span of 26 years, *A. adenophora* has now become widespread in central and western region of CHAL. There was substantial increase in the area invaded by *A. adenophora* between 1990 and 2018. The percentage coverage of *A. adenophora* in CHAL increased from 0.22% in 1990 to 4.55% in 2018. A study in China which analysed the invasion patterns of *A. adenophora* for a period of 60 years (1960-2003) reported that *A. adenophora* spread at a rate of 20 km per year from 1960-1990 and after 1990 the average expansion rate was 13.2 km per year (Wang and Wang, 2006). Spatial patterns of *A. adenophora* may have a foundation in the historical and current landuse. An analysis of land use/land cover change in CHAL from 1990-2010 reported that there was substantial loss of forest area in Middle Mountains to infrastructure development, resettlement, urban expansion and agricultural expansion (WWF, 2013) which might have facilitated the invasion spread of *A. adenophora*. Frequent landscape fragmentation, environmental disturbances and anthropogenic activities along roadsides have facilitated the propagation of invasive plants in forest fragments (Ohlemuller *et al.,* 2008; Waddell *et al.,* 2020). In addition, river and road networks are also found to enhance the spread of *A. adenophora* being the main dispersal pathway (Dong *et al.,* 2008; Sang *et al.,* 2010).

In current condition, the weed is found to be more abundant in Middle Mountains. In China, within the elevation range of 800-2500 m asl, the weed was more abundant (Wang & Wang, 2006). Furthermore, a recent study in Nepal also identified Middle Mountains having more suitable area for most of the invasive species (Shrestha & Shrestha, 2019). Infrastructure development, increasing abandoned land in midhills have made the region vulnerable to invasion.

Comparing the results of Landsat and Worldview-2 imageries using accuracy assessment, better accuracy of Worldview-2 images having high spatial resolution was evident. Similar result was obtained in other researches (Sankey *et al.,* 2014; Stych *et al.,* 2019). Sankey *et al.* (2014) reported an overall accuracy of 88% in Worldview-2 image which dropped by about 20% in Landsat image. Furthermore, while comparing the Worldview-2 and Landsat 8 images for the classification of forests affected by bark beetle outbreak, Stych *et al.,* (2019) also reported an overall accuracy of 86% in Worldview-2 image which dropped by 15% in Landsat 8 images. Though, multispectral sensors have high accuracy in comparison to medium spatial resolution sensors, their application is limited to small areas only (Matongera *et al.,* 2017). In addition, they are expensive to acquire and also have small swath width so, freely available images like Landsat have been frequently used that has potential to detect the species over space and time (Resasco *et al.*, 2007; Bishop *et al.,* 2019). Therefore, methods using freely available sensors in remote sensing that can also detect the invasion pattern in date back time is highly recommendable to get an overview of the spread of invasive species.

5.3 Plant functional traits

Functional traits provide ecological insight in the functioning of the species. In this study, the mean value of SLA in the study site ranged from 217 ± 67to 337 ± 86 cm^2/g which is close to value at the lower range reported by Wright *et al.* (2004) in a study of 2548 species. Similarly, LNC in our study ranged from 1.64% to 3.24% which lies within the mid-range of that reported by Wright *et al.* (2004). The mean value of SLA and LNC ranged from 6.667 to 714.285 cm^2/g and 0.2 to 6.4% respectively in a study of 2548 species (Wright *et al.,* 2004).

Many studies have compared the leaf traits between native and invasive species and have postulated that in comparison to natives, invasive species have higher SLA and LNC (van Kleunen *et al.,* 2010; Gallagher *et al.,* 2014). Invasiveness is promoted by higher values in trait hierarchy; trait means are more important than plasticity (Godoy *et al.,* 2012). However, it is not always necessary to have higher values for invasive species. In comparison to native *Eupatorium japonicum*, *A. adenophora* had lower SLA but higher photosynthetic rate and high LNC (Feng, 2008). Though this study did not compare the functional traits with other native species, such comparison would further help in generalizing the idea for the invasion success of *A. adenophora.*

Intraspecific variation emerges as a fundamental component of functional trait structure along wide environmental gradients (Vila-Cabrera *et al.,* 2015). SLA decreased with increasing elevation along the wide elevation gradient in this study. Recent metadata analysis carried out by Midolo *et al.* (2019) that includes 109 species also reported a decrease in SLA with increasing elevational difference. It has been suggested that low temperature limits cell expansion which leads to a large number of small cells per unit area and more cell wall and cell layers per unit area which results in decrease in SLA (Atkin *et al.,* 2006; Poorter *et al.,* 2008). Though weak positive correlation was reported between SLA and temperature (Rosbakh *et al.,* 2015), besides temperature, other environmental factors also play an important role in trait variation. LNC content decreased with the increase in elevation along elevation gradient in both the sites in our study which is similar to the previous researches (Shi *et al.,* 2012; Zhao *et al.,* 2016). Low leaf nitrogen may be due to the effect of low temperature on plant physiology or soil biogeochemistry (Reich & Oleksyn, 2004). Low temperature reduces the rates of decomposition and mineralization of organic matter resulting in low soil nitrogen availability (Reich & Oleksyn, 2004). Furthermore, low temperature may also limit root nutrient uptake which ultimately results in low leaf nitrogen (Reich & Oleksyn, 2004). Thus, reduced metabolic rate of plants and limitation of nutrient availability due to low temperature might have resulted in low LNC at high elevations. However, from the study of 1900 plant species across China, Han *et al.* (2011) suggested that precipitation explained more variation in LNC than temperature. LNC decreased with the increasing SLA in this study. Similar inverse relationship between SLA and LNC was reported in annual grasses growing in Mediterranean climate in southern France (Garnier *et al.,* 1997).

Populations with shorter growing seasons flower earlier ensuring the sufficient time required for development of the seed (Winn & Gross, 1993). In the same way, long growing season ensures vegetative growth of the plant to enable them for more seed production. It was found that the number of floral heads per ramet and seed output decreased with the increasing elevation in the study site. Similar decreasing trend was observed in a perennial plant, *Solidago macrophylla* (Asteraceae) along the elevation gradient (800-1550m) in New Hampshire, USA (Kelly, 1998). The lower number of floral heads with the increasing elevation might be explained as a response to harsher growing condition and seasons at high elevation. On contrary, seed size increased with the increasing elevation which was consistent with Li & Feng (2009) who also observed larger sized seeds of *A. adenophora* at higher elevations in China. Similar result was obtained in the study carried out in China (Li & Feng, 2009). Larger-sized seeds at higher elevations have been also reported in another invasive weed *Centaurea solsitialis* (Hierro *et al.,* 2019). Similar to the seed size, seed mass also increased with the increasing elevation. Increased seed size and seed mass improves plant fitness under unfavourable growing conditions (Westoby *et al.,* 2002; Moles & Westoby, 2004). Thus, large seeds with high nutrient storage could be critical for *A. adenophora* to cope with the stressful environment at higher elevations mainly during the earlier stages of seedling establishment stages. However, other researches have reported a negative relationship between seed size or seed mass and elevation (Guo *et al.,* 2010; Wang *et al.,* 2014). The decreasing seed number and increasing seed size as well as seed mass along the elevation gradient noted in this study suggests a probability of tradeoff between seed size and seed number or seed mass. Such tradeoff between seed size and seed mass has been reported in other species as well (Alexander *et al.*, 2009; Olejnicjak *et al.,* 2018). Seed mass is positively associated with seedling survival (Daws *et al.,* 2005). Thus, the increase in seed size or seed mass at the cost of seed number might have helped *A. adenophora* to withstand harsh environmental condition at upper elevations at its early stage of seedling establishment. However, the current sampling at the uppermost does not represent the weed's highest distribution limit in Nepal and the Himalaya. Therefore, further study considering uppermost elevational range could answer whether or not the trade-off continues between seed size-number-mass.

Previous researches have reported that the weed exhibited strong phenotypic plasticity to salinity (light intensity), phosphorus, latitude as well as elevation (Feng *et al.,* 2007; Feng, 2008; Zhao *et al.,* 2012). Phenotypic variability was observed in the traits of *A. adenophora* along the elevational gradient in this study as well but the probability of genetic or local adaptation can't be ruled out. For determining local adaptation, reciprocal transplant experiment is one of the useful tools (Zhao *et al.,* 2012). Therefore, further research using this tool might generate valuable information for explaining the invasion success of *A. adenophora.* Therefore, these results suggest that phenotypic plasticity might have helped the *A. adenophora* to occupy wide elevational range exposed to different climatic environments.

5.4 Impact on vegetation and soil characteristics

The results of our study demonstrate a strong negative impact of *A. adenophora* invasion on species richness, species diversity and species composition. *A. adenophora* produces large number of light weight seeds with high adaptability (Fu *et al.,* 2018) that enables it to grow vigorously and spread rapidly suppressing the native species. In addition, phytotoxic chemicals exudated by the roots, stem and leaves of *A. adenophora* also inhibit the growth of native plant species (Fu *et al.,* 2018; Poudel *et al.,* 2019; Thapa *et al.,* 2020). Furthermore, longer vegetative growth phase, higher above/belowground ratio, higher biomass accumulation, and cover than native species as reported by Gao *et al.* (2013) might also have assisted the weed to develop monodominant invaded stands reducing the species richness and species diversity. Studies from other countries have also reported the decrease in native species cover, density, richness and diversity due to invasion by *A. adenophora* (Ding *et al.,* 2007; Dogra *et al.,* 2010; Gao *et al.,* 2013; Fu *et al.,* 2018; Wu *et al.,* 2020). Furthermore, these findings are also consistent with studies on other invasive species which indicate strong effects of invasive species on different ecosystem properties (Ehrenfeld, 2003; Levine *et al.*, 2003; Timsina *et al.,* 2011; Ruwanza, 2020).

The results demonstrate large differences in the vegetation composition of invaded and non-invaded sites. The strong negative impact observed on species diversity and composition could be explained by the fact that *A. adenophora* grows so fast and spreads easily as seeds are transported by vectors like wind, water and animals. The seeds easily establish in open and disturbed areas. Three main factors are responsible for the rapid spread and invasion of *A. adenophora*, i.e., allelopathic effect, phenotypic plasticity and invasion-mediated changes in soil microbial community (Poudel *et al.,* 2019). At the present study sites, *A. adenophora* replaced species of genus like *Desmodium*, *Indigofera, Cyanotis* and grasses like *Cyperus* and *Pycreus*. Other studies have also noticed the replacement of native species like *Artimisia indica*, *Solanum surratense*, *Urtica dioica* (Baral *et al.,* 2017).

The result showed variation in different soil nutrients in invaded and non-invaded sites along the elevation gradient at two sites. Soil carbon and soil nitrogen was higher in invaded plots in comparison to non-invaded plots in both sites which coincides with that of the study by Niu *et al.* (2007). This can be attributed to the higher amount of above ground biomass of *A. adenophora* in invaded plots in comparison to the non-invaded plots. *A. adenophora* in invaded plots was taller than the native species in the non-invaded plots which might have led to higher soil organic carbon and nitrogen in invaded plots. Furthermore, as *A. adenophora* secretes high amount of water soluble phytotoxins (Poudel *et al.,* 2019), exudation of these phytochemicals might cause soil organisms to die adding more nitrate to the soil (Rizvi & Rizvi, 1992). While, soil potassium differed at one elevation belt of one site i.e., Simbhanjyang, no difference in soil phosphorus concentration was noticed at both the sites. This result contrasted with that of Niu *et al*. (2007) who observed an increase in soil nitrate, soil ammonia, available phosphorus and potassium in *A. adenophora* invaded soil. Wu *et al*. (2020) also reported an increase in soil $NaHCO_3$-extractable inorganic P and organic P after *A. adenohora* invasion. This variation in impact of *A. adenophora* in two sites may be attributed to difference in habitat condition as impact of invasive alien species are found to vary with spatial scale, ecological complexity and habitat condition (Vila *et al.,* 2011; Nijs *et al.,* 2012; Kunzi *et al.,* 2015). Furthermore, soil nutrient concentration increases when the site is initially in low nutrient condition whereas opposite trend is noticed in the site initially with high nutrient (Dassonville *et al.,* 2008). Change in soil nutrient concentration is attributed to the change in soil microbial community (Hawkes *et al.,* 2005). *A. adenophora* is reported to alter soil microbial community favoring its invasion (Niu *et al.,* 2007; Li *et al.,* 2009). Therefore, soil nutrient change might be the result of soil microbe's alteration. However, further research studying the relationship between soil microbial community alteration and nutrient composition is desirable. This result highlights the most important impact of onsite variation of nutrient in soils of invaded and noninvaded plots. In addition, the same species may have differential impacts depending upon local conditions.

5.5 Stem galling by a biocontrol agent *Procecidochares utilis*

Elevation significantly affects gall abundance, with the highest gall abundance at a mid-elevation range of 1940-2000 m asl along the elevation gradient in central Nepal (240-2965 m asl) exhibiting a unimodal pattern. This result concurred with those reported for dung beetles (Escobar *et al.*, 2005), *Palgiometriona* species (Flinte *et al.*, 2011), and the rosette-galling midge (Crutsinger *et al.*, 2013). The hump-shaped pattern might be explained by the "Abundant-center hypothesis," which proposes that species abundance is the highest at the center of its range where the most favorable biotic and abiotic conditions occur, and gradually decline toward the edges (Hengeveld & Hack, 1982; Brown, 1984). However, these assumptions have been frequently questioned because there are complex biogeographical processes like interspecific interactions, changing environmental conditions, temporal variation in abundance as well as life history traits of species acting together that determine the spatial abundance of the species along the environmental gradients (Dallas *et al.*, 2017; Santini *et al.*, 2019). Nevertheless, these studies did not include gall inducers, therefore, we can say that the pattern of distribution of species abundance may be contextual and differ with taxa. The survey of distribution pattern of *A. adenophora* along the elevation gradient of 300-2500 m asl in western Himalaya also revealed a unimodal pattern of distribution, its probability of occurrence peaking at 1320 m asl (Datta *et al.*, 2017). Although our study did not take weed abundance into account, previous research suggests that host plant abundance also influences the gall abundance (Boaventura *et al.*, 2018).

The largest mean gall diameter (15.05 ± 0.29 mm) measured in the present study is similar to that observed in South Africa (15.05 mm, Bennett & Van Staden, 1986). We found that gall diameter varied with elevation at a site with wide elevation gradient (Simbhanjyang) and it was the highest at the mid-elevation. This is consistent with previous works (Hartmann, 1984; Smith *et al.*, 2011). Marchosky and Craig (2004) also reported a strong environmental influence on size of gall. The tritrophic interaction between plant genotype, insect genotype and the environment determine the gall size of gall inducers (Weis & Abrahamson, 1986; Weis & Gormon, 1990). Variation in abiotic factors like temperature, humidity, soil factors etc. along the elevation gradient act as bottom-up forces that cascade upward, creating differences in the evolution of interaction of plants, galling insects, and their natural enemies (Craig *et al.*, 2007; Rasmann *et al.*, 2014). These differences created in interaction of multitrophic levels might have led to the variation in gall size. Furthermore, the largest gall diameter was found in mid-elevation range. As gall abundance also peaked at mid-elevation, this suggests that mid elevation sites may have an optimal condition for the growth of *P. utilis*. Increase in gall diameter with the increasing number of larvae per gall has been also reported previously (Marchosky & Craig, 2004; Tabuchi & Amano, 2004). Furthermore, while gall size is positively related to galling insect survival and fitness as well (Sopow & Quiring, 2001; Marchosky & Craig, 2004), we can assume that larvae of *P. utilis* might have the greatest fitness at mid-elevation.

Galling by *P. utilis* reduces the vegetative and reproductive growth of *A. adenophora* (Erasmus *et al.*, 1992; Buccellato *et al.*, 2012, 2019). We also found that stem diameter, a growth-related variable, was affected negatively by the gallfly. Gall development alters the sink-source relationship in their host plants which can reduce the photosynthetic capacity in remaining ungalled tissues placing overall metabolism of the host plant in stress (Raman & Abrahamson, 1995; Florentine *et al.,* 2005). Reduction in stem diameter from galling has also been reported in other plants like *Solidago altissima* L. (Asteraceae) by *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae) (Civettini *et al.*, 1999), *Milicia excelsa* (Welw) C.C. Berg (Moraceae) by *Phytolyma lata* Scott. (Hemiptera: Psylidae) (Iroko gall fly) (Agyeman *et al.*, 2009), *Genista monspessulana* L. (Fabaceae) (invasive French broom) by *Lepidapion argentatum* Gerstaecker (Coleoptera) (weevil) (Bitume *et al.*, 2019).

The innermost layer of the galls formed by *P. utilis* is transformed into a layer of nutritive tissue containing six to seven layers of cells with dense cytoplasm, large nuclei, and is highly mitotic (Bennett & Van Staden, 1986). Results indicate that *P. utilis* increases the amount of all three nutrient concentrations (N, P and K) inside galls in comparison to stem below gall and normal stem without gall. This implies that galls acted as mobilizing sinks by drawing nutrients from other parts of the host plant as reported by previous studies (McCrea *et al.*, 1985; Larsom & Witham, 1991; Marini-Filho & Fernandes, 2012). Success of a biocontrol agent largely depends upon its ability to harm the host plant, creating a nutrient sink (Harris & Shorthouse, 1996). This explains the potential of *P. utilis* for its detrimental effects on host plants. In addition, van Staden and Benett (1991), through radiochemical experiments, have already demonstrated that galls induced by *P. utilis* act as strong sinks and are able to manipulate photoassimilates. Furthermore, our results corroborate the nutrition hypothesis which states that gall tissues represent a higher nutritional food source than plant tissues without gall (Price *et al.*, 1986, 1987).

Nitrogen is one of the limiting nutrients that plays a vital role in growth, development, reproduction, and survival of herbivores (Mattson, 1980; Awmack & Leather, 2002). Our result that gall tissues had higher nitrogen concentration in comparison to normal stem tissues is consistent with the finding of other researchers (Cuevas-Reyes *et al.*, 2011; Li *et al.*, 2017). Similarly, phosphorus is one of the other main essential nutrients for the growth and development of herbivores (Perkins *et al.*, 2004; Cease *et al.*, 2016). We found that phosphorus concentration was also higher in gall tissues in comparison to ungalled tissues and this pattern is similar to the previous studies (Tsao & Whaley, 1950; Abrahamson & McCrea, 1986; Cuevas-Reyes *et al.*, 2011). Bagatto and Shorthouse (1994) postulated that a higher concentration of potassium in gall cells helps to maintain high negative osmotic potential, thereby increasing the turgor pressure, which helps in spontaneous bursting of the exit hole of the gall so that the insect can easily come out of the gall. In contrast, ungalled tissues have lower concentration of potassium and they do not require turgidity. This is further supported by our results where we observed that the potassium content in galled tissues was higher than ungalled tissues.

Although this study considered elevation gradients as the major influential factor for the abundance and size of galls, there are other factors such as host plant density, age, architectural complexity, soil nutrients and seasonality which are reported to affect the insect gall distribution (Cuevas-Reyes *et al.*, 2011; Stokes & Stiling, 2013; Silva *et al.*, 2015; Boaventura *et al.*, 2018). Similarly, gall size is also determined by the interaction among three different factors as plant genotype, insect genotype, and environment, and evidence also suggests that gall size (diameter) has evolved in response to selection by natural enemies (Weis & Abrahamson, 1985, 1986; Weis & Gormon, 1990). Since, the negative effect of parasitoids on *P. utilis* have already been noticed in several countries including Nepal (Muniappan *et al.,* 2009), abundance and size of gall as well as number of larvae per gall could also be regulated by the presence of native parasitoids. Therefore, incorporating all the bottom-up effects (e.g., host plant quality, quantity and morphology), top-down effects (e.g., natural enemies like parasitoids) and the interaction between them would provide valuable knowledge on insect gall distribution, gall size and population dynamics of galling insects which ultimately determines the success of a galling insect as a biocontrol agent against the weeds (Poudel *et al.,* 2020b).

CHAPTER 6

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This thesis depicts several important aspects of ecology of *Ageratina adenophora* that included its distribution (past, present and future), role of functional traits in its widespread invasion, its impact and the factors affecting the insect gall abundance formed by a biocontrol agent *Procecidochares utilis* and the structural and chemical characteristics of insect gall. Based on observed data and literature review, all of the hypotheses have been justified with generation of empirical data. *A. adenophora* is spreading rapidly since 1990s and an increase of 1-2 % in current suitable areas is expected in future climate scenarios. This finding supported the first hypothesis that the climatically suitable areas for the weed would expand in future with changing climate. Among four physiographic regions, Middle Mountains and among three protected areas, Langtang National Park had the highest percentage of suitable area for the weed under current as well as future climate scenarios. On contrary, the physiographic region, High Himalaya and the conservation area, Manaslu Conservation area currently has the lowest proportion of climatically suitable area. With global warming, *A. adenophora* will expand its distribution to higher elevation range which will amplify the consequences of climate change that this area has been already witnessing. This information can be utilized by resource managers and scientific community as an alert to prevent further spread of the weed in CHAL.

All of the plant functional traits showed variation along the elevation gradient. The decrease in SLA and LNC along short elevation gradient with increasing elevation may be attributed to the adaptation of the species to cold temperature that limits cell expansion and reduces decomposition of soil organic matter in the soil which ultimately lowered down the LNC. Furthermore, number of floral heads per ramet and number of seeds per floral head decreased with the increasing elevation. This decreasing trend of these traits supported the hypothesis that plant fitness of IAPS of tropical origin decreases with increasing elevation. However, seed size and seed mass increased with elevation demonstrating tradeoff between seed number and seed size or seed mass. Larger seeds with high nutrient storage might help the weed in its earlier stages of establishment. Thus, phenotypic plasticity observed in functional traits along the elevation gradient might have helped the weed to occupy wide elevation gradient with different climatic conditions. Since sharp decrease was not noted, it can be concluded that the weed might further move upslope in future.

Ageratina adenophora growing in wide range of habitats caused significant reduction in species richness, species diversity as well as changed the species composition. In addition to aboveground vegetation, the weed also altered the chemical characteristics of the soil. These findings have also supported the third hypothesis that *A. adenophora* reduces species diversity, species richness and alters species composition and soil chemical characteristics. This modification in aboveground vegetation and soil characteristics could lead to changes in other ecological processes and ultimately hamper the ecosystem functioning. Therefore, appropriate management actions are needed that could prevent further impact of the weed.

The abundance of galls formed by *P. utilis* peaked at the mid elevation and declined towards higher and lower elevation regions exhibiting a unimodal pattern justifying the fourth hypothesis that activity of stem galling insect peaks at mid-elevation. Overall, this study suggests that mid elevation range represents the optimal condition for the galling insect growth within the elevation range studied and galls induced by *P. utilis* on *A. adenophora* act as strong nutrient sinks.

Thus, this study suggests that *Ageratina adenophora* is spreading rapidly since past with probabilities of further expansion in future. Variability in its functional traits is assisting the weed to invade wide range of habitats depicting the probability of further upslope movement. With its widespread invasion, the weed has caused reduction in species richness, altered species composition and soil characteristics hampering the ecosystem. Therefore, there is a need of formulating effective, prompt management strategies by resource managers to prevent further spread of the weed and minimize its impacts. In addition, as this study found mid-elevation range the most suitable condition for a biocontrol agent, *Procecidochares utilis*, there is a need of adoption of other control measures for the weed at higher and lower elevational ranges.

6.2 Recommendations

6.2.1 Future research agendas

- As occurrence data of introduced range was only used while building ecological niche models, use of data from both native and invasive range would provide more accurate estimate of potential niche of the species.
- Reciprocal Transplant experiment can be conducted further to explore the role of local adaptation in invasion success of *A. adenophora*.
- Impact being site specific, more impact assessment studies in different regions would help to know the status of invasion impacts caused by the weed and to take necessary actions.
- Since our study considered only abitoic factor (elevation) in shaping the distribution of galls and its size, future studies incorporating the effect of other abiotic factors like soil nutrition, seasonality and biotic factors like natural enemies on gall abundance and size would increase the understanding of gall dynamics.

6.2.2 Management recommendations

Following recommendations are made from the present research work:

- Middle Mountain region needs more urgent attention of scientific community and land resource managers to implement effective management plans to prevent the further spread of the weed.
- Control and monitoring effort for the management of this weed to areas near river and road networks is recommended.
- Siwalik and High Himalaya as well as Manaslu Conservation Area which has less suitable areas in current condition could gain climatically suitable areas in future climate scenarios, therefore, regular monitoring and inspection of these regions is recommended to prevent further expansion of the weed.
- For Middle Mountain and Langtang National Park which contain large areas at risk of invasion, Integrated management approach can be adopted and implemented with regular monitoring of the climatically suitable areas to prevent further invasion impact of the weed.

CHAPTER 7

7. Summary

Invasive alien plant species (IAPS) are one of the major causes of global change with substantial negative impacts on ecosystem structure, ecosystem services, human health and socio-economy. Among several IAPS, *Ageratina adenophora* is one of the noxious invasive weeds that has already invaded 40 countries of the world. The weed has caused significant negative ecological as well as economic impact in the invaded regions. Therefore, the present study was conducted to explore the distribution of the weed (past, present and future), role of functional traits in its invasion success, ecological impacts and influence of elevation in shaping the abundance of a biocontrol agent of the weed in central Nepal.

Maxent model was used to predict the climatically suitable areas of *A. adenophora* under current and three future climate change trajectories (RCP 2.5, RCP 4.5, RCP 8.5) in two different time periods (2050 and 2070) in Chitwan-Annapurna Landscape (CHAL) of central Nepal using occurrence locations of the weed, climatic and topographic variables. For analysing the change in invaded area of *A. adenophora* from the year 1990-2018 in CHAL, Landsat images were used and knowledge-based classification technique was applied. Seven rules (elevation, maximum temperature, minimum temperature, average rainfall, landuse, normalized difference vegetation index (ndvi) and digital number) were assigned classifying them into suitable and unsuitable classes on the basis of geographic locations recorded. For estimating the invaded area in smaller areas of interest (Dhading, Kaski and Tanahun), both high resolution (worldview-2) and low resolution (landsat) images were used and comparison was made between the two images. The role of six functional traits (Specific leaf area (SLA, Leaf nitrogen content (LNC), number of floral heads per ramet, total seeds per floral head, seed size and seed mass) was analysed for the wide spread of *A. adenophora* along the elevation gradient of 240-2965 m asl. Two sites of central Nepal (Simbhanjyang and Chandragiri) were taken into consideration for the study of impacts of *A. adenophora* on vegetation and soil characteristics. The role of elevation on the abundance of a biocontrol agent, *Procecidochares utilis*, size of gall formed by it, its capacity to act as nutrient sink and impact on stem diameter of the weed was analysed.

The most important variable that governed the distribution of *A. adenophora* was the minimum temperature of the coldest month. About 38% of CHAL area is climatically suitable for the weed in current condition. Among three physiographic regions, Middle Mountain had more climatically suitable areas. With climate change in future, an increment of 1-2% of climatically suitable areas of the weed is expected. All protected areas (Annapurna Conservation area, Langtang National Park and Manaslu conservation area) and three physiographic regions (Siwaliks, High Mountain and High Himalaya) would gain climatically suitable areas in future climate scenarios. Furthermore, *A. adenophora' s* upper elevational limit would expand by 31-48 m asl in future climate scenarios. While analyzing the spatial pattern of invasion since 1990 to 2018, it was found that the spread of *A. adenophora* started from the eastern part of Nepal and with the time duration, 0.22% of invaded area in 1990 has now reached to 4.55 % of CHAL. In comparison to Landsat images, worldview-2 images had higher accuracy and were and proved to be more reliable for estimating invaded area. Thus, identified probable climatically suitable areas of the weed will act as an alert for policy makers to adopt precautionary measures to prevent further spread of weed into new areas.

Along the elevation gradient, *Ageratina adenophora* exhibited trait variability. SLA, LNC, number of floral heads per ramet and total seeds per floral head decreased with the increasing elevation along the elevation gradient of 500-2500 m asl. However, different pattern was observed in the traits along the elevation gradient of 1500-2530 m asl in Chandragiri. Seed size and seed mass increased with the increasing elevation at all three sites. The strategy of the weed to adapt to low temperatures at higher elevations was revealed by the trade-off between seed size-seed number and seed mass. This indicates the probability of the weed to expand further upslope.

Invasion by *Ageratina adenophora* reduced the species richness and species diversity in the invaded sites. Shannon and Simpson diversity indices in non-invaded sites were about 1.9 and 1.6 times higher than in the invaded sites respectively. It also altered the species composition significantly. A difference of 49% was observed between invaded and non-invaded sites as explained by Sorenson similarity index. Besides, vegetation, *A. adenophora* invasion increased the carbon and nitrogen concentration of soil. However, potassium and phosphorus concentration did not differ between invaded and non-invaded sites. This result indicates that impact of *A. adenophora* could be site specific. However, more impact assessment study including various other regions would provide better understanding of the impact and help in adopting management measures in future.

The abundance of a biocontrol agent, *Proecidochares utilis* peaked at mid elevation distribution range (1950-2000 m asl) of *A. adenophora*. Also, the gall size was largest at mid-elevation lowering towards the margins. *P. utilis* significantly reduced the diameter of the weed and gall formed by it showed nutrient sink capacity for all the three nutrients studied (N, P & K). Besides, elevation, other abiotic factors as well as biotic factors might also affect gall population dynamics. Therefore, incorporating both biotic as well as abiotic factors would help in understanding the ecology of a biocontrol agent that would ultimately assist in management decisions.

Thus, ecological niche models as well as functional trait studies both predicted the upslope migration of the weed in future invading new areas. Therefore, there is a need of effective preplanning and timely action by the resource managers and policy makers to prevent further spread of the weed in new areas in future. In addition, the negative impacts of the weed on species richness, diversity, composition and soil characteristics as demonstrated by this study have also emphasized on taking prompt actions and adopting management interventions to mitigate the impacts of the weed. Furthermore, though the activity of gall fly was highest at mid-elevational range, there is a need of adoption of other control measures for the successful management of the weed in higher and lower elevational areas.

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APPENDICES

Appendix 1: Bioclimatic variables used for modeling suitable habitat of *Ageratina adenophora*

Code	Variables name
ALT	Altitude
ASPECT	Aspect
SLOPE	Slope
BIO 1	Annual Mean Temperature
BIO 2	Mean Diurnal Range (Mean of Monthly (Max temp - Min Temp))
BIO 3	Isothermality (BIO 2/BIO 7) (*100)
BIO 4	Temperature Seasonality (standard deviation * 100)
BIO 5	Max Temperature of Warmest Month
BIO 6	Min Temperature of Coldest Month
BIO 7	Temperature Annual Range (BIO 5 - BIO 6)
BIO 8	Mean Temperature of Wettest Quarter
BIO 9	Mean Temperature of Driest Quarter
BIO 10	Mean Temperature of Warmest Quarter
BIO 11	Mean Temperature of Coldest Quarter
BIO 12	Annual Precipitation
BIO 13	Precipitation of Wettest Month
BIO 14	Precipitation of Driest Month
BIO 15	Precipitation Seasonality (Coefficient of Variation)
BIO 16	Precipitation of Wettest Quarter
BIO 17	Precipitation of Driest Quarter
BIO 18	Precipitation of Warmest Quarter
BIO 19	Precipitation of Coldest Quarter

Appendix 2: Correlation matrix of 19 bioclimatic and 3 topographic variables

Layer	Altitude	bio1	aspect	slope	bio10	bio11	bio12	bio13	bio14	bio15	bio16	bio17	bio18	bio19	bio2	bio3	bio4	bio5	bio6	bio7	bio8	bio9
altitude	1																					
bio1	-1.0	1																				
aspect	0.0	0.0	1																			
slope	0.4	-0.4	0.0	1																		
bio10	-1.0	1.0	0.0	-0.4	1																	
bio11	-1.0	1.0	0.0	-0.4	1.0	1																
bio12	-0.9	0.8	0.0	-0.4	0.8	0.8	1															
bio13	-0.9	0.8	0.0	-0.4	0.8	0.8	1.0	1														
bio14	-0.4	0.4	0.0	-0.1	0.4	0.4	0.3	0.3	1													
bio15	-0.7	0.7	0.0	-0.3	0.7	0.7	0.8	0.8	0.1	1												
bio16	-0.9	0.8	0.0	-0.4	0.8	0.8	1.0	1.0	0.3	0.8	1											
bio17	0.1	-0.1	-0.1	0.0	-0.1	-0.1	-0.1	-0.1	0.6	-0.5	-0.1	1										
bio18	-0.7	0.7	0.0	-0.3	0.7	0.7	1.0	1.0	0.3	0.7	1.0	-0.1	1									
bio19	0.4	-0.4	-0.1	0.1	-0.4	-0.4	-0.4	-0.4	0.4	-0.7	-0.4	0.9	-0.4	1								
bio2	0.5	-0.5	0.0	0.0	-0.4	-0.5	-0.5	-0.5	-0.5	-0.2	-0.5	-0.3	-0.6	-0.1	1							
bio3	0.2	-0.2	0.1	0.4	-0.2	-0.1	0.0	0.0	-0.2	0.0	0.0	-0.5	0.1	-0.3	-0.2	1						
bio4	0.4	-0.4	0.0	-0.2	-0.4	-0.5	-0.5	-0.5	-0.3	-0.2	-0.5	0.1	-0.6	0.2	0.8	-0.6	1					
bio5	-1.0	1.0	0.0	-0.5	1.0	1.0	0.8	0.8	0.4	0.7	0.8	-0.1	0.7	-0.4	-0.4	-0.2	-0.4	1				
bio6	-1.0	1.0	0.0	-0.4	1.0	1.0	0.9	0.8	0.4	0.7	0.8	-0.1	0.7	-0.4	-0.5	-0.1	-0.5	1.0	1			
bio7	0.3	-0.3	0.0	-0.2	-0.3	-0.3	-0.4	-0.4	-0.3	-0.1	-0.4	0.0	-0.5	0.0	0.9	-0.6	1.0	-0.2	-0.4	1		
bio8	-1.0	1.0	0.0	-0.4	1.0	1.0	0.8	0.8	0.4	0.7	0.8	-0.1	0.7	-0.4	-0.5	-0.2	-0.4	1.0	1.0	-0.3	1	
bio9	-1.0	1.0	0.0	-0.4	1.0	1.0	0.8	0.8	0.4	0.7	0.8	-0.1	0.7	-0.4	-0.5	-0.2	-0.4	1.0	1.0	-0.3	1.0	1

Appendix 3: Landsat ETM+ images used in this study, including path and row number, acquisition date and data sources for different year

Data Source	Year	Path/row	Acquisition date	Instrument	Source
Landsat 5	1992	142/040	1992/11/15	Thematic Mapper (TM)	https://earthexplorer.usgs.gov/
		142/041	1992/11/15		
		143/040	1992/11/06		
		141/041	1992/11/08		
		142/040	1991/11/29		
Landsat 5	1999	141/040	1999/10/27	Thematic Mapper (TM)	
		141/041	1999/10/27		
		142/041	1999/12/05		
		143/040	1999/12/28		
		142/040	1999/12/05		
Landsat 5	2009	142/041	2009/10/29	Thematic Mapper (TM)	
		143/041	2009/11/05		
		142/040	2009/10/29		
		141/040	2009/11/23		
		143/040	2009/11/05		
		141/041	2009/11/23		
Landsat 8	2018	141/041	2018/06/25	Operational Land Imager (OLI)	
		141/040	2018/06/25		
		142/040	2017/06/13		
		142/041	2016/06/10		
		143/040	2017/06/04		

Appendix 4: Worldview-2 imagery data sources

Districts	Raster	Acquisition date
Dhading	10APR24051303-M2AS_R1C1-011412008050_01_P001.TIF	2010
	10APR24051303-M2AS_R1C2-011412008050_01_P001.TIF	2010
	10APR24051303-M2AS_R2C1-011412008050_01_P001.TIF	2010
	10APR24051303-M2AS_R2C2-011412008050_01_P001.TIF	2010
	10APR24051303-M2AS_R2C3-011412008050_01_P001.TIF	2010
	18APR14053230-M2AS_R1C1-011412018020_01_P001.TIF	2018
	18APR14053230-M2AS_R1C2-011412018020_01_P001.TIF	2018
	18APR14053230-M2AS_R2C1-011412018020_01_P001.TIF	2018
	18APR14053230-M2AS_R2C2-011412018020_01_P001.TIF	2018
	18APR14053230-M2AS_R2C3-011412018020_01_P001.TIF	2018
	18APR14053230-M2AS_R3C2-011412018020_01_P001.TIF	2018
	18APR14053230-M2AS_R3C3-011412018020_01_P001.TIF	2018
Kaski	17DEC09052020-M2AS-058496162010_01_P002.TIF	2017
Tanahun	09FEB14050434-M2AS_R1C1-011412008150_01_P001.TIF	2009
	09FEB14050434-M2AS_R1C2-011412008150_01_P001.TIF	2009
	09FEB14050434-M2AS_R1C3-011412008150_01_P001.TIF	2009
	09FEB14050434-M2AS_R1C4-011412008150_01_P001.TIF	2009
	09FEB14050434-M2AS_R2C3-011412008150_01_P001.TIF	2009
	09FEB14050434-M2AS_R2C4-011412008150_01_P001.TIF	2009

Appendix 5: Annual rainfall and temperature data

Station ID	Station name	District	Latitude	Longitude	Annual rainfall (mm) (1970-2016)	Tmax (°C) (1970-2016)	Tmin (°C) (1970-2016)
715	Khanchikot	Arghakhanchi	27.93333	83.15	1595.88	22.32	15.20
605	Baglung	Baglung	28.26667	83.6	1705.26	29.89	18.57
927	Bharatpur	Chitawan	27.66667	84.43333	1896.56	33.16	22.49
902	Rampur	Chitawan	27.61667	84.41667	1868.95	32.74	21.85
809	Gorkha	Gorkha	28	84.61667	1514.29	28.40	18.82
806	Larke Samdo	Gorkha	28.66667	84.61667	600.43	28.56	18.57
725	Tamghas	Gulmi	28.06667	83.25	1719.81	24.85	15.73
814	Lumle	Kaski	28.3	83.8	5122.36	22.17	14.90
811	Malepatan (Pokhara)	Kaski	28.11667	84.11667	3474.70	29.00	18.30
804	Pokhara Airport	Kaski	28.21667	84	3606.14	28.67	18.80
866	Pokhara (Reg. Off.)	Kaski	28.21667	83.98333	2651.04	29.47	19.79
802	Khudi Bazar	Lamjung	28.28333	84.36667	3000.31	28.89	18.24
905	Daman	Makwanpur	27.6	85.08333	1518.94	20.65	11.35
906	Hetaunda N.F.I.	Makwanpur	27.41667	85.05	2216.22	31.05	20.48
816	Chame	Manang	28.55	84.23333	677.52	19.32	7.72
633	Chhoser	Mustang	29.18333	83.98333	160.77	18.27	4.67
623	Dhiee	Mustang	29.1	84	76.72	20.64	7.84
601	Jomsom	Mustang	28.78333	83.71667	186.78	20.46	9.30
607	Lete	Mustang	28.63333	83.6	986.58	19.07	9.87
612	Mustang (Lomangthang)	Mustang	29.18333	83.96667	111.23	16.59	4.01
604	Thakmarpha	Mustang	28.75	83.7	296.81	19.52	8.87
609	Beni Bazar	Myagdi	28.35	83.56667	1390.91	30.48	18.71
616	Gurja Khani	Myagdi	28.6	83.21667	1583.39	20.00	8.52
706	Dumkauli	Nawalparasi	27.68333	84.21667	2214.45	32.82	22.86
708	Parasi	Nawalparasi	27.53333	83.66667	1649.10	35.31	22.60
728	Semari	Nawalparasi	27.53333	83.75	1758.03	34.36	22.26
1007	Kakani	Nuwakot	27.8	85.25	2565.50	21.70	13.56
1004	Nuwakot	Nuwakot	27.91667	85.16667	1683.71	29.45	19.22
1057	Pansayakhola	Nuwakot	28.01667	85.11667	2755.97	21.10	12.39
702	Tansen	Palpa	27.86667	83.53333	1241.37	27.33	17.77
614	Kushma	Parbat	28.21667	83.7	2284.75	30.66	18.78
1055	Dhunche	Rasuwa	28.1	85.3	1642.30	22.59	13.40
1001	Timure	Rasuwa	28.28333	85.38333	752.91	24.91	14.31
805	Syangja	Syangia	28.1	83.88333	2670.25	29.52	18.63
810	Chapkot	Syangja	27.88333	83.81667	1673.41	31.18	20.51
832	Dandaswara	Syangja	28.08333	83.91667	2845.36	26.49	16.16
808	Bandipur	Tanahun	27.93333	84.41667	1633.39	27.68	18.78
817	Damauli	Tanahun	27.96667	84.28333	1520.04	32.59	21.07
815	Khairini Tar	Tanahun	28.03333	84.1	2025.16	31.04	20.73
303	Jumla	Jumla	27.28333	82.16667	617.0706897	23.38	9.23
310	Dipal Gaun	Jumla	26.26667	83.21667	710.7225	24.66	9.27
409	Khajura (Nepalgunj)	Banke	26.1	81.78333	1264.286957	33.27	22.48
416	Nepalgunj (Reg. Off.)	Banke	28.06667	81.61667	1227.825581	33.32	23.68
420	Nepalgunj Airport	Banke	28.1	81.66667	1416.975	33.49	22.56
716	Taulihawa	Kapilbastu	27.55	83.06667	1366.064444	33.4	22.22
918	Birganj	Parsa	27	84.86667	312.94	36.24	22
1009	Chautara	Sindhupalchok	27.78333	85.71667	417.2174603	26.88	16.58
1016	Sarmathang	Sindhupalchok	27.95	85.6	709.805	18.69	10.46
1027	Bahrabise	Sindhupalchok	27.78333	85.9	588.6857143	29.94	15.92
1030	Kathmandu Airport	Kathmandu	27.7	85.36667	311.5291667	27.27	16.39
1039	Panipokhari (Kathmandu)	Kathmandu	27.73333	85.33333	322.0651163	27.24	16.99

Appendix 6: MicroKjeldahl method for the determination of leaf/soil nitrogen content

MicroKjeldahl method includes three steps – digestion, distillation and titration.

Digestion

Pre oven dried leaves were crushed and again kept in an oven at 60°C for 24 hours. 0.25 g crushed leaves and 2 g catalyst (mixture of potassium sulphate, anhydrous copper sulphate and selenium in the ratio 100:10:1) was added in Kjeldahl digestion flask. For soil sample, 0.5 g shade dried soil sample was taken in Kjeldahl digestion flask and 3.5 g Potassium sulphate plus 0.4 g anhydrous copper sulphate was added in Kjeldahl digestion flask. Then 6 ml conc. H_2SO_4 was added. Then the flask was heated gently at 150°C on heating mantle with gentle shaking for about 30 minutes until the bubble cease to ooze out. Then the temperature was increased to 400°C and was set to digestion for 30-45 minutes until clear light green coloration appeared. The flask was then allowed for cooling down for about 20 minutes. 60 ml of distilled water was added gradually to it with constant shaking to avoid bubbling inside the flask for proper mixing of digested material with water. Similar procedure was done for two blank samples without plant/soil material.

Distillation

The digested material was transferred to Kjeldahl distillation flask and heated at 30°C – 40°C in a heating mantle till the digested material becomes lukewarm. Once the mixture became warm, 30 ml of 40% NaOH was poured into the flask and the temperature was raised to 70°C – 100°C. About 40-50 ml of the distillate containing ammonia was collected in the beaker containing 10 ml boric acid indicator.

Titration

The distillate was titrated against 0.1 N HCl solution until faint pink color was obtained. The amount of HCl consumed was noted. The leaf/soil nitrogen content was calculated using the formula,

$$\text{Leaf/soil Nitrogen Content (LNC) (\%)} = \frac{14 \times \mathrm{N} \times (\mathrm{S} - \mathrm{B})}{\mathrm{M}} \times 100$$

Where, S = Vol. of HCl (ml) consumed by the sample

B = Vol. of HCl (ml) consumed by blank

N = Normality of HCl (0.1 N)

M = Oven dried Mass of leaf/soil sample taken

Appendix 7: Walkely and Black rapid titration method for the determination of soil organic carbon

0.5 g of soil was taken in a dry 250 ml conical flask. 5 mL of 1N $K_2Cr_2O_7$ and 10 ml of conc. H_2SO_4 was added to it one after another. The mixture was gently swirled to ensure proper mixing and allowed to stand for 30 minutes for digestion. Then 100ml of distilled water and 5ml of ortho-phosphoric acid was added simultaneously to the mixture. Lastly 0.5 ml of diphenylamine indicator solution was added to the mixture, stirred for few seconds till the whole mixture get converted to dark blue color and then titrated against 0.5N Ferrous ammonium sulphate solution. The end point of titration was noted when the dark blue color of the mixture turned to bright green color. Simultaneously, a standardized blank without soil was run. The initial and final reading of ferrous ammonium sulphate solution in burette was noted. Then the percentage of OC in soil was calculated by using formula shown below:

$$\text{Estimated OC \% in soil} = N \times \frac{(B - C)}{\text{Weight of soil (g)}} \times 0.003 \times 100$$

Where,

N = Normality of ferrous ammonium sulphate

B = Blank reading

C = Titration reading with soil

Finally, the estimated organic carbon percentage was multiplied by a factor 1.3 based on assumption that there was 77% recovery of organic matter in this procedure and total organic carbon measure was obtained as:

Total Organic Carbon % = estimated Organic Carbon % × 1.3

Appendix 8: Method for estimating soil phosphorus

Available phosphorus of soil samples was estimated by using procedures as described in Trivedy and Goel (1986). Turger's extract was made by mixing 2 g of soil with 200 ml of 0.002 N H_2SO_4 and the mixture was shaken (photo 15) in a vibrator (model: KCHVIBRAX-VXR) at speed of 1200/min for half an hour. Then the total suspension (200 ml) was filtered by using Whatmann No.1 filter paper to get a clear soil solution. Filtration was repeated until the filtrate was clear. Then after, 50 ml of the filtrate was taken in a clean beaker and 2 ml of ammonium molybdate solution was added to it followed by 5 drops of $SnCl_2$ solution. A blue color was observed in the mixture after addition of $SnCl_2$ solution. Then the reading of the solution was taken at 690 nm on a spectrophotometer using distilled water as blank solution with same amount of chemicals. The reading was taken after 5–12 minutes of the addition of $SnCl_2$. Similarly, for standard cuwrve, various dilutions of the standard phosphate solution at the interval of 0.1 mg P/L were made and their absorbance at 690 nm was noted. Finally, a curve of absorbance and concentration of various dilutions of phosphorus was made and the equation for the curve was estimated by using MS Excel (Figure A1).

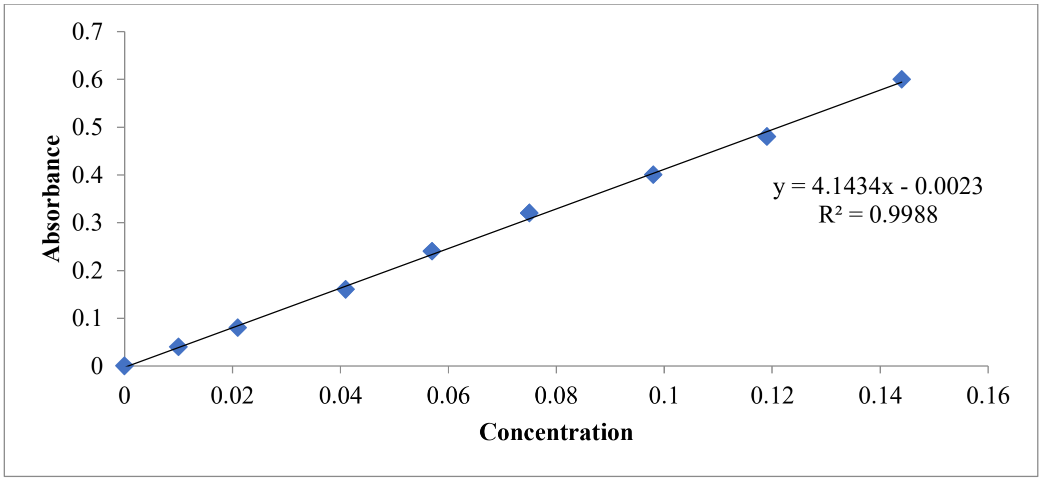


Figure A1: Line graph showing calibration curve of available phosphorus

Thus, obtained equation from standard curve was used to estimate the concentration of available phosphorus. Finally, the percentage of available phosphorus in soil was calculated by using following formula:

$$\%\ \text{available phosphorus} = \frac{\text{mg P/L soil solution}}{50}$$

Where,

mg P/L soil solution was obtained with the help of standard curve.

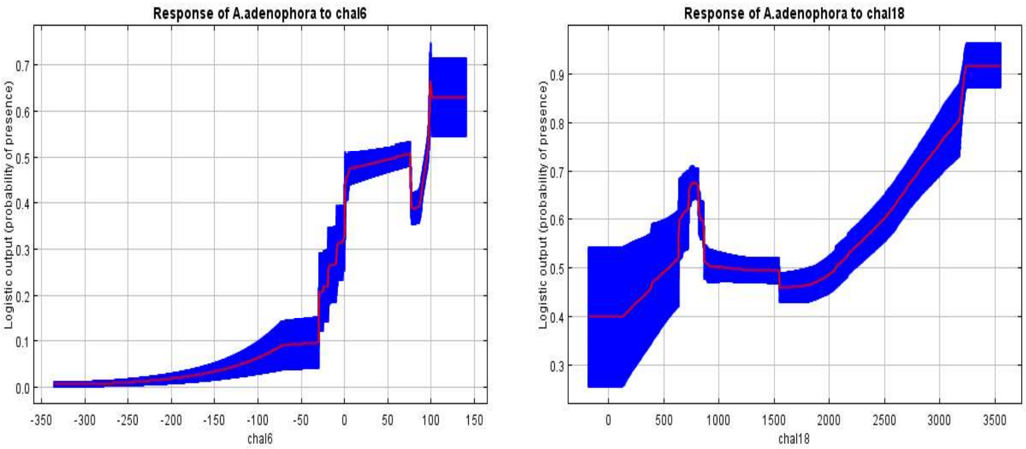
Appendix 9: Method for estimating soil potassium

Exchangeable Potassium content of soil samples was determined by flame photometer method (Trivedy and Goel, 1986). Soil extract was prepared by mixing 2 g of soil sample with 20 ml of 1 N ammonium acetate solution. Then the mixture was shaken in a vibrator of KCH-VIBRAX-VXR model at speed of 1200/min for fifteen minutes. Afterwards, the total suspension of 20 ml was filtered by using Whatmann no.1 filter paper to get a clear soil solution. Filtration was repeated until the filtrate was clear. Thus, obtained clear filtrate was transferred to a clean rinsed test tube and its flame photometer reading was noted at filter of 768 nm.

Appendix 10: Model evaluation matrices

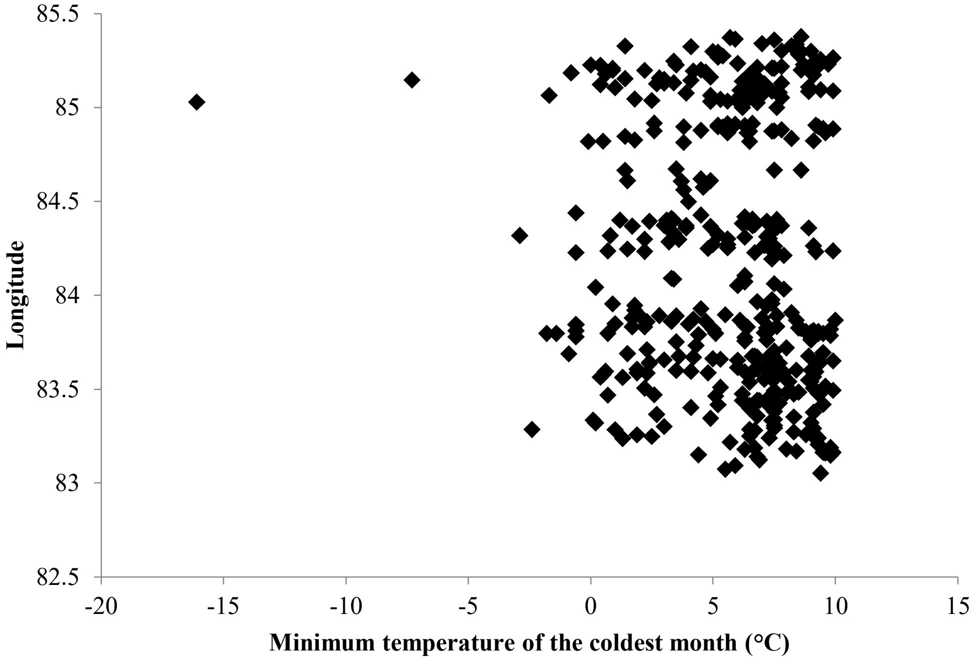
Measures	Value
Mean Training AUC	0.85
Mean Test AUC	0.8
TSS	0.52

Appendix 11: Marginal response curves of the 2 highly contributing predictor variables (A) bio 6, minimum temperature of coldest month, and (B) bio 18, precipitation of the warmest quarter



(A) (B)

Appendix 12: Scatter plot of longitude of occurrence points and minimum temperature of the coldest month for current climate



Appendix 13: Summary result of DCA of Chandragiri

	DCA1	DCA2	DCA3	DCA4
Eigen values	0.7047	0.3761	0.3169	0.2439
Decorana values	0.7171	0.3695	0.3299	0.2914
Axis lengths	5.1018	4.384	3.5736	2.9529

Appendix 14: Summary results of DCA of Simbhanjyang

	DCA1	DCA2	DCA3	DCA4
Eigen values	0.6272	0.4009	0.2755	0.2426
Decorana values	0.6277	0.4041	0.2945	0.2603
Axis lengths	3.7797	3.1329	3.3102	3.3937

Appendix 15: List of plants obtained in invaded and non-invaded plots of Simbhanjyang

SN	Noninvaded plots	Invaded plots	Common to both plots
1	*Acmella calva* (DC.) R.K. Jansen	*Athyrium foliolosum* (Wall.) Moore ex Sm.	*Achyranthes bidentata* Blume
2	*Acystopteris sp.*	*Camellia kissi* Wall.	*Adenostemma lavenia* (L.) Kuntze
3	*Adiantum caudatum* L.	*Eleusine indica* (L.) Gaertn.	*Ageratum houstonianum* Mill.
4	*Ageratum conyzoides* L.	*Fagopyrum dibostrys* (D. Don) H. Hara	*Alnus nepalensis* D. Don
5	*Aleuritopteris albomarginata* (Clarke) Ching	*Hedychium sp.*	*Anaphalis busua* (Buch.-Ham. ex D.Don) DC.
6	*Arisaema tortuosum* (Wall.) Schott	*Holmskioldia sanguinea* Retz.	*Anaphalis contorta* D.Don Hook. f.
7	*Arthraxon hispidus* (Thunb.) Makino	*Leucas lanata* Benth.	*Anaphalis triplinervis* (Sims) C. B. Clarke
8	*Bauhinia purpurea* L.	*Lindernia ruelliodes* (Collsm.) Pennelt.	*Artemisia indica* Willd.
9	*Bothriochloa glabra* (Roxb.) A. Camus	*Microstegium ciliatum* (Trin.) A.Camus	*Arundinella nepalensis* Trin.
10	*Cheilanthes sp.*	*Paspalum distichum* L.	*Berberis aristate* DC.
11	*Cirsium verutum* (D.Don) Spreng.	*Persicaria hydropiper* (L.) Spach.	*Bidens Pilosa* L.
12	*Clinopodium umbrosum* (M.Bieb.) K.Koch	*Persicaria nepalensis* (Meisn.) H.Gross	*Boehmeria platyphylla* D.Don
13	*Coccinia cordifolia* (L.) Cogn.	*Persicaria posumbu* (Buch.-Ham. ex D. Don) H. Gross	*Capillipedium assimile* (Steud.) A. Camus
14	*Colocasia esculenta* (L.) Schott	*Phyllanthus reticulatus* Poir.	*Carex filicina* Nees
15	*Conyza sp.*	*Pouzolzia sanguinea* (Blume) Merr.	*Carex sp.*
16	*Conyza stricta* Willd.	*Pteridium revolutum* (Blume) Nakai	*Castanopsis indica* (Roxb. Ex Lindl.) A.DC.
17	*Conyza sumatrensis* Retz. (E.H. Walker)	*Pteris biaurita* L.	*Cautleya gracilis* (Sm.) Dandy
18	*Cyanotis vaga* (Lour.) Schult. & Schult.f.	*Rhododendron arboretum* Sm.	*Cayratia trifolia* Linn.Domin
19	*Cyperus cyperoides* (L.) Kuntze	*Rosa brunonii* Lindl.	*Centella asiatica* (L.) Urb.
20	*Deparia petersonii* (Kunze) M.Kato	*Schima wallichi* (DC.) Korth.	*Cissampelos pareira* L.
21	*Desmodium elegans* DC.	*Strobilanthes lachenensis* C.B.Clarke	*Cynodon dactylon* (L.) Pers.
22	*Desmodium heterocarpon* (L.) DC.	*Themeda hookeri* (Griseb.) A. Camus	*Cyrtococcum patens* (L.) A.Camus

23	*Desmodium laxiflorum* DC.	*Thylepteris procera* (D.Don) Fraser-Junk	*Drymaria cordata* (L.) Willd. ex Roem. & Schult.
24	*Desmodium microphyllum* (Thunb.) DC.	*Urtica dioica* L.	*Equisetum arvense* L.
25	*Desmodium motorium* (Houtt.) Merr.	*Zanthoxylum acanthodium* DC.	*Eragrostis nigra* Nees ex Steud.
26	*Desmodium multiflorum* DC.		*Eulalia mollis* (Griseb.) Kuntze
27	*Diascorea bulbifera* L.		*Galium asperifolium* Wall.
28	*Dichrocephala integrifolia* (L.fil.) Kuntze		*Gaultheria fragrantissima* Wall.
29	*Eragrostis uniloides* (Retz.) Nees ex Steud.		*Gonostegia hirta* (Blume) Miq.
30	*Fimbristylis dichotoma* (L.) Vahl		*Imperata cylindrica* (L.) P. Beauv.
31	*Fimbristylis quinquangularis* (Vahl) Kunth		*Kyllinga nemoralis* (J.R. Forst. & G. Forst.) Dandy
32	*Fragaria indica* Andrews		*Lantana camara* L.
33	*Fragaria nubicola* Lindl.		*Microstegium nudum* (Trin.) A. Camus
34	*Galium acutum* Edgew.		*Nephrolepis cordifolia* (L.) C. Persl
35	*Gaultheria nummularioides* D.Don		*Oplismenus burmannii* (Retz.) P. Beauv.
36	*Gentiana capitata* Buch.-Ham. ex D. Don		*Oxalis corniculate* L.
37	*Hydrocotyle nepalensis* Hook.		*Phyllanthus parvifolius* Buch.-Ham. ex D. Don
38	*Ipomoea quamoclit* L.		*Pilea glaberrima* (Blume) Blume
39	*Justicia procumbens* L.		*Pinus roxburghii* Sarg.
40	*Kylinga brevifolia* Rottb.		*Plectranthus mollis* (Aiton) Spreng.
41	*Lygodium japonicum* (Thunb.) Sw.		*Prinsepia utilis* Royle
42	*Micromeria biflora* (Buch.-Ham. ex D. Don) Benth.		*Pteris normalis* D.Don
43	*Mimosa pudica* L.		*Pyracantha crenulata* (D. Don) M. Roem.
44	*Neillia thyrsiflora* D.Don		*Rubia manjith* Roxb.
45	*Oplismenus compositus* (L.) P. Beauv.		*Rubus ellipticus* Sm.
46	*Persicaria barbata* (L.) H. Hara		*Rubus foliolosus* D.Don
47	*Persicaria chinensis* (L.) Nakai		*Scutellaria discolor* Colebr.
48	*Phyllanthus urinaria* L.		*Setaria palmifolia* (J.Koenig)

		Stapf
49	*Pinus roxburghii* Sarg.	*Stellaria vestita* Kurz
50	*Plantago major* L.	*Strobilanthes oligocephala* T. Anderson ex C.B. Clarke
51	*Poaceae2*	*Thelypteris dentata* (Forssk.) E.P.St. John
52	*Polygonum hydropiper* (L.)	*Tinospora sinensis* (Lour.) Merr.
53	*Potentilla sp.*	*Triumfetta bartramia* L.
54	*Pycreus diaphanous* (Schrad. ex Schult.) S.M. Huang	*Unknown sp.2*
55	*Pycreus sanguinolentus* (Vahl) Nees	*Urena lobata* L.
56	*Ranunculus hirtellus* Royle	
57	*Selaginella sp.*	
58	*Setaria parviflora* (Poir.) Kerguelen	
59	*Solanum xanthocarpum* Schrad. & Wendl.	
60	*Solena heterophylla* Lour.	
61	*Spermacoce mauritiana* Gideon.	
62	*Sporobolus diander* P. Beauv.	
63	*Swertia chirayita* (Roxb.) H.Karst	
64	*Taraxaccum officinale* Weber ex Wiggins	
65	*Torenia asiatica* L.	
66	*Trifolium repens* L.	
67	*Unknown sp1*	
68	*Viola Pilosa* Blume	
69	*Vitis sp.*	
70	*Wikstroemia canescens* Wall. ex Meisn.	
71	*Woodfordia fruticose* (L.) Kurz	

Appendix 16: List of plants obtained in invaded and non-invaded plots of Chandragiri

SN	Noninvaded plots	Invaded plots	Common to both plots
1	*Adenostemma Lavenia* (L.) Kuntze	*Ainsliaea latifolia* (D.Don) Sch.Bip	*Achyranthes bidentata* Blume
2	*Adiantum caudatum* L.	*Argyreia hookeri* C.B. Clarke	*Acmella calva* (DC.) R.K. Jansen
3	*Anaphalis triplinervis* (Sims) C. B. Clarke	*Betula alnoides* Buch.-Ham. ex D. Don	*Aconogonum mole* (D.Don) H.Hara
4	*Anisomeles sp*	*Conyza japonica* (Thunb.) Less.	*Aestilbe rivularis* Buch.-Ham. ex D. Don
5	*Apluda mutica* L.	*Conyza sumatrensis* (Retz.) E.H. Walker	*Agrostis pilosula* Trin.
6	*Arisaema tortuosa* (Wall.) Schott	*Coriaria nepalensis* Wall.	*Alnus nepalensis* D. Don
7	*Arthraxon hispidus* (Thunb.) Makino	*Dennstaedtia appendiculata* (Wall. ex Hook.) J.Sm.	*Anaphalis busua* (Buch.-Ham. ex D.Don) DC.
8	*Athyrium pectinate* (Wall. ex Hope) C. Presl	*Dumasia villosa* DC.	*Anaphalis contorta* D.Don Hook. f.
9	*Bracharia distachya* (L.) Stapf	*Eleusine indica* (L.) Gaertn.	*Anemone vitifolia* Buch.-Ham. ex DC.
10	*Bryonia laciniosa* L.	*Galium acutum* Edgew.	*Arisaema sp*
11	*Campanula colarata* Wall.	*Galium asperifolium* Wall.	*Artemisia indica* Willd.
12	*Campanula pallida* Wall.	*Justicia procumbens* L.	*Arundinaria falcata* Nees
13	*Cannabis sativa* L.	*Lobelia pyramidalis* Wall.	*Athyrium foliolosum* (Wall.) Moore ex Sm.
14	*Chenopodium album* L.	*Phyllanthus sp.*	*Athyrium sp.*
15	*Codonopsis viridis* Wall.	*Salix sp.*	*Berberis aristate* DC.
16	*Colebrookea oppositifolia* Sm.	*Scrophularia urticifolia* Wall. ex Benth.	*Bidens Pilosa* L.
17	*Colquhounia coccinea* Wall.	*Solena heterophylla* Lour.	*Boehmerria platyphylla* D. Don
18	*Conyza canadensis* (L.) Cronquist	*Spiraea micrantha* Hook.fil.	*Boeninghausiana albiflora* (Hook.) Rchb. ex Meisn.
19	*Conyza stricta* Willd.		*Borreria alata* (Aubl.) DC.
20	*Cyanotis cristata* (L.) D. Don		*Capillipedium assimile* (Steud.) A. Camus
21	*Cyanotis vaga* (Lour.) Schult. & Schult.f.		*Carex filicima* Nees
22	*Cyperus cyperinus* (Retz.) Valck.Sur.		*Carex sp.*

23	*Cyperus cyperoides* (L.) Kuntze	*Castanopsis indica* (Roxb. ex Lindl.) A. DC.
24	*Cyrtococcum patens* (L.) A. Camus	*Cautleya gracilis* (Sm.) Dandy
25	*Desmodium multiflorum* DC.	*Clinopodium umbrosum* (M.Bieb.) K.Koch
26	*Dicrocephala integrifolia* (L.fil.) Kuntze	*Cynodon dactylon* (L.) Pers.
27	*Diplocyclos palmatus* (L.) C. Jeffrey	*Cynodon radiatus* Roth.
28	*Drynaria mollis* Bedd.	*Debregeasia longifolia* (Burm.fil.) Wedd.
29	*Dryopteris sp.*	*Deparia petersonii* (Kunze) M.Kato
30	*Elatostema sp.*	*Desmodium elegans* DC.
31	*Equisetum arvense* L.	*Dioscorea deltoidei* Wall. ex Griseb.
32	*Erigeron alpinus* L.	*Digitaria ciliaris* (Retz.) Koeler
33	*Fimbrystylis aestivalis* (Retz.) Vahl.	*Drymaria cordata* (L.) Willd. ex Roem. & Schult.
34	*Fimbrystylis dichotoma* (L.) Vahl	*Eragrostis nigra* Nees ex Steud.
35	*Fragaria nubicola* Lindl.	*Fragaria indica* Andrews
36	*Galinsoga parviflora* Cav.	*Galium elegans* Wall. ex Roxb.
37	*Gerbera gossipiana* (Royle) Beauverd.	*Gaultheria fragrantissima* Wall.
38	*Glyceria tongolensis* C.B. Clarke	*Gentiana capitata* Buch.-Ham. ex D. Don
39	*Gynura nepalensis* DC.	*Geranium nepalense* Sweet
40	*Hedera nepalensis* K.Koch	*Girardinia diversifolia* (Link) Friss
41	*Hemiphragma heterophyllum* Wall.	*Globba sp.*
42	*Hydrocotyle sibthorpioides* Lam.	*Gonostegia hirta* (Blume) Miq.
43	*Hypericum uralum* Buch.-Ham. ex D.Don	*Hedychium sp.*
44	*Hypoestes trifloral* (Forrsk.) Roem. & Schult.	*Hydrocotyle nepalensis* Hook.

45	*Indigofera bracteate* Baker	*Impatiens racemose* DC.
46	*Indigofera cylindracea* Baker	*Impatiens sp.*
47	*Indigofera pulchella* Roxb.	*Imperata cylindrica* (L.) P. Beauv.
48	*Isodon lopanthoides* (Buch.-Ham. ex D.Don) H. Hara	*Ipomoea purpurea* (L.) Roth
49	*Jasminum humile* L.	*Katoella beddome*
50	*Jasminum mesnyi* Hance	*Kholmii sp.*
51	*Katoella pulchra* (D.Don) Fraser-Jenk.	*Kyllinga brevifolia* Rottb.
52	*Lindenbergia grandiflora* (Buch.-Ham. ex D.Don) Benth.	*Kyllinga nemoralis* J.R. Forst. & G. Forst.) Dandy.
53	*Lindenbergia indica* (L.) Vatke	*Lepisorus sp.*
54	*Lycopodium japonicum* Thunb.	*Leucas ciliata* Benth.
55	*Maesa chisia* Buch.-Ham. ex D.Don	*Microstegium nudum* (Trin.) A. Camus
56	*Microstegium ciliatum* A. Camus	*Miscanthus nepalensis* (Trin.) Hack.
57	*Ocimum sanctum* L.	*Myriactis nepalensis* Less.
59	*Onychium lucidum* (D.Don) Spreng.	*Neillia thyrsiflora* D.Don
60	*Ophiopogon intermedius* D.Don	*Neillia rubiflora* D.Don
61	*Paspalidium flavidum* A. Camus	*Oplismenus burmannii* (Retz.) P. Beauv.
62	*Persicaria chinensis* (L.) Nakai	*Oplismenus compositus* (L.) P. Beauv.
63	*Phragmites karka* (Retz.) Trin. ex Steud.	*Oxalis corniculate* L.
64	*Pilea glaberrima* (Blume) Blume	*Paspalum distichum* L.
65	*Pinus roxburghii* Sarg.	*Persicaria barbata* (L.) H. Hara
66	*Pouzolzia sanguinea* (Blume) Merr.	*Persicaria capitata* (Buch.-Ham. ex D.Don) H.Gross
67	*Pteridium revolutum* (Blume) Nakai	*Polystichum sp.*
68	*Pteris aspercaulis* Wall.	*Pteris cretica* L.
69	*Pycreus diaphanus* (Schrad. ex Schult.) S.M. Huang	*Ranunculus diffuses* DC.

70	*Pycreus sanguinolentus* (Vahl) Nees	*Rhododendron arboretum* Sm.
71	*Quercus glauca* Thunb.	*Rhus javanica* L.
72	*Randia tetrasperma* (Wall. ex Roxb.) Benth. & Hook.f. ex Brandis	*Rubia manjith* Roxb.
73	*Roscoea purpurea* Sm.	*Rubus ellipticus* Sm.
74	*Rubus paniculatus* Sm.	*Rumex nepalensis* Spreng.
75	*Scutellaria discolor* Colebr.	*Sanicula elata* Buch.-Ham. ex D.Don
76	*Selaginella sp.*	*Scutellaria repens* Buch.-Ham. ex D.Don
77	*Setaria parviflora* (Poir.) Kerguelen	*Smilax sp.*
78	*Sigesbeckia orientalis* L.	*Sonchus wightianus* DC.
79	*Smilax elegans* Wall. ex Kunth.	*Stellaria vestita* Kurz
80	*Solanum nigrum* L.	*Strobilanthes nutans* (Nees) T. Anderson
81	*Solanum xanthocarpum* Schrad. & Wendl.	*Strobilanthes atropurpurea* Nees
82	*Stellaria graminea* L.	*Strobilanthes lachenensis* C.B.Clarke
83	*Stellaria monosperma* Buch.-Ham. ex D. Don	*Swertia chirayita* (Roxb.) H.Karst.
84	*Swertia alata* Royle ex D. Don	*Tetrastigma serrulatum* (Roxb.) Planch.
85	*Thalictrum foliolosum* DC.	*Trifolium repens* L.
86	*Unknown sp*	*Vigna sp.*
87	*Valeriana hardwickei* Wall.	*Viola Pilosa* Blume
88	*Veronica persica* Poir.	*Zanthoxylum acanthodium* DC.
89	*Viola betonicifolia* Sm.	
90	*Viola canescens* Wall.	
91	*Woodwardia fruticosa* (L.) Kurz	

Appendix 17: Data sheet used for the collection of data in plant functional traits study

Ecological Impact of *Ageratina adenophora*

Field data sheet

SN: Site no.: Way point: Aspect:

Latitude: Longitude: Elevation:

Locality: Plot size:

Disturbance (0-3): Grazing ( ) / Fire ( ) / Others: ____

Important notes:

SN	**Number of heads per ramet**	**SN**	**Number of heads per ramet**
1.		6.	
2.		7.	
3.		8.	
4.		9.	
5.		10.	

Appendix 18: Data sheet used for the collection of data in study of impact of *A. adenophora* on native species

Ecological Impact of *Ageratina adenophora*

Field data sheet

SN: Plot no.: Plot type: Inv./Non-inv. Way point: Aspect:

Slope: Latitude: Longitude: Elevation (m):

Locality: Land use:

Disturbance (0-3): Grazing ( ) / Fire ( ) / Others: ____

Ageratina: Cover (%) #Ramets: Plot size:

Species composition

SN	**Species**	**Cover (%)**	**SN**	**Species**	**Cover (%)**

Appendix 19: Data sheet used for the collection of data in study of gall fly as a biocontrol agent

Stem galling of *Ageratina adenophora*

SN: Site no.: Plot no.: Way point: Aspect:

Slope: Latitude: Longitude: Elevation (m):

Locality: Land use: Plot size:

Disturbance (0-3): Grazing ( ) / Fire ( ) / Others: ____

Number of galls per 100 ramets:

SN	**Gall length**	**Gall breadth**	**Stem diameter**		**S.N**	**Gall length**	**Gall breadth**	**Stem diameter**	
			Galled stem	**Ungalled stem**				**Galled stem**	**Ungalled stem**

Appendix 20: List of publications

1. Poudel, A.S., Jha, P.K., Shrestha, B.B. & Muniappan, R. (2019). Biology and management of the invasive weed *Ageratina adenophora* (Asteraceae): current state of knowledge and future research needs. *Weed Research*, 59(2), 79-92.

2. Poudel, A.S., Shrestha, B.B., Jha, P.K., Baniya, C.B., & Muniappan, R. (2020). Stem galling of Ageratina Adenophora (Asterales: Asteraceae) by a biocontrol agent *Procecidochares utilis* (Diptera: Tephritidae) is elevation dependent in central Nepal. *Biocontrol Science and Technology*, 30(7), 611-627.

3. Poudel, A. S., Shrestha, B. B., Joshi, M. D., Muniappan, R., Adiga, A., Venkatramanan, S., & Jha, P. K. (2020). Predicting the Current and Future Distribution of the Invasive Weed *Ageratina adenophora* in the Chitwan–Annapurna Landscape, Nepal. *Mountain Research and Development*, *40*(2), R61.

Appendix 21: Manuscripts under publication process

1. Poudel, A.S., Shrestha, B.B., Jha, P.K., & Muniappan, R. Impact of *Ageratina adenophora* on the vegetation and soil characteristics in Chitwan-Annapurna Landscape, Nepal.
2. Poudel, A.S., Shrestha, B.B., Jha, P.K., & Muniappan, R. Functional traits variation of *Ageratina adenophora* along elevation gradient in central Nepal.
3. Poudel, A.S., Shrestha, B.B., Jha, P.K., & Muniappan, R. Spatio-temporal distribution of *Ageratina adenophora* in Chitwan-Annapurna Landscape, Nepal.

Conference and Seminar attended

Oral presentation

1. Poudel, A.S., Shrestha, B.B., & Muniappan, R. 2018. Plant functional traits variation of an invasive weed *Ageratina adenophora* along elevation gradient in Chitwan-Annapurna Landscape, Nepal. Third International Conference on Mountains in the changing world. (9-10th October, 2018) Kathmandu, Nepal.

2. Poudel, A.S., Shrestha, B.B., Poudel, K., Jha, P.K. & Muniappan, R. 2019. Predicting the distribution of an invasive weed *Ageratina adenophora* in Central Nepal using satellite images. XIX International Plant Protection Congress (IPPC) 2019. (10th- 14th November, 2019) Telangana, India.

3. Poudel, A.S., Shrestha, B.B., Jha, P.K., & Muniappan, R. 2019. Predicting the potential distribution of an invasive weed *Ageratina adenophora* under climate change scenarios in Chitwan-Annapurna Landscape, Nepal. National Climate Conference. (31st Dec 2018-1st January 2019) Guphadaanda, Sindhupalanchowk.

Poster presentation

1. Poudel, A.S., Shrestha, B.B., & Jha, P.K. 2017. *Ageratina adenophora* (Asteraceae) invasion: current state of knowledge and prospect for future research. International Conference on Biodiversity, Climate change and Livelihood, 2017. (10-12th January, 2017) Kathmandu, Nepal.

2. Poudel, A.S., Shrestha, B.B., Jha, P.K., & Muniappan, R. 2018. Functional traits variation of an invasive weed *Ageratina adenophora* (Sprengel) R. King and H. Robinson along elevation gradients in central Nepal. 10th HOPE Meeting with Nobel Laureates. Japan. (11th-15th March, 2018) Yokohama, Japan.

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Biology and management of the invasive weed *Ageratina adenophora* (Asteraceae): current state of knowledge and future research needs

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Summary

Biological invasion is increasing worldwide and the management of invasive species is becoming an important priority for vegetation managers. Success of invasive species management depends on a thorough understanding of the biology of the organism in question and the effectiveness of current management efforts, in order to identify the best practices for management improvement. In this review, we synthesised current biological knowledge of a noxious invasive weed *Ageratina adenophora* to identify knowledge gaps and assessed management efforts to identify best practices. Finally, we proposed some priority areas for future research to fill knowledge gaps and improve management. Our analysis showed that *A. adenophora* has already invaded 40 countries, mainly in Asia, Oceania, Africa and Europe. Phenotypic plasticity, allelopathic interference and invasion-mediated changes in the soil microbial community are the proposed mechanisms that facilitate rapid spread of this weed. However, allelopathy as a mechanism of invasion success of this weed has not been supported by ecologically meaningful experiments. Though mechanical, chemical and biological control measures have been used, their success remains limited and the weed continues to spread in new regions. Among seven biological control agents examined to date, gall fly (*Procecidochares utilis*) and leaf spot fungus (*Passalora ageratinae*) have been effective in limited areas to suppress growth of this weed. Some perennial native grasses (e.g. *Setaria sphacellata* and *Lolium perenne*) have shown potential to competitively suppress *A. adenophora*. In conclusion, understanding the invasion mechanisms, exploring further to identify effective biological control agents, combined with approaches of ecological restoration, could help in the management of this weed.

Keywords: Biological invasion, Crofton weed, control measures, *Procecidochares utilis*, research gaps.

Poudel AS, Jha PK, Shrestha BB & Muniappan R (2019). Biology and management of the invasive weed *Ageratina adenophora* (Asteraceae): current state of knowledge and future research needs. *Weed Research*. https://doi.org/10.1111/wre.12351

Introduction

The problem of invasive alien species has escalated in recent years across all taxonomic groups and geographic areas (Seebens *et al*., 2016). Furthermore, about 17% of global land area and 16% of global biodiversity hotspots are highly vulnerable to invasions (Early *et al*., 2016). Plant invasions reduce biodiversity and alter ecosystem

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process, leading to economic losses and ecological imbalances (Holmes *et al*., 2009; Vilà *et al*., 2011; Pysek *et al*., 2012).

Among several invasive plants, *Ageratina adenophora* (Sprengel) R. King and H. Robinson (Asteraceae; synonym: *Eupatorium adenophorum* Spreng.; common name: crofton weed) is one of the most problematic weeds in Asia, Oceania and Africa with serious ecological impacts and economic losses (Wu *et al*., 2004; Ding *et al*., 2007; Muniappan *et al*., 2009; Baral *et al*., 2017). The genus *Ageratina* has at least 197 species that are mostly native of the tropical and subtropical regions in the Americas (King & Robinson, 1970 as cited by Clewell & Wooten, 1971). A congener *Ageratina riparia* (Regel) R.M. King & H. Rob. is also an invasive weed in Hawaii (USA) and Oceania (Nakao *et al*., 1975). *Ageratina adenophora* possesses several traits which are the characteristics of an 'ideal' weed (*sensu* Baker, 1965), such as the production of numerous seeds (Parsons & Cuthbertson, 2001), adaptation to short- and long-distance seed dispersal (Wang *et al*., 2011), discontinuous germination and prolonged seed viability (Shen *et al*., 2011), high stress tolerance (Li *et al*., 2008; Rivera *et al*., 2017), high phenotypic plasticity (Zhao *et al*., 2012) and competitive ability through special means such as allelopathy (Inderjit *et al*., 2011). It was ranked first among problematic invasive weeds in Nepal (Tiwari *et al*., 2005) and one of the two invasive weeds prioritised by local communities for management in natural ecosystems (Shrestha *et al*., 2018). Though different management strategies have been employed, their success has remained low and the weed has continued to invade new regions (e.g. Del Guacchio, 2013). This probably reflects a gap in understanding the invasion mechanism of the weed and low effectiveness of the current control measures. Periodic review of published references is essential to synthesise the current state of knowledge and identify gaps for future research. There are a few reviews published on *A. adenophora* that have explored the potential invasion mechanisms, impacts and control strategies implemented for this weed in China (e.g. Sun *et al*., 2004; Yang *et al*., 2017). However, besides China, the weed is problematic in Africa, Australia and other Asian countries (Muniappan *et al*., 2009). In this review, we examined all the relevant publications globally to (i) analyse distribution of *A. adenophora* in its introduced range, (ii) identify knowledge gaps through the synthesis of current biological knowledge, (iii) assess management efforts to identify best practices, and (iv) identify priority areas for future research to fill knowledge gaps and improve management of this invasive weed.

Geographic distribution, habitat and dispersal pathway

Ageratina adenophora was first introduced outside of its native range (México) as an ornamental plant to the United Kingdom (Europe) in 1826 (Auld & Martin, 1975) and then to Hawaii (USA) in 1860 (Muniappan *et al*., 2009) and Australia in 1875 (Auld & Martin, 1975). It is believed to have been introduced as a garden plant in India in 1924 (Tripathi *et al*., 2012), but herbarium specimens were collected as early as 1914 (based on digital image serial number 225216, deposited at Central National Herbarium, Calcutta). It has commonly been believed that the weed spread naturally to China during the 1940s from Myanmar along the international highway (Wang & Wang, 2006; Dong *et al*., 2008). It was first recorded from New Zealand in 1931 (Webb, 1987), Nepal in 1952 (Tiwari *et al*., 2005), South Africa in 1958 (Henderson, 2006) and recently Italy in 2013 (Del Guacchio, 2013). Currently, it has naturalised in 40 countries from tropical to temperate regions of the world (Fig. 1, Table S1) with the possibility of further spread in the introduced ranges (Wang & Wang, 2006; Tererai & Wood, 2014). Though the weed is native to the neotropics, it currently can be found from the northern temperate (Germany, latitude 53°N) to southern temperate regions (New Zealand, latitude −38°S; GBIF, 2018). After more than 100 years of introduction outside its native range, the invasive nature of *A. adenophora* was first reported in Australia during the 1940s, in Hawaii during the 1950s and in California (USA) during the 1960s (Auld, 1972). Currently, it is considered one of the worst invasive weeds in Asia, Africa and Oceania (Muniappan *et al*., 2009), while the invasiveness and its impact appear relatively low in Europe (Del Guacchio, 2013).

Current occurrence data of *A. adenophora* have been used to predict the potential areas of invasion in China, Western Himalaya and South Africa using ecological niche modelling. Subtropical zones of southern and south-central China have been predicted to be highly vulnerable to invasion by *A. adenophora* (Wang & Wang, 2006). The most influential factor governing the expansion of this weed in China under future climate change scenarios is reported to be the temperature during winter (Wang *et al*., 2017). In Western Himalaya, the suitable habitat of *A. adenophora* has been predicted to increase under future climate change scenarios (Lamsal *et al*., 2018; Thapa *et al*., 2018). It is expected to expand upslope from its current distribution reaching up to 3547 m a.s.l. by 2070 (Thapa *et al*., 2018). These studies have revealed the presence of climatically suitable areas outside the current distribution and predicted expansion of climatically suitable areas

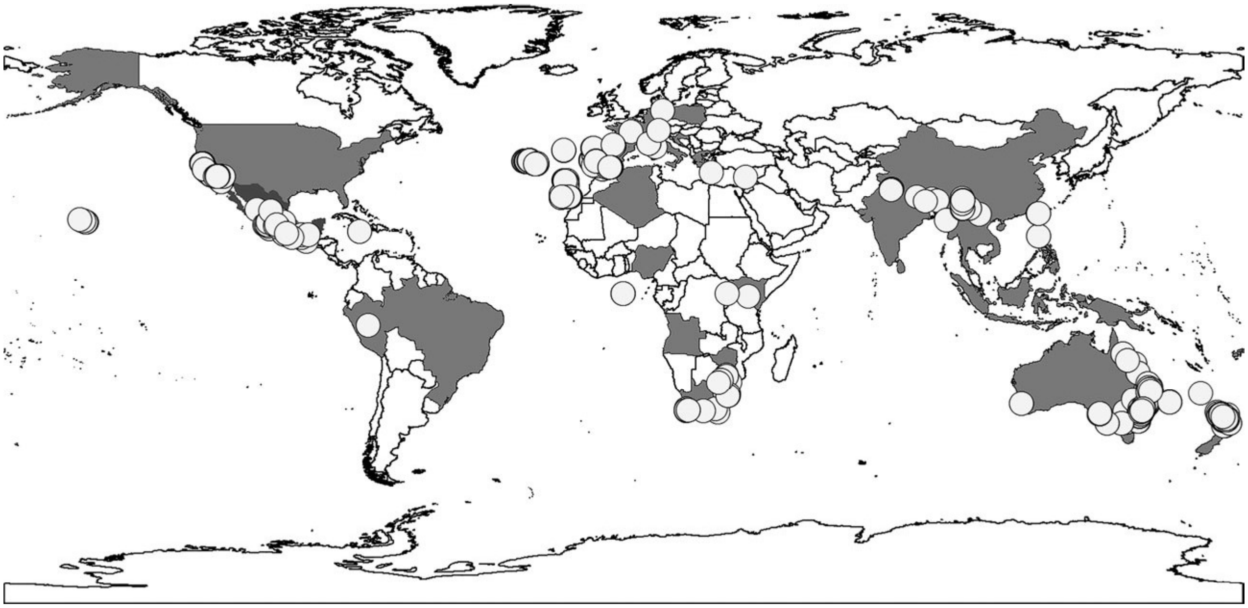

Fig. 1 A worldwide map of distribution of *Ageratina adenophora*. Countries shaded black indicate its native range, whereas countries shaded grey represent its introduced range. Black circles represent the occurrence locations available in GBIF (Source: CABI, 2018; GBIF, 2018; GRIIS, 2018).

of *A. adenophora* in future. Similarly, Tererai and Wood (2014) predicted that the areas with warm temperature and high precipitation are climatically suitable for invasion in South Africa.

Spatio-temporal trends in China showed that *A. adenophora* has spread from subtropical zones to much cooler and drier areas at higher elevation (Zhu *et al.*, 2007). In Nepal, the weed is now found up to 3280 m a.s.l. where there is snow for >1 month during winter Siwakoti *et al.* (2016). Epigenetic modifications might have contributed in conferring cold tolerance to this plant, leading to spread into the cooler regions of China (Xie *et al.*, 2015). In addition to this, the apomictic nature of the weed favours geographic parthenogenesis (a phenomenon in which asexual species often occupy larger and more northern distribution than their sexual counterparts) and might also have contributed to the wider and more northern distribution (Horandl, 2006; Vrijenhoek & Parker, 2009). A high proportion of niche expansion has been observed in *A. adenophora* that resulted from the change in the realised niche following the release from biotic constraints, rather than evolutionary change in the fundamental climatic niche (Dellinger *et al.*, 2016). Understanding the roles of epigenetic modifications and the apomictic nature of the species should be topics for future research to understand the niche expansion of this weed.

Ageratina adenophora colonises roadsides, railway embankments, banks of water bodies, crop fields, grasslands, plantations and forests (Tiwari *et al.*, 2005; Liu *et al.*, 2007; Baral *et al.*, 2017). It can grow both in full sun and highly shaded areas (Di Tomaso *et al.*, 2013), but growth performance is better in sun than in shade. Elevation ranges of the distribution of *A. adenophora* appear to be similar in native and introduced ranges. In its native range (México), it is found from 520 to 3200 m a.s.l. (Sang *et al.*, 2010), whereas in the introduced range, it has been reported between 330 and 2500 m a.s.l. in China (Wang & Wang, 2006) and 400 and 3280 m a.s.l. in Nepal (Siwakoti *et al.*, 2016; Shrestha, 2016). Similar elevation ranges in native and introduced ranges suggest the potential role of pre-adaptation for the invasion success of this weed (Sakai *et al.*, 2001; Schlaepfer *et al.*, 2010). The probability of its occurrence is, however, not uniform within this elevation range. It peaks at 1320 m a.s.l. in Western Himalaya and declines steeply to higher and lower elevations (Datta *et al.*, 2017). In general, the areas with warm temperature and high precipitation are suitable for its invasion (Tererai & Wood, 2014), while the dry valleys with high temperature (maximum temperatures >35°C) and low precipitation (<800 mm year^{-1}) are unsuitable for this weed (Sang *et al.*, 2010). There was complete failure of seed germination in regions characterised by hot summers (mean maximum temperatures *c.* 37°C) with dry spells (Datta *et al.*, 2017).

As mentioned above, *A. adenophora* was introduced as an ornamental plant in gardens, from where it escaped to natural habitats, spread rapidly and became established as an invasive weed. In the introduced ranges, roads and watercourses (e.g. river, streams) are the major dispersal corridors of this weed (Lu & Ma, 2006; Dong *et al*., 2008; Horvitz *et al*., 2014). Multiple factors, such as the dispersal of tiny and light seeds by water, wind, animals and vehicles, removal of native/resident vegetation during construction works and a heterogeneous landscape with a mosaic of suitable microhabitats, contribute to the rapid spread of this species (Wang *et al*., 2011). These factors have contributed to upslope as well as downslope dispersal in hilly and mountain landscapes. In plains areas, flood also facilitates long-distance dispersal (Wang *et al*., 2011).

Biology of *Ageratina adenophora*

Morphology

Ageratina adenophora is a multistem perennial herb with a woody rootstock, growing up to a height of 2 m. Individuals from introduced ranges have higher plant height, stem diameter, leaf length, leaf breadth and leaf area in comparison with individuals from native ranges (Feng *et al*., 2009). Longer vegetative growth phase, higher biomass accumulation, higher above/belowground ratio and higher coverage than native species enable this weed to establish monoculture stands (Gao *et al*., 2013). High invasiveness is also due to the production of a large number of seeds (i.e. 10 000 seeds per plant per season) which can disperse over long distances by wind (due to presence of pappus) or water (Parsons & Cuthbertson, 2001).

Reproduction and seed ecology

Ageratina adenophora primarily reproduces by seeds which are produced apomictically without fertilisation (Parsons & Cuthbertson, 2001). It is likely that there is failure of meiosis due to the autotriploid nature (with 51 chromosomes) of the plant, highly abnormal meiosis and high pollen sterility (Khonglam & Singh, 1980; Lu *et al*., 2008; Bala & Gupta, 2014). The seed length, breadth and mass vary from 1.37 to 1.69 mm, 0.29 to 0.51 mm, and 28.4 to 52.4 µg, respectively, and seed breadth and mass increase with increasing elevation between 640 and 2430 m a.s.l. (Li & Feng, 2009). The plant also reproduces vegetatively by stem fragments (Parsons & Cuthbertson, 2001). The relative importance of different reproductive modes appears to depend on environmental conditions. Under low light, it reproduces primarily by seeds, whereas under high-intensity light, it reproduces primarily by vegetative clones (Yu & Ma, 2006). Under favourable conditions, the weed can produce 7000–10 000 seeds per plant, of which 70–85% seeds are viable (Parsons & Cuthbertson, 2001). When the viable seeds are buried in the soil, due to unfavourable environmental condition, *ca*. 70% of them enter enforced dormancy and the remaining proportion into secondary dormancy, thereby increasing the longevity of seeds (Yadav & Tripathi, 1982). The seeds on the soil surface survive for only one germination season (about 5 months), whereas at soil depths of 5–10 cm they remain viable for 4 years (Shen *et al*., 2011). In stands dominated by *A. adenophora*, 57% of seeds are found in 0–2 cm soil layer, 24% in 2–5 cm and 19% in 5–10 cm layer of soil (Shen *et al*., 2006). Seed germination occurs in 3 days after planting (Shen *et al*., 2011) and in a wide range of temperatures (10–25°C), 25°C being the optimum (Lu *et al*., 2006). In Petri dish experiments, maximum germination was reported at pH 5.7 (Lu *et al*., 2006). Seeds of *A. adenophora* are considered moderately photoblastic with only 17% germination in the dark (Lu *et al*., 2006). However, higher seed germination was reported under low light (25–28% of full light) than under full light (Wang *et al*., 2006, 2012).

Eco-physiology

Ageratina adenophora has a C_3 photosynthetic pathway (Liu *et al*., 1988). Seedlings of this weed can tolerate shading up to 10% of daylight intensity, making it a good competitor in shade (Auld & Martin, 1975). In low light conditions, it increases biomass allocation to leaves and has high specific leaf area (SLA) and attained highest relative growth rate at 55% of relative irradiances (Sun *et al*., 2006). When compared with native species across different irradiances (10%, 23%, 40% and 100%), *A. adenophora* exhibited higher relative growth rate, biomass, leaf area and cover (Zheng *et al*., 2009; Fu *et al*., 2010). However, the performance of the weed decreased with the decreasing irradiance, raising the possibility of inhibiting its invasion at lower irradiances (Zheng *et al*., 2009). Further research studying its growth performance in <10% irradiance might answer this possibility. *Ageratina adenophora* has hypostomatic leaves with a stomatal density of 314 mm^{-2} (Rivera *et al*., 2017). The hypostomatic leaf, which accounts for the minimisation of water loss, is one of the indicator traits of stress avoidance strategy of this weed (Rivera *et al*., 2017). In low moisture conditions, the plant shows flexibility in stomatal function and water utilisation, allocating more resources to leaf, stem and seed production and reducing the allocation to roots (Yadav & Tripathi, 1985; Zu *et al*., 2005). Resource allocation also varies between populations. Individuals from the introduced range allocated more nitrogen in photosynthesis than to cell walls in comparison with individuals from the native range. This increased the plant growth and vigour of the populations from the introduced range (Feng *et al*., 2009). This provided evidences in support of the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey & Notzold, 1995). In addition, individuals from introduced populations showing higher light-saturated photosynthetic rate, higher photosynthetic energy use efficiency and shorter payback time of leaf construction cost than the native population also provide a mechanistic explanation on how the decreased allocation to defence increased allocation to growth (Feng *et al*., 2011).

Ageratina adenophora prefers temperatures in the range of 10–25°C (Tererai & Wood, 2014). However, in the introduced range, freezing tolerant (−5°C) populations have been also reported (Li *et al*., 2008). The evolution of this freezing tolerant population is attributed to the epigenetic variation in ICE1 demethylation (Xie *et al*., 2015). Experimental warming increased the biomass production and canopy of *A. adenophora,* as well as caused lower mortality in comparison with its native neighbours (He *et al*., 2012). This suggests that global warming may enhance invasion of *A. adenophora* by increasing its growth and making it stress tolerant. Mild fire increases the population density and longevity of individuals of this weed (Tripathi & Yadav, 1987). Different vegetative and reproductive traits of *A. adenophora* (the plant height, biomass, flower number per plant, branch number, seed production, seed germination rate) varied with latitude or elevation, whereas in reciprocal transplant experiments, no variation was seen, suggesting that phenotypic plasticity rather than local adaptation have enabled this weed to invade a wide range of habitats and climatic regions (Zhou *et al*., 2009; Zhao *et al*., 2012; Datta *et al*., 2017). Apomixis and pre-adaptation might be the possible reasons behind the lack of local adaptation in this weed (Datta *et al*., 2017).

Phytochemicals and allelopathy

Ageratina adenophora synthesises a number of secondary metabolites. Until now, eight monoterpenoids, three diterpenoids, six triterpenoids, 26 sesquiterpenes, six steroids, eight flavonoids, 25 phenolic acid derivatives, as well as other compounds such as fatty acids, alcohol, anhydrides and ketones, are known from this plant (Ma *et al*., 2015). These secondary metabolites are present either in aerial parts or in roots of *A. adenophora*, aerial parts being the major reservoir (Table S2).

A number of laboratory bioassay involving Petri dish experiments have been conducted to assess the role of allelopathy in invasion success of *A. adenophora* (Tripathi *et al*., 1981; Baruah *et al*., 1994). Out of 23 references related to allelopathy of this weed, 21 were based on Petri dish experiments. However, these bioassays have little or no ecological significance because in field conditions various biotic and abiotic environmental conditions determine the fate of allelochemicals (Inderjit & Weston, 2000). Considering these limitations, only a few recent studies have used ecologically meaningful experimental design to assay allelopathy. Inderjit *et al*. (2011) showed that volatile organic compounds (VOCs) from litter of *A. adenophora* had stronger inhibitory effects to species from non-native ranges of the weed (China and India) than to species from native ranges (México) and that the VOCs differed quantitatively between the native and invasive ranges. This partly explains the role of VOCs in invasion success of *A. adenophora,* supporting the Novel-Weapon hypothesis of invasion (Callaway & Ridenour, 2004). Understanding the amount and persistence of specific potential allelochemicals is also prominent in allelopathy research. Four phytotoxins which are found in very low concentration in soil invaded by *A. adenophora* degrade rapidly in 1 week (Yang *et al*., 2016). In addition, research suggested that soil microflora also reduce the allelopathic influence of *A. adenophora* by degrading allelochemicals released by the weed (Zhu *et al*., 2011). Even if the allelochemicals are degraded by microbial activity within a short duration, there may be some lag period between degradation and release of allelochemicals that may be sufficient enough to suppress the growth of native species. Therefore, future research aiming to reveal invasion mechanism of this weed should focus on understanding the lag period between the release and degradation of allelochemicals.

Genetic variation

Studies on genetic structure and genetic variability of invasive weeds provide a useful insight in understanding its invasion history, dispersal routes and patterns (Sakai *et al*., 2001). Limited research has been carried out in this field with respect to *Ageratina adenophora*. The chloroplast genome of *A. adenophora* is 150 689 bp in length which is found to be smaller than in other members of Asteraceae (Nie *et al*., 2012). Small genome size is often associated with traits favouring invasion (Suda *et al*., 2015). However, there is no information about the nuclear genome size of *A. adenophora* until now. Because reduction in genome size is considered to enhance the phenotypic variation in fitness-related traits of invasive species (Lavergne *et al*., 2010), further research on genome size of different populations of *A. adenophora* (native and introduced ranges) may help to explain the role of genome size of *A. adenophora* in its invasion.

Research carried out by using intersimple sequence repeat (ISSR) markers and amplified fragment length polymorphism (AFLP) method in China exhibited genetic variations in the population of *A. adenophora* (Gui *et al.*, 2008; Huang *et al.*, 2009). This genetic diversity or genetic distance increased with increasing geographic distance and elevation (Gui *et al.*, 2008; Huang *et al.*, 2009). More genetic heterogeneity within populations of *A. adenophora* explains the possibility of multiple introduction in China (Huang *et al.*, 2009). Furthermore, genetic diversity in apomictic populations can be attributed to the accumulation of mutations within clonal lineages, chromosomal arrangements, facultative sexuality within apomicts and multiple evolutionary origins from genetically variable sexual ancestors (Horandl & Paun, 2007; Vrijenhoek & Parker, 2009).

Impacts of invasion

Ecological and economic impacts

Ageratina adenophora has caused significant ecological impacts in the invaded regions. The weed has invaded forests, grasslands and wetlands where it forms monodominant stands and reduces cover and diversity of native plant species (Ding *et al.*, 2007; Niu *et al.*, 2007a; Gao *et al.*, 2013; Baral *et al.*, 2017). In forest understory, it suppresses growth of tree seedlings and displaces native plant species (Fu *et al.*, 2018). Due to its ability to suppress seedling establishment of tree species, it is commonly known as *Banmara* in Nepal that literally means ‘forest killer’ (Tiwari *et al.*, 2005). The relationship between cover of *A. adenophora* and native plant species richness varies with temporal and spatial scales. At small spatial scales (25 m^2) and early stages of invasion, communities with less native plant species richness are highly susceptible to invasion by this weed, whereas at large spatial scales (e.g. province), successful invasion of this plant is positively correlated with diversity of native species (Lu & Ma, 2005). Invasion by this weed has reduced crop production in agriculture land and forage production in the pastures (Parsons & Cuthbertson, 2001; Baral *et al.*, 2017). In addition, invasion by this weed also alters the structure of carabid (ground beetle) communities (Gu *et al.*, 2008).

Invasion by *A. adenophora* also changes the biochemical characteristics of soil. Invasion-induced changes include increase in number of vesicular-arbuscular mycorrhizal fungi, aerobic and anaerobic bacteria, fungi–bacteria ratio, as well as available nutrients such as nitrogen, phosphorus, potassium and organic carbon (Niu *et al.*, 2007a). These changes in structure and diversity of the soil microbial community create a new hospitable environment for the weed itself, resulting in the positive soil-biota feedback and hostility for the native species in the soil (Niu *et al.*, 2007a; Li *et al.*, 2009). It is also thought that bacteria having antagonistic activity might have helped this weed to escape its natural enemies and harmful soil-borne diseases (Niu *et al.*, 2007b). Furthermore, microbe-driven high nitrogen availability in soil also enhances biomass accumulation of *A. adenophora,* favouring its invasion (Bajpei & Inderjit, 2013). The alteration of soil microbial community is mainly driven by the allelochemicals present in the leachates of *A. adenophora* (Liu *et al.*, 2010). Along with these ecological impacts, it has reportedly caused economic losses to agriculture, forestry and livestock husbandry (Wu *et al.*, 2004; Baral *et al.*, 2017). However, these economic losses are mostly anecdotal and the quantitative assessment of the economic losses due to *A. adenophora* invasion can be important topics for future research.

Health impacts

Ageratina adenophora has a poisonous effect on domestic animals, such as horses. It is known to cause respiratory disease in horses and may result in death, if horses continue to feed upon it for prolonged periods (O’Sullivan, 1979). The plant in the flowering stage is more toxic than in juvenile stages (O’Sullivan, 1985). Health hazards of this weed to humans and other livestock have not been reported. Instead, rural communities in Nepal use leaf leachates of this plant to treat cuts and wounds (Shrestha *et al.*, 2018), implying an antiseptic effect.

Control and management

Various measures have been implemented to control *A. adenophora*; they can be grouped into mechanical, cultural, chemical and biological methods. No single approach has been effective, but the integration of these approaches combined with ecosystem-based management has been found to be effective in minimising its impacts.

Mechanical control

Mechanical control can be done where the invaded sites are easily accessible. Hand pulling or digging with a mattock, along with the crown removal for the prevention of regrowth, is preferable for small invaded areas. For large areas, slashing followed by ripping or ploughing and sowing desirable competitive pasture species is thought to be an effective method for control (Parsons &

Cuthbertson, 2001; Trounce & Dyason, 2003; Di Tomaso *et al.*, 2013; CABI, 2018). However, these mechanical methods are practically impossible in mountain landscapes such as Himalayas, due to difficult terrain.

Cultural control

Cultural control includes any non-mechanical and non-chemical practices that help to suppress the weed establishment, reproduction, dispersal and survival. In Nepal, people harvest *A. adenophora* and use it for cattle bedding and composting (Tiwari *et al.*, 2005; Baral *et al.*, 2017; Shrestha *et al.*, 2018). The biomass of this weed is also used for making bio-briquettes (Baral *et al.*, 2017). In China, *A. adenophora* is used as a substrate to produce domestic fungus, as well as to make dye (Sun *et al.*, 2004). These practices of biomass exploitation may reduce seed output, but the effect is often localised and may not contribute to the control of this weed in the landscape.

Chemical control

Chemical control of *A. adenophora* can be performed by spraying herbicides such as glyphosate, fluroxypyr, 2,4-D amine, picloram + 2,4-D, picloram + triclopyr, dicamba + MCPA and metsulfuron methyl (Parsons & Cuthbertson, 2001; Di Tomaso *et al.*, 2013). They are most effective when the plant is in the vegetative stage and growing actively. *Ageratina adenophora* is capable of tolerating low doses (12, 24 or 60 g a.i. ha^{-1}) of picloram, but a high dose (120 or 240 g a.i. ha^{-1}) of this herbicide causes structural deformities of mitochondria and chloroplast, ultimately leading to death of this weed (Liu *et al.*, 2014). There are only a few studies that have compared effectiveness of different herbicides. Auld (1972) reported 2,4-D amine (0.6% solution) as more cost-effective than sodium chlorate (5%) when applied in autumn. Paraquat and glufosinate exhibit fast control of *A. adenophora* with short duration of efficacy, whereas the efficacy of picloram and triclopyr lasted for 360 days maintaining the efficacy at 90% (Zhu *et al.*, 2013). Chemical control using these herbicides is often considered costly, temporary and hazardous. However, a combination of slashing and herbicide applications followed by sowing competitive forage species is recommended to restore productivity of infested land (Trounce & Dyason, 2003).

Biological control

Introducing natural enemies as a biological control agent can be environmentally friendly, cost-effective and sustainable for controlling invasive species. Seven biological control agents have been proposed so far for the control of *Ageratina adenophora* (Table 1). Among them, *Procecidochares utilis* Stone, a gallfly native of México, was first introduced to Hawaii in 1945 (Bess & Haramoto, 1972). From Hawaii, it was transported to Australia and then to New Zealand, South Africa, India, Nepal, China and Thailand (Winston *et al.*, 2014). This gallfly was intentionally introduced to six countries [*viz*. Hawaii (USA), Australia, New Zealand, South Africa, Thailand and India], whereas it dispersed naturally from India to Nepal and China (Muniappan *et al.*, 2009). Generally, stem galling leads to stunted growth, low seed output. High gall intensity also results in plant mortality (Erasmus *et al.*, 1992; Muniappan *et al.*, 2009). The impact of this gallfly varies in different regions (Table 1). In Hawaii, the fly had significant effects on sites with low to moderate moisture, whereas in high rainfall areas, the fly had negligible effect on the weed (Bess & Haramoto, 1972). However, in other countries, the noticeable effect of this fly was not seen, which is ascribed to high levels of parasitism by native parasitoids (Muniappan *et al.*, 2009; Heystek *et al.*, 2011).

Another biological control agent is a leaf spot fungus *Passalora ageratinae* Crous & A.R. Wood [previously referred to as *Cercospora eupatorii* Peck*, Phaeoramularia eupatorii-odorati* (Yen) Liu and Guoor, *Mycovellosiella eupatorii-odorai* (Yen) Yen], which has also been established in seven countries (Table 1). This fungus is a native of México and was first reported in gallfly released sites in Queensland, Australia, in 1954, where it was accidentally introduced with a shipment of the gallfly from Hawaii (Dodd, 1961). This fungus might have also been accidentally introduced along with the gallfly in other countries (Muniappan *et al.*, 2009). The leaf spot fungus reduces photosynthetic rate, transpiration and chlorophyll content, thus reducing plant height and number of leaves and flowers (Yang & Guo, 1991; Guo *et al.*, 1992 as cited by Zhang *et al.*, 2008). However, these effects have only marginal impacts on the plant and could not slow down the rapid spread in the countries reported. A laboratory experiment has reported an additive interaction between a gallfly and a leaf spot fungus, increasing the stress on *A. adenophora* (Buccellato *et al.*, 2012). Synergistic effects of these two biological control agents need to be evaluated under field conditions.

Rust fungus *Baeodromus eupatorii* (Arthur) Arthur, leaf-feeding insect *Oidaematophorus beneficus* Yano and Heppner, Australian stem borer *Dihammus argentatus* Auriv. (a native of Australia) are also potential biocontrol agents. *Baeodromus eupatorii* is a native of México and was released in Australia in 2014 and caused extensive defoliation in *A. adenophora* within 6–12 months of

Table 1 Status of different biological control agents in different countries

Agent	Country	Source of introduction	Year of release/ first report	Established	Impact	References
Baeodromus eupatorii (Arthur) Arthur (Fungi: Pucciniosiraceae)	Australia	Mexico*	2014	Unknown	Unknown	CSIRO (2016)
Dihammus argentatus Auriv (Coleoptera: Cerambycidae)	Australia	Australia*	1950	Unknown	unknown	Dodd (1961), Muniappan *et al.* (2009)
Doryluss orientalis Westwood (Hymenoptera: Formicidae)	China	China*	2009	Unknown		Niu *et al.* (2010)
Oidaematophorus beneficus Yano & Heppner (Lepidoptera: Pterophoridae)	Hawaii, USA	Mexico*	1973	Yes	Slight	Winston *et al.* (2014)
Passalora ageratinae Crous & A.R. Wood (Fungi: Mycosphaerellaceae)	Hawaii, USA	Unknown source	1945	Yes	Unknown	Muniappan *et al.* (2009)
	Australia	Unknown source →Hawaii, USA	1954	Yes	Slight	Dodd (1961), Muniappan *et al.* (2009), Heystek *et al.* (2011)
	New Zealand	Unknown source →Hawaii, USA →Australia	1958	Yes	Unknown	Muniappan *et al.* (2009)
	India	Unknown source→Hawaii, USA → Australia→NewZealand	1963	Yes	Unknown	Muniappan *et al.* (2009), CABI (2018)
	Nepal	Unknown source →Hawaii, USA→ Australia→New Zealand→India	1973	Yes	Unknown	Muniappan *et al.* (2009)
	China	Unknown source→Hawaii, USA→ Australia→New Zealand→India→ Nepal	Unknown	Yes	Unknown	Muniappan *et al.* (2009), Heystek *et al.* (2011), Winston *et al.* (2014)
	South Africa	Unknown source → Hawaii, USA →Australia	1987	Yes	Slight	Muniappan *et al.* (2009), Heystek *et al.* (2011), Winston *et al.* (2014)
Procecidochares utilis Stone (Diptera: Tephritidae)	Hawaii, USA	Mexico*	1945	Yes	Variable	Bess & Haramato (1972), Winston *et al.* (2014)
	Australia	Mexico*→Hawaii, USA	1952	Yes	Slight	Winston *et al.* (2014)
	New Zealand	Mexico*→Hawaii, USA→Australia	1958	Yes	Slight	Muniappan *et al.* (2009), Winston *et al.* (2014)
	India	Mexico*→Hawaii, USA→ Australia→New Zealand	1963	Yes	Slight	Muniappan *et al.* (2009), Winston *et al.* (2014)
	Nepal	Mexico*→Hawaii, USA→ Australia→ New Zealand → India	1973 Fortutious introduction	Yes	Slight	Winston *et al.* (2014)
	China	Mexico*→Hawaii, USA→ Australia→NewZealand→India → Nepal	1984 Fortutious introduction	Yes	Slight	Winston *et al.* (2014)
	South Africa	Mexico* → Hawaii, USA → Australia	1984	Yes	Medium	Winston *et al.* (2014)
	Thailand	Mexico* → Hawaii, USA	1991	Yes	Slight	Winston *et al.* (2014)
Xanthaciura connexionis Benjamin (Diptera: Tephritidae)	Hawaii, USA	Mexico*	1955	No	No	Winston *et al.* (2014)

*Native source of the biological control agents; → refers to the route of transfer.

releases, indicating its high potential for control (CSIRO, 2016). However, research is ongoing in Australia to examine whether it is an effective biocontrol agent. The leaf-feeding insect *O. beneficus* was introduced from México to Hawaii for the control of *Ageratina riparia,* but later it was also reported in *A. adenophora* (Nakao *et al*., 1975; Conant, 1998). However, feeding damage of this insect on *A. adenophora* is accidental (Conant, 1998). *Xanthaciura connexionis* Benjamin is a fly native to México that was released in Hawaii in 1955 (Winston *et al*., 2014). Australian stem borer, *D. argentatus* is native to Australia, not México, and is a potential biological control agent of *A. adenophora* (Dodd, 1961). Similarly, *Doryluss orientalis* Westwood is a natural enemy of *A. adenophora* found in China (Niu *et al*., 2010). This polyphagous ant killed the whole plant by breaking off the nutrient exchange between shoots and roots in an experiment conducted in south-west China (Niu *et al*., 2010). Nevertheless, further research is needed to test the suitability of this ant to be used as a biocontrol agent. Thus, the control efficacy of these four agents is still unproven. It appears that none of the biological control agents used so far is effective when used individually. Their use in combination may be effective due to synergistic effect, but this aspect has not been evaluated and should be a topic for future research.

In recent decades, priority is being given to biological control of *A. adenophora* but Zhu and Sang (2008) revealed that herbivory by these agents could make this invader stronger. They found experimentally that defoliation of *A. adenophora* increased its competitive ability and reduced the growth of native species. Thus, they concluded that defoliation not only stimulated the compensatory growth responses but might have also stimulated the exudation of allelopathic chemicals from *A. adenophora* that helped to suppress the native neighbours.

Ecosystem-based management

Ecosystem-based approaches are gaining popularity for the management of invasive species; these include sustainable management, conservation and restoration of ecosystems for the benefit of all organisms (Strifling, 2011). Habitats with high diversity and cover of native species have strong resistance to invasion by *A. adenophora,* while disturbed habitats favoured invasions (Lu & Ma, 2005, 2006; Wan *et al*., 2010). Furthermore, the negative effects of *A. adenophora* can be reduced by increasing density of native plants (Thapa *et al*., 2016). Perennial grasses such as *Setaria sphacelata* (Schum) Stapf ex Massesey cv. Narok and *Lolium perenne* L. have potential to competitively suppress the growth of *A. adenophora* (Zhu *et al*., 2007; Jiang & Li, 2008). As high possibility of replacement control by competitive grasses has been found, adoption of this approach might help in control of this weed in seminatural ecosystems such as pastures.

Future directions for research and management

From the review of previous studies, we conclude that though a number of studies have been carried out to address invasion biology, impacts and management strategies, most of the studies were limited to China. In a systematic review, Yu *et al*. (2016) found 93% of studies on *A. adenophora* originated from China. Further studies covering all invaded biogeographic regions through international collaborations should provide valuable ecological insights into invasion mechanisms and potentially broadening our understanding of other biological invasions (Yu *et al*., 2016). The roles of phenotypic plasticity or genetic adaptation capability need to be further explored in different introduced ranges to understand their relative contribution to the invasion success of this weed (Zhao *et al*., 2012). Since previous studies on genetic variation in *A. adenophora* populations were undertaken only in China, similar studies in populations from other introduced ranges will help to explain the genetic basis of invasion success. There is a paucity of studies quantifying the impacts of the weed in different ecosystems, so there is a need to address this aspect as well. As most of the previous research aiming to establish allelopathy as a mechanism of invasion of this weed were based on laboratory bioassays without ecologically meaningful results, future research linking the roles of allelopathy and novel chemicals under field conditions needs to be evaluated. Most importantly, we suggest that management must be directed towards the use of biological control agents combined with approaches of ecological restoration for control of this weed in natural environments.

Conclusions

Ageratina adenophora has already invaded 40 countries of the world outside its native range and still has the potential of further spread. There is geographic bias in published research with most (>90%) of the past studies on this species being undertaken in China. Focusing on other invaded regions in Asia and Africa will help to better understand the invasion process of this weed. Biological traits exhibiting phenotypic plasticity and invasion-induced changes of soil microbial community contribute to its invasiveness. Though allelopathy has been considered as a mechanism used by this weed to suppress native species, field evidence of this mechanism is yet to be proven. There are only handful of studies carried out on the genetic structure, genetic variation and genome size of this weed. Similarly, there is very little published on the economic impacts of *A. adenophora*. Generally, biological control is considered cost-effective, but none of the seven biological control agents of this weed are effective in reducing abundance and preventing further spread of this weed. The possibility of using native plant species to competitively suppress *A. adenophora* is being evaluated under laboratory conditions, demonstrating the potential of controlling this weed and restoring invaded habitats. Overall, future research should be directed to field-based allelopathic assessments, studies on genetic structure and variation at large spatial scales, quantitative assessment of economic impacts, and investigation of efficacy of synergistic effects of multiple biological control agents combined with ecosystem-based management approach for the control of this weed.

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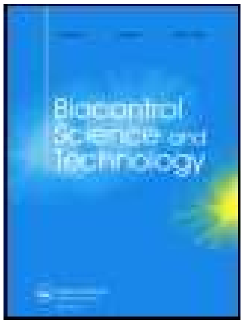
Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Occurrence of *Ageratina adenophora* in its native and introduced range

Table S2. Chemical constituents from different parts of *Ageratina adenophora*



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Stem galling of *Ageratina adenophora* (Asterales: Asteraceae) by a biocontrol agent *Procecidochares utilis* (Diptera: Tephritidae) is elevation dependent in central Nepal

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ABSTRACT

Gall inducers are widely used as biocontrol agents to suppress the vegetative and reproductive growth of invasive weeds. *Procecidochares utilis* Stone (Diptera: Tephritidae) is a gall forming fly that was released as a biocontrol agent against the noxious invasive weed *Ageratina adenophora* (Sprengel) R. King and H. Robinson (Asterales: Asteraceae). However, the effectiveness of *P. utilis* in controlling *A. adenophora* has been reported to vary according to geographic regions, with very low effectiveness in the Himalaya. In this study, we measured the abundance of *P. utilis* stem galling on *A. adenophora* along the elevation gradient (240–2965 m asl) in central Nepal. We found that elevation had a significant effect on gall abundance as well as gall size. Gall abundance and size peaked at mid-elevation (1940–2000 m asl). Stem galling by the fly reduced the stem diameter of the weed and all the three nutrients (N, P and K) in galls were significantly higher than in ungalled stems. Our study indicates that elevation is an important abiotic factor that strongly influences the *P. utilis* stem gall abundance, as well as gall size, and galls formed by *P. utilis* act as nutrient sinks. These findings provide insights in insect gall ecology, which may help the successful biocontrol of *A. adenophora*.

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Biological control; galling insects; gall abundance; gall morphology; nutrient sinks

Introduction

Control of invasive weeds using biocontrol agents is a growing strategy worldwide (Schwarzlander et al., 2018). Among different biocontrol agents, herbivorous especially gall-inducing insects are used in weed biocontrol since they retard the vegetative growth and reproduction of invasive weeds (Bitume et al., 2019; Erasmus et al., 1992; Florentine et al., 2005). Gall inducers are valuable biocontrol agents because they often have a narrow host range, and low probability of non-target impacts (Harris & Shorthouse, 1996). Interactions among gall-inducing insects used as a biocontrol agent, the host plant, and the environment determine the population dynamics of the gall inducers

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(Price & Hunter, 2015). Therefore, understanding the factors influencing population dynamics of gall inducers is central to the successful management of weeds (Clerck-Floate & Bourchier, 2000; McEvoy, 2018).

Infestation by galling insects results in the formation of plant galls that provide nutrition, protection, and shelter to the residing insects (Raman, 2007; Shorthouse et al., 2005). An estimated 132,930 species of gall-inducing insects have been reported globally (Espirito-Santo & Fernandes, 2007). Among these, *Procecidochares utilis* Stone (Diptera: Tephritidae) is a stem galling fly on one of the noxious invasive weed *Ageratina adenophora* (Sprengel) R. King and H. Robinson (Asterales: Asteraceae). The *P. utilis* fly is one of the seven biocontrol agents against *A. adenophora* that is established in eight countries outside of its native range (Poudel et al., 2019; Table S1). In Hawaii, *P. utilis* had significant negative impact on the plant but was not successful in high rainfall areas (Bess & Haramoto, 1959, 1972). Partial success in the control of *A. adenophora* infestation was achieved in NewZealand (Fowler et al., 2000). In Australia, *P. utilis* reduced the vegetative and reproductive vigour of *A. adenophora*, halting its rapid spread (Page & Lacey, 2006; Winston et al., 2014). Studies indicate that persistent and high population of galls are necessary to suppress the plants (Bess & Haramoto, 1959; Page & Lacey, 2006). Therefore, knowledge on *P. utilis* stem gall abundance is central to the successful management of the weed.

In Nepal Himalaya, *A. adenophora* is distributed across a wide elevation range (164–3280 m asl) (GBIF, 2019; Siwakoti et al., 2016). Environmental change along the elevation gradient produces variation in the suitability of the host plant, which affects the growth and development of insects residing inside it (Hodkinson, 2005; Korner, 2007). This ultimately shapes the distribution and size of galling insects (Hodkinson, 2005). Interactions among plant genotype, insect genotype, and the environment determine the gall size (Weis & Abrahamson, 1986; Weis & Gorman, 1990). There is evidence that galling insect survival and fitness depends upon gall size (Egan et al., 2011; Marchosky & Craig, 2004; Sopow & Quiring, 2001). Generally, maggots in small-sized galls are more susceptible to parasitism by parasitoids than in the large-sized galls (Marini-Filho & Fernandes, 2012; Weis et al., 1985). Also, the number of galling insects present inside the gall is positively correlated with the gall size (Sopow & Quiring, 2001; Tabuchi & Amano, 2004). However, knowledge of the role of elevation on gall inducers is poor. Thus, studies on the effect of environmental factors on gall abundance and size are of great ecological importance and will be useful for understanding the survival and fitness of galling insects.

The complex relationship between gall inducers and host plants has been well recognised and a number of hypotheses have proposed the adaptive nature of gall induction (Hartley & Lawton, 1992; Price et al., 1987; Stone & Schonrogge, 2003). The nutrition hypothesis states that plant galls are adaptations that provide insects with higher nutritional food sources in comparison to ungalled plant tissue (Price et al., 1986; Price et al., 1987; Stone & Schonrogge, 2003). The ability of the gall inducers as a biocontrol agent to harm host plants mainly depends upon the efficiency of galls to act as nutrient sinks (Harris & Shorthouse, 1996). Thus, establishment of sinks in galls deprives the host plant of resources that could have been otherwise utilised by the plant in growth and reproduction, exerting negative impacts upon the host plant fitness (Abrahamson & Weis, 1997; Marini-Filho & Fernandes, 2012; McCrea et al., 1985).

To our knowledge, the effects of elevation on the performance of *P. utilis* has not been reported. Some studies have indicated that galling by *P. utilis* causes reduction in shoot height, biomass, and reproductive potential of the weed (Buccellato et al., 2012; Buccellato et al., 2019; Erasmus et al., 1992), while its effect on stem diameter of the weed has not been explored. Furthermore, the ability of galls to act as nutrient sinks has not been examined in *A. adenophora*. The aim of the present study was therefore to examine the effect of elevation on gall abundance and gall size, investigate the nutrient sink efficiency of galls, and assess the impact of the gall fly on stem diameter of the weed. An increased understanding on insect gall ecology will assist in the use of this biocontrol agent against *A. adenophora*.

Materials and methods

Field sampling

A distribution survey of galls was carried out during November–December 2018 along the elevation gradients in two sites: Eklefaant-Simbhanjyang (hereafter called Simbhanjyang) (Dhading and Makwanpur districts) and Chandragiri (Kathmandu district) (Figure 1; Table S2). Two additional sites (Rasuwa and Chitwan districts) were also surveyed that represented the highest and lowest elevational range of *A. adenophora* distribution in Nepal Himalaya.

For data collection, three quadrats (10 m × 10 m, and 50 m apart from each other) were sampled in each elevation belt. One hundred ramets were counted in each quadrat and the gall abundance was noted as the number of ramets with galls (Smith et al., 2011). From

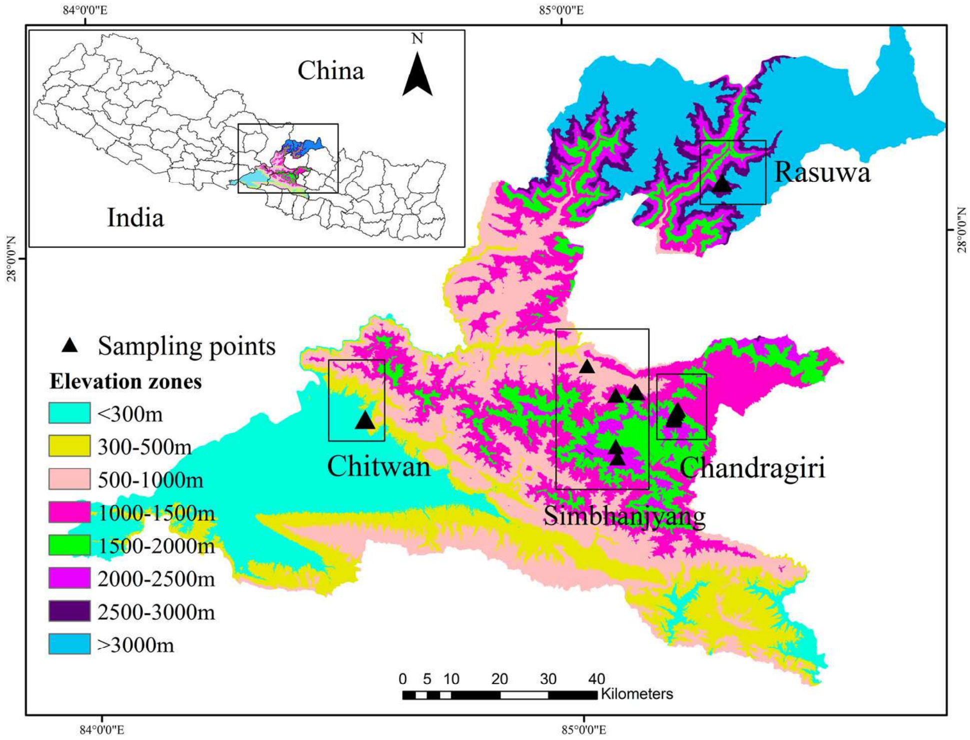

Figure 1. Map of central Nepal showing elevation zones and sampling sites.

each quadrat, 20 ramets having fresh galls without the emergence hole were selected. The length and breadth of each gall were measured using digital vernier calipers (WT 4171). The diameter of stems with galls was measured 1 cm below the gall (Marini-Filho & Fernandes, 2012). The ramet of the same bunch (i.e. genet) without a gall was considered to be a normal stem. The diameter of the normal stem was also measured at the same height as that of the galled stem. Galls and stem section of 5 cm in length (just 1 cm below the gall) were excised from the galled ramets. A similar stem section (5 cm) was also cut out from the normal stem at the same height as that of the galled stem (Tooker et al., 2008). In this way, from one genet, three samples were collected: gall, stem below gall (galled stem), and normal stem (ungalled stem). The samples were kept in a paper bag and allowed to dry in the shade until taken to the laboratory. From each elevation, altogether 60 (20 ramets × 3 sites) samples of gall, stem below gall, and normal stem were collected.

Laboratory analysis

From each gall sample, the gall flies in different stages were counted and removed carefully. Then the galls, galled, and ungalled samples were oven-dried at 80° C for 24 h (Florentine et al., 2005). The dried samples were ground into powder with an electric grinder. The samples from three different quadrats of the same elevation belt were mixed so as to have an adequate amount of plant materials for nutrients analysis. Then they were kept in the fridge (4° C) until further analysis. The samples were analysed for three essential nutrients: nitrogen (N), phosphorus (P), and potassium (K). Nitrogen was analysed using the Micro-Kjeldahl method (Horneck & Miller, 1998). Wet method using sulphuric acid and hydrogen peroxide was used for digestion of plant samples for the determination of phosphorus and potassium concentration (Campbell & Plank, 1998). Then, phosphorus was estimated calorimetrically using the vanado-molybdate method (Juo, 1978) and potassium by using a flame photometer (Horneck & Hanson, 1998). Nitrogen estimation was done in Regional Soil Testing Laboratory at Hetauda, whereas phosphorus and potassium were estimated in the Agricultural Technology Centre in Kathmandu.

Statistical analysis

All the statistical analyses were carried out in R version 3.4.4 (R Core Team, 2019). Values of gall abundance (number of galls per 100 ramets), nutrient concentrations, gall diameter and diameter of the stem with and without galls were evaluated for normality using Shapiro–Wilk test and for homogeneity of variance using Bartlett test. To examine the variation in gall abundance (response variable) with elevation (predictor variable), a generalised linear model with linear as well as quadratic terms was fitted using the Poisson distribution with logit function in R. We used Akaike Information criteria (AIC) to select the best model. For this, the data of galls from all sites were used to get a full distributional range of the fly ($n = 48$). The Pearson's correlation and a simple linear regression were used to analyse the relationship between number of larvae per gall and gall diameter. Similar analyses were used to examine the relationship between gall diameter and diameter of the stem with gall. Differences in gall diameter across different elevations were compared using analysis of variance (ANOVA) followed by post hoc Tukey test. To assess the impact of galling on stem diameter of the weed, Kruskal–

Wallis test was used. The nutrient data of samples from Simbhanjyang and Rasuwa was normal; therefore, nutrient data of these sites were compared using analysis of variance (ANOVA) followed by post hoc Tukey test. However, nutrient data of samples from Chandragiri were not normal; therefore, was subjected to Kruskal–Wallis test with Bonferroni's correction. The relationship between the three nutrients was analysed by using Pearson's correlation test.

Results

Effect of elevation on P. utilis *gall abundance*

The relationship between elevation and gall abundance ($n = 48$) was unimodal ($R^2 = 0.78$, $P < .0001$; Figure 2). Elevation explained about 78% of the variance in abundance of galls. The abundance of galls per 100 ramets were the highest in plots between 1940–2000 m asl,

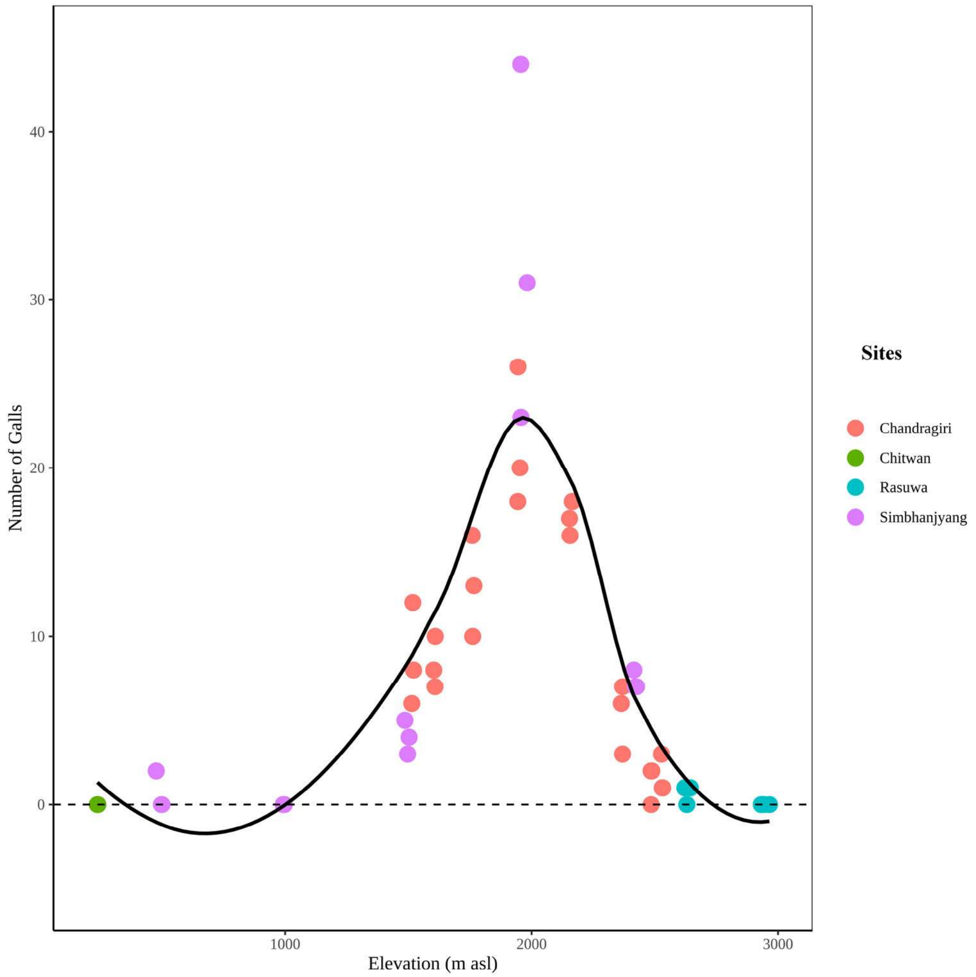

Figure 2. Relationship between gall abundance and elevation in central Nepal ($P < .001$, $R^2 = 0.78$). The line is fitted on the basis of generalised linear model of second order.

which is close to the mid-elevation of the entire elevation range of the distribution of *A. adenophora* (240–2965 m asl) in Nepal. Galls were absent at the highest (Dhimsha of Rasuwa district, 2965 m asl) and lowest (Jutpaani of Chitwan district, 240 m asl) elevational distribution range of *A. adenophora*.

Effect of elevation on P. utilis *gall size*

The gall diameter in Simbhanjyang was 13.33 ± 2.38 mm, whereas it was 14.50 ± 2.14 mm in Chandragiri. Change in gall diameter with elevation was different between the two sites. The gall diameter increased with the increasing elevation up to 2000m asl and then decreased after that in Simbhanjyang ($F_{1,236} = 57.31$, $P < .001$), where the elevation difference between the lowest and the highest sites was 1920 m asl (Figure 3(A)). However, in Chandragiri, with narrow elevation difference (980 m asl), the gall diameter did not vary significantly along the elevation gradient ($F_{1,344} = 5.134$, $P = .07$; Figure 3(B)).

The number of larvae per gall in the study area ranged from one to six. The number of larvae per gall increased with increasing gall diameter ($R^2 = 0.43$, $P < .001$ (Simbhanjyang); $R^2 = 0.67$, $P < .001$ (Chandragiri); Figure 4). Furthermore, the gall diameter was also significantly and positively correlated with stem diameter ($R^2 = 0.03$, $P < .001$ (Simbhanjyang); $R^2 = 0.08$, $P < .001$ (Chandragiri); Figure S1).

Effect of gall on stem diameter

The mean diameter of galled stems was significantly lower than stems without galls ($\chi^2 = 7.4$, d.f. = 1, $P < .006$ (Simbhanjyang); $\chi^2 = 24.2$, d.f. = 1, $P < .001$ (Chandragiri); Figure 5) in both of the sampling sites. The diameter of galled stem was 1.1 times smaller than the stems without galls (ungalled stem) in both the sites.

Nutrient concentrations

In Simbhanjyang, nitrogen concentration was higher in gall samples than in stems below galls and the normal stems without galls ($F_{2,42} = 61.69$, $P < .001$), whereas phosphorus concentration was significantly higher in gall samples than in normal stems without galls ($F_{2,42} = 3.958$, $P < .001$). However, potassium concentration did not differ among the samples ($F_{2,42} = 2.597$, $P = .0864$; Figure 6(A)). In contrast, all three nutrients (N, P_2O_5 and K_2O) were higher in galls than in stem below galls and normal stem without galls in Chandragiri ($\chi^2 = 40.913$, d.f = 2, $P < .001$ (N): $\chi^2 = 33.811$, d.f = 2, $P < .001$ (P_2O_5); $\chi^2 = 37.375$, d.f = 2, $P < .001$ (K_2O); Figure 6(B)). In Rasuwa (the highest elevation sampled), all the three nutrients were significantly higher in galls than in normal stem ($F_{2,6} = 483.7$, $P < .001$ (N); $F_{2,6} = 15.56$, $P < .001$ (P_2O_5); $F_{2,6} = 12.42$, $P < .001$ (K_2O); Figure 6(C)). However, the potassium concentration in gall samples did not differ with that of stem below gall while the difference was significant for nitrogen and phosphorus.

The concentration of all these three nutrients, i.e. nitrogen, phosphorus and potassium were significantly and positively correlated with each other in both sites (Table 1).

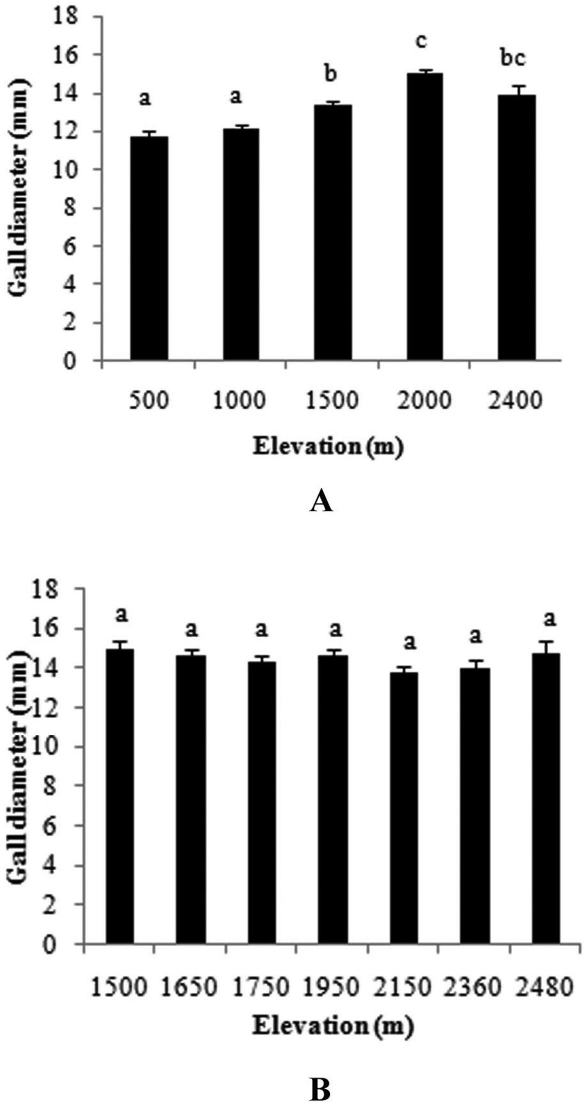

Figure 3. Gall diameter along the elevation gradient in central Nepal. (A) Simbhanjyang and (B) Chandragiri. Different letters above the bar denote significant differences among the samples from different elevation belts ($P < .001$, Tukey-HSD test).

Table 1. Correlation between mineral nutrients.

Gradients	Nutrients	
Chandragiri	P_2O_5	K_2O
K_2O	0.54***	
N	0.6***	0.64***
Simbhanjyang	P_2O_5	K_2O
K_2O	0.73***	
N	0.44***	0.41***

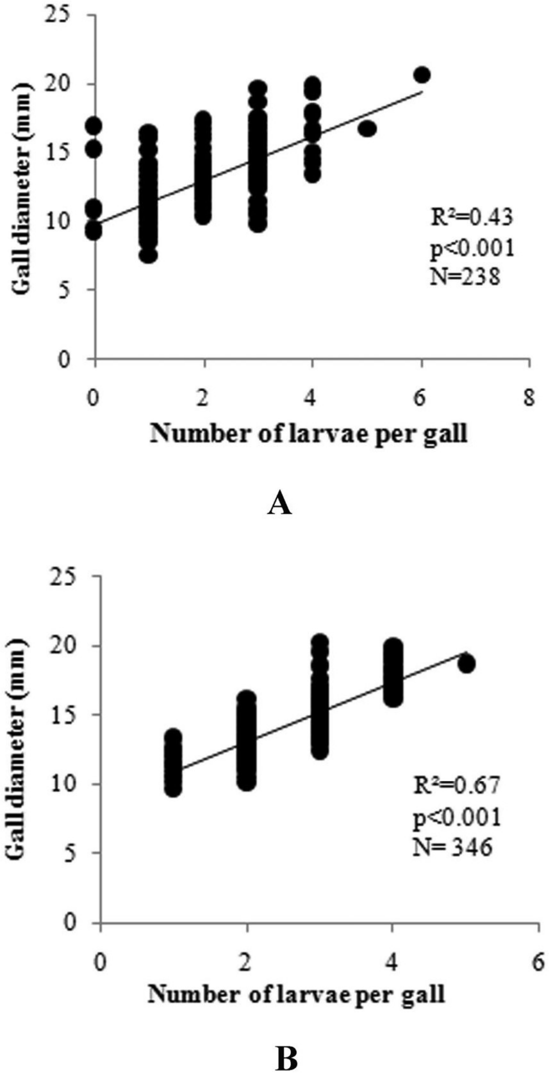

Figure 4. Relationship between number of larvae per gall and gall diameter in two sites in central Nepal. (A) Simbhanjyang and (B) Chandragiri.

Discussion

To, the best of our knowledge, this is the first study that examined the effect of elevation on *P. utilis* gall abundance. We found that gall abundance varied with elevation in a unimodal pattern, with the highest gall abundance at a mid-elevation range along the elevation gradient in central Nepal (240–2965 m asl). A similar pattern was reported for dung beetles (Coleoptera: Scarabaeidae) (Escobar et al., 2005), *Palgiometriona* species (Coleoptera: Chrysomelidae) (Flinte et al., 2011), and *Rhopalomyia solidaginis* (Diptera: Cecidomyiidae) (Crutsinger et al., 2013). This mid-domain effect can be explained by the 'Abundant-centre hypothesis,' which posits that species abundance is the highest at the centre of its range where the most favourable biotic and abiotic conditions occur, and gradually

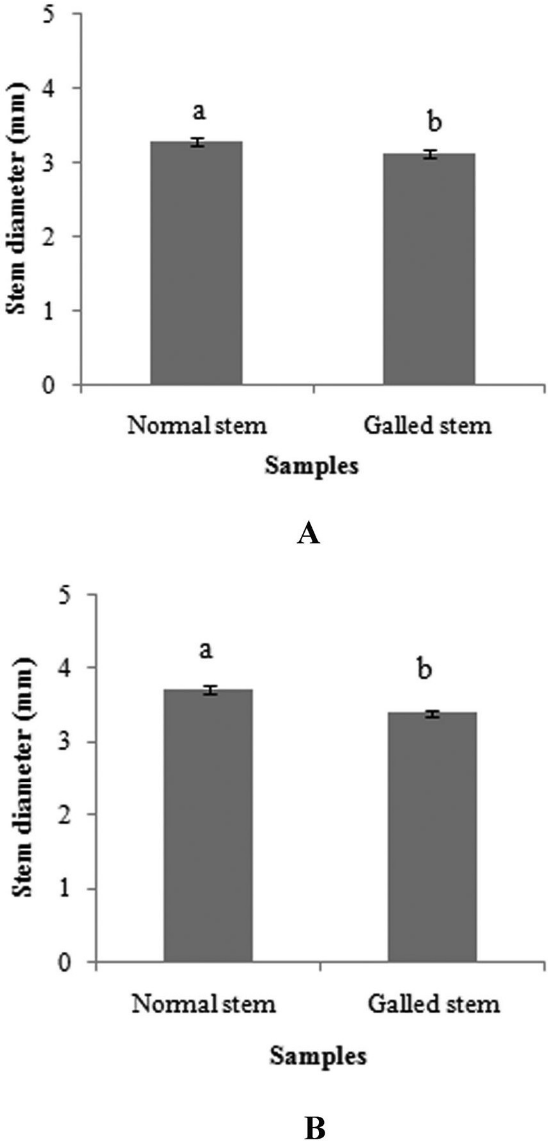

Figure 5. Diameter (mean ± S.E) of normal stem and galled stem in two sites of central Nepal. (A) Simbhanjyang, (B) Chandragiri. Means denoting different letter are significantly different ($P < .05$, Kruskal Wallis Test).

decline toward the edges (Brown, 1984; Hengeveld & Haeck, 1982). However, these assumptions have been frequently questioned because there are complex biogeographical processes like interspecific interactions, changing environmental conditions, temporal variation in abundance as well as life history traits of species acting together that determine the spatial abundance of the species along the environmental gradients (Dallas, Decker, & Hastings, Hastings, 2017; Santini et al., 2019). Nevertheless, these studies did not include gall inducers; therefore, we argue that the pattern of distribution of species abundance may be contextual and differ with taxa. The survey of distribution pattern of *A. adenophora*

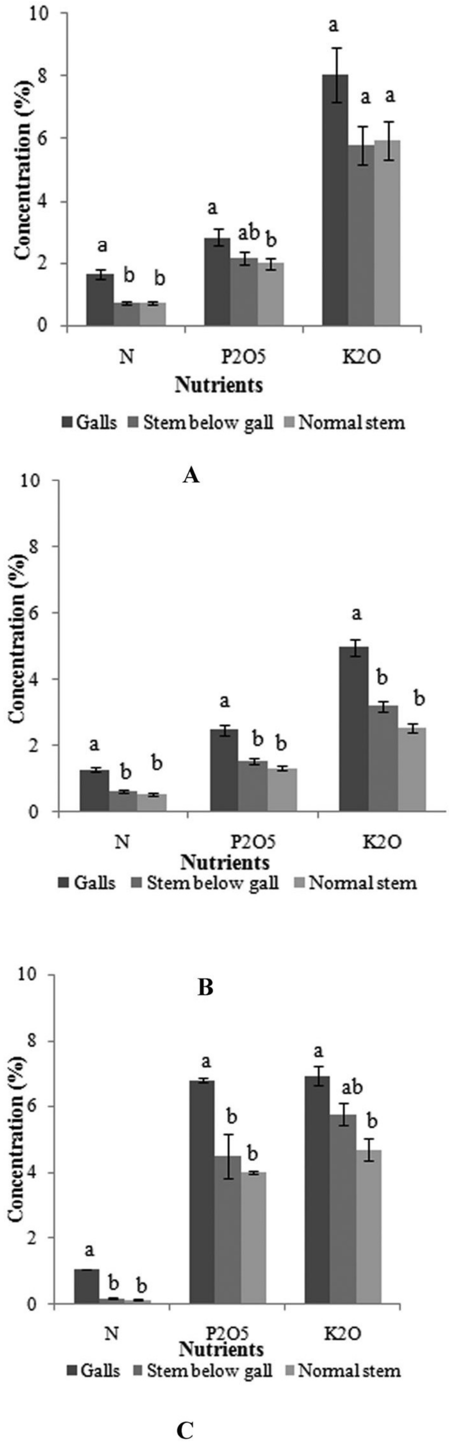

Figure 6. Nutrient concentrations (mean ± S.E) within gall samples, stem below gall and normal stem without gall in three sites of central Nepal. (A) Simbhanjyang, (B) Chandragiri, (C) Rasuwa. Means followed by different letter are significantly different ($P < .001$, Tukey-HSD test for normal data (Simbhanjyang and Rasuwa), whereas Kruskal–Wallis test with bonferroni's correction for non-normal data).

along the elevation gradient of 300–2500 m asl in western Himalaya also revealed a unimodal pattern of distribution, its probability of occurrence peaking at 1320 m asl (Datta et al., 2017). Although our study did not take weed abundance into account, previous research suggests that increased host plant abundance increases the gall abundance (Boaventura et al., 2018). This suggests the possibility of greater host plant abundance at the mid-elevation range in central Nepal.

Our results indicated that gall diameter varied along the elevation gradient, peaking at mid-elevation range when the elevation gradient of the study area is sufficiently wide. This is consistent with previous works (Hartmann, 1984: Smith et al., 2011). Marchosky and Craig (2004) reported a strong influence of environmental variation on the size of gall. The tritrophic interaction between plant genotype, insect genotype and the environment determines the gall size (Weis & Abrahamson, 1986; Weis & Gorman, 1990). Variation in abiotic factors like temperature, humidity, soil factors etc. along with the elevation gradient act as bottom-up forces that cascade upward, creating differences in the evolution of interaction of plants, galling insects, and their natural enemies (Craig et al., 2007; Rasmann et al., 2014). These differences in interaction among different trophic levels might have led to the variation in gall size. The largest gall diameter (15.05 ± 0.29 mm) measured in the present study is similar to that observed in South Africa (15.05 mm, Bennett & van Staden, 1986). As both gall abundance and gall size peaked at mid-elevation, this suggests that mid-elevation sites may have an optimal condition for the growth of *P. utilis*. Our observation that gall diameter increased with the increasing number of larvae per gall is also consistent with previous findings (Marchosky & Craig, 2004; Tabuchi & Amano, 2004). Furthermore, while gall size is positively related to survival and fitness of the galling insects (Marchosky & Craig, 2004; Sopow & Quiring, 2001), it can be assumed that larvae of *P. utilis* might have the greatest fitness at mid-elevation. However, this assertion needs further evaluation.

Galling by *P. utilis* reduces the vegetative and reproductive growth of *A. adenophora* (Buccellato et al., 2012, 2019; Erasmus et al., 1992). We also found that stem diameter, a growth-related variable, was reduced by the gallfly. Gall development alters the sink-source relationship in their host plants, which can reduce the photosynthetic capacity in the remaining ungalled tissues, placing overall metabolism of the host plant in stress (Raman & Abrahamson, 1995; Florentine et al., 2005). Reduction in stem diameter from galling has also been reported in other plants like *Solidago altissima* L. (Asterales: Asteraceae) by *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae) (Civettini et al., 1999), *Milicia excelsa* (Welw) C.C. Berg (Morales: Moraceae) by *Phytolyma lata* Scott. (Hemiptera: Psylidae) (Iroko gall fly) (Agyeman et al., 2009), *Genista monspessulana* L. (Fabales: Fabaceae) (invasive French broom) by *Lepidapion argentatum* Gerstaecker (Coleoptera: Brentidae) (weevil) (Bitume et al., 2019).

Gall inducers are able to intercept or redirect nutrients from other parts of the host plant to galls, making galls metabolic sinks (Abrahamson & McCrea, 1986; Bagatto & Shorthouse, 1991; Li et al., 2017; Marini-Filho & Fernandes, 2012; McCrea et al., 1985). Our result supports this assertion. The concentration of all three nutrients (N, P and K) in galls were higher than in stem section below the gall and normal stem without gall. This implies that galls acted as mobilising sinks by drawing nutrients from other parts of the host plant as reported by previous studies (Larson & Whitham, 1991; Marini-Filho & Fernandes, 2012; McCrea et al., 1985). Furthermore, an anatomical study reveals the fact that during gall formation, the innermost layer of the galls formed by *P. utilis* is transformed into a layer of nutritive tissue containing six to seven layers of cells with dense cytoplasm, large nuclei, and is highly mitotic (Bennett & van Staden, 1986). Besides, nutrient sink, a radiochemical experiment demonstrates that *P. utilis* gall also acts as strong sinks of photoassimilates (van Staden & Bennett, 1991). Formation of gall with strong sink capacity is often associated with significant harm to the host plants (Harris & Shorthouse, 1996). Therefore, our data and previous studies suggest that stem galling by *P. utilis* can have significant negative impact on *A. adenophora*. Our results corroborate the nutrition hypothesis which states that gall tissues have higher concentration of nutrients than the tissues without gall (Price et al., 1986, 1987).

Nitrogen is one of the limiting nutrients that plays a vital role in growth, development, reproduction, and survival of herbivores (Awmack & Leather, 2002; Mattson, 1980). Our result that gall tissues had higher nitrogen concentration in comparison to normal stem tissues is consistent with the previous findings (Cuevas-Reyes et al., 2011; Li et al., 2017). Similarly, phosphorus is another essential nutrient for the growth and development of herbivores (Cease et al., 2016; Perkins et al., 2004). We found that phosphorus concentration was also higher in gall tissues in comparison to ungalled tissues and this pattern is similar to the previous studies (Abrahamson & McCrea, 1986; Cuevas-Reyes et al., 2011; Tsao & Whaley, 1950). A high concentration of potassium in gall cells as we reported helps to maintain high negative osmotic potential, thereby increasing the turgor pressure, which helps in spontaneous bursting of the exit hole of the gall so that the insect can easily come out of the gall (Bagatto & Shorthouse, 1994).

Although this study considered elevation gradients as the major influential factor for the abundance and size of galls, there are other factors such as host plant density, age, architectural complexity, soil nutrients, and seasonality that are reported to affect the insect gall distribution (Boaventura et al., 2018; Cuevas-Reyes et al., 2011; Silva et al., 2015; Stokes & Stiling, 2013). Similarly, gall size is also determined by the interaction among plant genotype, insect genotype, and environment, and evidence also suggests that gall size (diameter) has evolved in response to selection by natural enemies (Weis & Abrahamson, 1985, 1986; Weis & Gorman, 1990). Therefore, incorporating all the bottom-up effects (e.g. host plant quality, quantity, and morphology), top-down effects (e.g. natural enemies like parasitoids), and the interaction between them would provide valuable knowledge on insect gall distribution, gall size, and population dynamics of galling insects, which ultimately determines the success of a galling insect as a biocontrol agent against weeds.

Conclusion

Using the data collected from sites covering the entire elevation range of distribution of *Ageratina adenophora* and its galling insect in Nepal, we showed that the abundance of galls peaked at the mid-elevation and declined towards higher and lower elevation regions, exhibiting a unimodal pattern. Overall, our results suggest that mid-elevation represents the optimal condition for the galling insect growth within the elevation range studied and galls induced by *P. utilis* on *A. adenophora* act as strong nutrient sinks. Since our study considered only one abiotic factor (elevation) in shaping the distribution of galls and their size, future studies that incorporate the effect of other abiotic factors like soil nutrition, seasonality, and biotic factors like natural enemies (i.e. parasitoids) on gall abundance and size will improve our understanding of the gall dynamics. Besides biotic and abiotic factors, future investigations should also consider how the interaction among these factors determines the distribution and size of galls, which determines the success of a galling insect as a biocontrol agent.

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Disclosure statement

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Predicting the Current and Future Distribution of the Invasive Weed *Ageratina adenophora* in the Chitwan–Annapurna Landscape, Nepal

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With increasing globalization, trade, and human movement, the rate of alien species introduction has increased all around the globe. In addition, climate change is thought to exacerbate the situation by allowing range expansion of invasive species into new areas. Predicting the distribution of invasive species under conditions of climate change is important for identifying susceptible areas of invasion and developing strategies for limiting their expansion. We used Maxent modeling to predict the distribution of one of the world's most aggressive invasive weeds, Ageratina adenophora *(Sprengel) R. King and H. Robinson, in the Chitwan–Annapurna Landscape (CHAL) of Nepal under current conditions and 3 future climate change trajectories based on 3 representative concentration pathways (RCPs 2.6, 4.5, and 8.5) in 2 different time periods (2050 and 2070) using species occurrence data, and bioclimatic and topographic variables. Minimum temperature in the coldest month was the most important variable affecting the distribution of* A. adenophora. *About 38% (12,215 km^2) of the CHAL area is climatically suitable for* A. adenophora, *with the Middle Mountain physiographic region being the most suitable one. A predicted increase in current suitable areas ranges from 1 to 2% under future climate scenarios (RCP 2.6 and RCP 8.5). All protected areas and 3 physiographic regions (Siwaliks, High Mountain, High Himalaya) are likely to gain climatically suitable areas in future climate scenarios. The upper elevational distribution limit of the weed is expected to expand by 31–48 m in future climate scenarios, suggesting that the weed will colonize additional areas at higher elevations in the future. In conclusion, our results showed that a vast area of CHAL is climatically suitable for* A. adenophora. *Expected further range expansion and upslope migration in the future make it essential to initiate effective management measures to prevent further negative impacts of this invasive plant.*

Keywords: *climate change; ecological niche modeling; habitat suitability; invasive weeds; Maxent.*

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Introduction

Biological invasions, a major driver of global environmental changes, are posing serious threats to global biodiversity and ecosystem functioning (IPBES 2019). Climate change is likely to further amplify the risks of biological invasions (Walther et al 2009; Bradley, Wilcove, et al 2010). Biological invasions and climate change act synergistically, and this synergistic relation between the 2 parameters of global change has been identified as a major threat to biodiversity (Dukes and Mooney 1999; Walther et al 2009; Mainka and Howard 2010). The profound negative impacts of invasive plant species on the diversity of native species, soil dynamics, and ecosystem processes, which cause ecological and economic losses, are well known (Marbuah et al 2014; Villa and Hulme 2017; Castro-Diez et al 2019). These impacts are likely to be further exacerbated by climate change, enhancing traits that promote invasiveness and creating a more hospitable climate for invasive species to cross geographic barriers, thereby facilitating range expansion in new areas (Dukes and Mooney 1999; Stacowicz et al 2002; Walther et al 2009; Bradley, Blumenthal, et al 2010; Bellard et al 2013). In comparison to native plant species, parameters of global change, such as increased temperature and CO_2 enrichment, enhance the performance of invasive species, imposing a threat of further spread (Liu et al 2017). Therefore, predicting the distribution of invasive weeds under climate change scenarios and identifying the areas potentially at risk are urgent needs for effective management planning to minimize ecological and economic impacts.

A first step to identify the risk of invasions is to use ecological niche models (ENMs) to predict suitable ecological niches for a species across a landscape. These relate documented presence records of the focal species with the environmental or spatial characteristics of the potential sites (Elith and Leathwick 2009; Franklin 2009). The niche

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concept is central to ENMs and is based on Hutchinson's (1957) concept of fundamental and realized niches (Araujo and Guisan 2006). It is highly likely that an invasive species at an early stage of invasion occupies only a small fraction (ie realized niche) of the fundamental niche in the introduced range, and there is always a risk of invasion in the unoccupied part of the fundamental niche (Soberon and Nakamura 2009). However, controversies persist over which facets of the niche are projected by ENMs (Araujo and Guisan 2006; McInerny and Etienne 2012). ENMs have been gaining popularity and are widely used by ecologists in invasive species risk assessments (Qin et al 2016; Suarez-Mota et al 2016; Wan et al 2017; Shrestha et al 2018; Thapa et al 2018). Among different ENMs, Maxent is one of the most popular species distribution modeling tools. This model uses presence-only records and has been commonly used in building habitat suitability maps for invasive species (Phillips et al 2006; Merow et al 2013; West et al 2016; Lamsal et al 2018).

Among 124 countries, Nepal has the third highest threat to agriculture sectors from invasive species (Paini et al 2016). To date, 179 species of flowering plants are naturalized, and, among them, 26 species are reported to be invasive in Nepal (Shrestha 2019; Shrestha, Budha, et al 2019). The crofton weed, *Ageratina adenophora* (Sprengel) R. King and H. Robinson (Asteraceae), is one of the most noxious invasive weeds in many parts of Asia, Oceania, and Africa. It has had serious ecological impacts on native biodiversity and caused enormous economic losses (Poudel et al 2019). It is ranked as the most problematic invasive weed in Nepal (Tiwari et al 2005).

Though many studies have been carried out to investigate the potential distribution of *A. adenophora* on broader spatial scales in Nepal (Shrestha and Shrestha 2019), China (Wang and Wang 2006; Wang et al 2017), the Himalayas (Lamsal et al 2018; Thapa et al 2018), and South Africa (Tererai and Wood 2014), there is a lack of such studies on smaller scales where management strategies are implemented. One of the most important landscapes in Nepal is the Chitwan–Annapurna Landscape (CHAL), located in central Nepal. CHAL harbors rich biodiversity due to its wide elevation gradient (200–8091 m above sea level [masl]), diverse topography, and climatic variations (subtropical to alpine) (WWF 2013; MFSC 2016). Invasion by alien species has already been recognized as a major threat to biodiversity in CHAL (WWF 2013), and *A. adenophora* is the invasive weed most prioritized by the local communities for management in natural ecosystems due to its negative impacts on biodiversity and livelihoods (Shrestha, Shrestha, et al 2019). Therefore, there is an urgent need to recognize potential areas of distribution of *A. adenophora* in CHAL under the current climate and identify areas at risk of being invaded by this weed under future climate scenarios.

In this study, we used the Maxent modeling tool to predict the current and future potential distribution of *A. adenophora* in CHAL using occurrence records from different sources. The objective was to prepare habitat suitability maps for the weed under current climatic conditions and future climate scenarios (RCP 2.6, RCP 4.5, and RCP 8.5 in the years 2050 and 2070) to identify the key environmental factors influencing its distribution and areas at risk of invasion. Information on its potential distribution will be very useful for the scientific community and managers in developing future monitoring and management strategies to prevent further expansion of the weed in this landscape.

Methods

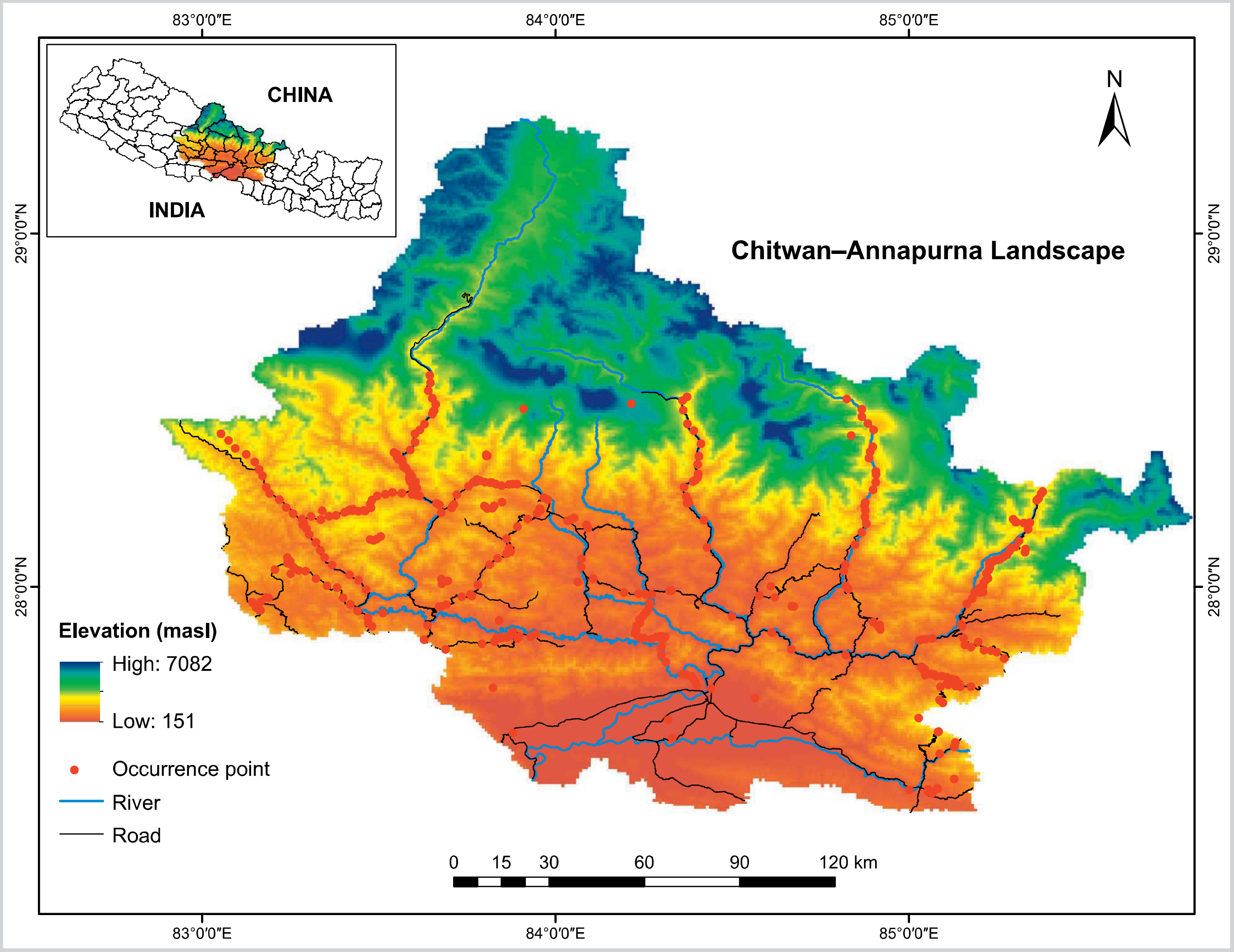
Study area

CHAL is located in central Nepal and covers 19 districts. This landscape has a wide elevation gradient, ranging from 200 to 8091 masl, and covers an area of 32,057 km^2 (WWF 2013). It spans 4 physiographic regions, namely, Siwalik, Middle Mountain, High Mountain, and High Himalaya. They have diverse climatic conditions, from subtropical in Siwalik to alpine in the High Himalaya, and a cold and dry climate in Trans-Himalayan regions. This geographic and climatic diversity shapes the habitat and environmental conditions for CHAL's rich biodiversity, which includes more than 104 species of mammals (Bhuju et al 2007), 500 species of birds (Baral and Inskipp 2005; Bhuju et al 2007), and 3430 species of plants, with high levels of endemism and genetic diversity (BPP 1995). Forests and grasslands are the main natural ecosystems, occupying 35.5% and 8.6% of the landscape, respectively, whereas 21.1% of the area is under agriculture (WWF 2013). The region has a population of 4.5 million people (CBS 2013). The average minimum and maximum temperatures are 5°C and 40°C, and the average annual rainfall ranges from 165 to 5244 mm (MFSC 2016). The landscape includes portions of 4 globally recognized ecoregions and comprises 3 national parks (Chitwan, Parsa, and Langtang) and 2 conservation areas (Annapurna and Manaslu) (MFSC 2016). Annapurna Conservation Area and Chitwan National Park are among the sites with a high number of visiting tourists (DNPWC 2018). Most of the lower and mid-hill forests of this region are at risk of fragmentation and conversion to other vegetation types due to climate change (Thapa et al 2015). In comparison to the eastern and western regions of Nepal, central Nepal, where CHAL is located, hosts higher numbers of naturalized plant species (Bhattarai et al 2014). The combination of diverse natural environment along with anthropogenic disturbances has made the region vulnerable to biological invasions (WWF 2013).

Study species

A. adenophora is native to Mexico but is established in 40 countries outside its native range (Poudel et al 2019). In Nepal, 26 invasive plant species are reported, 20 of which have been documented in the CHAL region, where *A. adenophora* is the most problematic weed in natural ecosystems, and its management is highly prioritized by local people (Tiwari et al 2005; Shrestha 2019; Shrestha, Shrestha, et al 2019). It was first reported in 1958, having been accidentally introduced from the eastern border of India to Nepal (Tiwari et al 2005). It covers a wide elevation gradient of 400–3280 masl in Nepal (Siwakoti et al 2016). The ability of *A. adenophora* to occupy a wide range of climatic habitats and spread rapidly can be attributed to its phenotypic plasticity, allelopathy, and ability to alter the soil microbial community to favor its further invasion (Poudel et al 2019). It has reduced forage supply, displaced native plant species, causing loss of biodiversity, and prevented forest regeneration in CHAL (WWF 2013; Shrestha, Shrestha, et al 2019).

FIGURE 1 Study area, with elevation zone, major rivers, road networks, and occurrence locations of *Ageratina adenophora* in the Chitwan–Annapurna Landscape, Nepal. (Source: Survey Department, Government of Nepal)



Species occurrence data

In total, 686 occurrence points were collected from different sources. We noted 245 occurrence points from secondary sources (Siwakoti et al 2016; Shrestha and Shrestha 2019). The remaining 441 occurrence points were collected by the first author during field visits in 2016–2019. Road networks are the major conduit for dispersal of *A. adenophora* (Dong et al 2008); therefore, occurrence data were mainly collected through field surveys along roadsides and trekking routes (Figure 1). This sampling bias was addressed by spatial filtering of the data. Duplicate records of occurrence points were deleted and spatially thinned using the spThin package (Aiello-Lammens et al 2015) in the R software (version 3.4.4) (R Core Team 2017), so that only 1 single location occurred in each 1 km^2 grid cell. Spatial filtering makes it possible to reduce overfitting to sampling bias in ENMs (Boria et al 2014). In total, 403 occurrence records obtained after filtering were used to build the models (Figure 1).

Environmental and bioclimatic variables

In December 2018, we downloaded 19 grid-based bioclimatic variables that represent annual trends, seasonality, and extreme climatic conditions from the WorldClim database (version 1.4) (www.worldclim.org; Hijmans et al 2005) at a spatial resolution of 30 arc-seconds ($\sim$1 km^2) (Appendix S1, *Supplemental material*, https://doi.org/10.1659/MRD-JOURNAL-D-19-00069.1.S1). Elevation was obtained from the Shuttle Radar Topographic Mission (SRTM) at 90 m spatial resolution. This was then resampled into 30 arc-second spatial resolution by using the nearest neighbor resampling technique in ArcGIS (version 10.3). Slope and aspect rasters of the study area were derived from the elevation data.

To predict climatically suitable areas in future climate scenarios, we chose projections from the Community Climate System Model (CCSM4) under b1 emission scenarios, which are based on the fifth phase of the Coupled Model Intercomparison Project5 (CMIP5) (Gent et al 2011). We selected 3 greenhouse gas (GHG) emission scenarios, also known as Representative Carbon Pathways (RCP 2.6, RCP 4.5, and RCP 8.5), for 2 different time periods (2050 and 2070) as adopted by the Intergovernmental Panel on Climate Change in its Fifth Assessment Report (AR5) (IPCC 2013). RCP 2.6, RCP 4.5, and RCP 8.5 represent the lowest, medium, and highest emission scenarios, corresponding to a 1.0°C, 1.4–1.8°C, and 2.0–3.7°C projected increase in global mean surface temperature, respectively (van Vuuren et al 2011; IPCC 2013).

The datasets were extracted for the study area (CHAL) using the Spatial Analyst Tool and the Extraction Tool in ArcGIS (version 10.3). These datasets were converted from

TABLE 1 Model evaluation matrices.

Measures	Value
Mean training AUC	0.85
Mean test AUC	0.80
TSS	0.52

raster format to ASCII files in ArcGIS. We repeated the procedure to prepare the predicted distribution maps for the 2 future climate scenarios (2050 and 2070).

Model building

Maxent (version 3.3.3) software was used to build the ENMs (Phillips and Dudik 2008). Maxent is one of the most commonly used habitat suitability modeling techniques. It uses presence-only data and is widely used for invasive species (Phillips et al 2006; Shrestha et al 2018; Maharjan et al 2019). Maxent works well with incomplete or limited data, so it can provide robust estimates of potentially suitable habitats for invasive species at small spatial scales (Jarnevich et al 2006; Jarnevich and Reynolds 2011; West et al 2016).

To reduce multicollinearity among predictor variables (19 bioclimatic and 3 topographic variables) and overfitting of the model, pairwise correlation analyses were performed in R, and highly correlated variables with a Pearson's correlation coefficient ≥ 0.8 were removed (Appendix S2, *Supplemental material*, https://doi.org/10.1659/MRD-JOURNAL-D-19-00069.1.S1) (Merow et al 2013). While selecting one variable from each pair of highly correlated variables ($r^2 > 0.8$), special attention was paid to existing biological and ecological insights into the species. Ultimately, 7 bioclimatic variables—isothermality (Bio 3), minimum temperature of the coldest month (Bio 6), temperature annual range (Bio 7), precipitation of the driest month (Bio 14), precipitation seasonality (Bio 15), precipitation of the warmest quarter (Bio 18), and precipitation of the coldest quarter (Bio 19)—and 2 topographic variables—aspect and slope—were used as predictors to build the habitat suitability model.

The Maxent model used 75% of the data for training and the remaining 25% for testing. We used a logistic format because it improves model calibration by estimating the probability of a species being present depending on environmental variables (Phillips and Dudik 2008). A convergence threshold of 10^{-5}, a maximum iteration value of 5000, 15 replications with a replicated run type subsample, and 10,000 random background points were used to build the model. As a threshold rule, we chose tenth percentile training presence on the basis of the area under the curve (AUC) and true skill statistic (TSS). Tenth percentile training presence omits the 10% of localities or training presence records with the lowest predicted values (Radosavljevic and Anderson 2014) and is highly conservative in estimating species tolerance with respect to each climatic variable (Svenning et al 2008). The remaining parameters were kept at their default values.

We imported the Maxent output, which is continuous data with values ranging from 0 to 1, into ArcGIS (version 10.3) and classified the map using the Reclassify Tool into 2 classes, suitable habitat and unsuitable habitat, on the basis of a tenth percentile training presence logistic threshold. In this way, a binary habitat suitability map was created for the current and all future climate scenarios. We also calculated the climatically suitable area for present and future climate scenarios, as well as changes in suitable areas in terms of gain, loss, and stable areas in the future under all scenarios in ArcGIS. Data for physiographic regions and protected areas were then clipped to projected maps with suitable areas for current and future climate scenarios to calculate the suitable areas in these physiographic regions and protected areas. Changes in the upper and lower elevational distribution range under future climate scenarios in comparison to current were quantified using the Extraction Tool and digital elevation model (DEM) raster for all maps, current and future, in ArcGIS.

Model evaluation

Threshold-independent (area under the receiver operating characteristic [ROC] curve [AUC]) and threshold-dependent (TSS) measures of model accuracy were used to evaluate model performance (Fielding and Bell 1997; Allouche et al 2006; Franklin 2009). AUC values range from 0–1.0, with 0.5–0.7 considered low, 0.7–0.9 moderate, and >0.9 high (Swets 1988; Manel et al 2001). The TSS value ranges from −1 to +1, where +1 indicates a perfect agreement, and 0 or less indicates a performance no better than random (Allouche et al 2006). Marginal response curves were used to visually investigate the relationship between environmental variables (predictors) and the predicted index of habitat suitability of *A. adenophora*. The relative contribution of different predictor variables to the Maxent model was assessed by the variable percentage contribution and jackknife procedures (Elith et al 2011). The jackknife test of variable importance helps to identify those variables with important individual effects (Elith et al 2011). Two jackknife tests were taken into account. The jackknife test of regularized training gain shows the training gain of a variable when used in isolation and the training gain of a variable when omitted, and it compares these values to the training gain of all variables. Similarly, the jackknife test of AUC based on the AUC of test data shows the predictive performance of the variable when used in isolation and the predictive performance of the variable when omitted, and it compares these values with the AUC value when all variables are used (Phillips 2017).

Results

Model performance and variable contribution

The current model for *A. adenophora* performed better than random, with a mean training AUC value of 0.85, mean test AUC value of 0.80, and a TSS value of 0.52 (Table 1). Responses of each predictor variable are shown in the response curve (Appendix S3, *Supplemental material*, https://doi.org/10.1659/MRD-JOURNAL-D-19-00069.1.S1). Out of 9 predictor variables used for model building, minimum temperature of the coldest month (Bio 6) contributed the most (48.7%), followed by precipitation of the warmest quarter (Bio 18). Aspect had the lowest contribution (2.9%) (Table 2). Maxent's jackknife test of variable importance also showed that minimum temperature of the coldest month (Bio 6) had the highest training gain and AUC,

TABLE 2 Relative contribution of the environmental variables to the Maxent model built for current climatic conditions.

Predictor variables[a)]	Percentage contribution	Permutation importance
Bio 6	48.7	50.1
Bio 18	19.4	8.1
Slope	6.6	9.5
Bio 7	5.9	8.0
Bio 15	5.7	3.4
Bio 19	3.8	5.0
Bio 3	3.5	8.8
Bio 14	3.4	2.7
Aspect	2.9	4.3

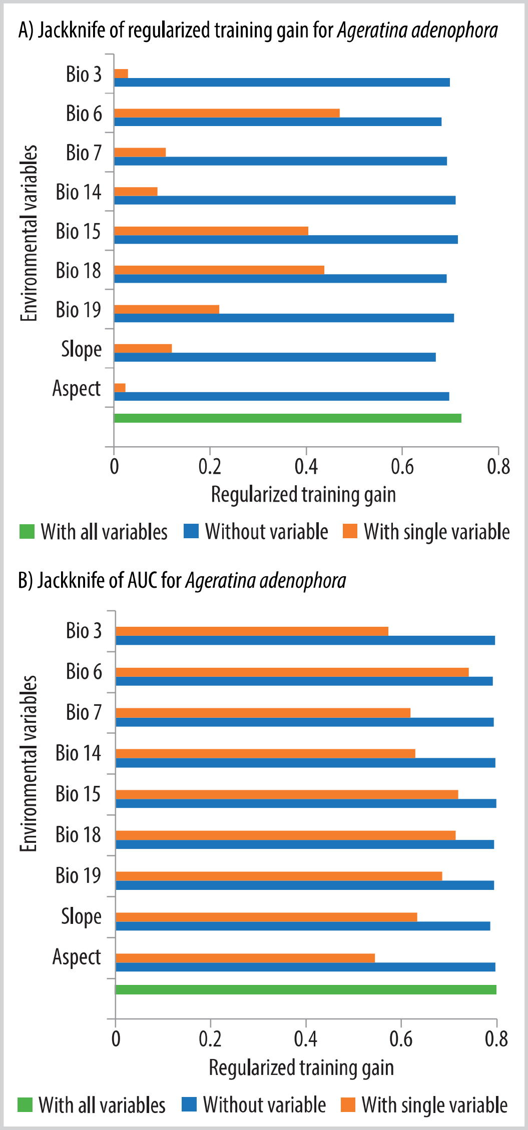
[a)] Bio 3, isothermality; Bio 6, minimum temperature of coldest month; Bio 7, temperature annual range (Bio 5–Bio 6); Bio 14, precipitation of driest month; Bio 15, precipitation seasonality (coefficient of variation); Bio 18, precipitation of warmest quarter; Bio 19, precipitation of coldest quarter.

followed by precipitation of the warmest quarter (Bio 18) when used in isolation (Figure 2). The response curve of the variable minimum temperature of the coldest month (Bio 6) showed that the probability that the weed will occur below 1°C was the lowest, increasing with increasing minimum temperature (Appendix S3, *Supplemental material*, https://doi.org/10.1659/MRD-JOURNAL-D-19-00069.1.S1). In addition, the minimum temperature of the coldest month of about 96% of the occurrence points used in model building was above 0°C (Appendix S4, *Supplemental material*, https://doi.org/10.1659/MRD-JOURNAL-D-19-00069.1.S1). Similarly, the response curve of precipitation of the warmest quarter (Bio 18) indicated that the probability the weed will occur increased with increasing precipitation in the warmest quarter above 500 mm (Appendix S3, *Supplemental material*, https://doi.org/10.1659/MRD-JOURNAL-D-19-00069.1.S1).

Current potential distribution

Currently, 38% (12,215 km^2) of the total area of CHAL is suitable for *A. adenophora* (Figure 3; Table 3). All districts of CHAL within the elevational range of 119–2824 masl had climatically suitable areas for this weed. The most suitable areas for *A. adenophora* were found to be in the Middle Mountain physiographic region (75%), followed by Siwalik (37%), High Mountain (29%), and High Himalaya (0.1%) (Table 4). High Himalaya is hardly suitable for the weed, with the fewest suitable areas found in the districts of Manang and Mustang. Nonetheless, all districts of the CHAL region were found to have climatically suitable areas. Because the Middle Mountain and High Mountain regions had the most suitable areas for the weed, we tried to observe the change in climatically suitable areas in 3 protected areas situated in these physiographic regions. Among the 3 protected areas—Annapurna Conservation Area, Langtang National Park, and Manaslu Conservation Area—Langtang National Park was predicted to have the highest percentage of area suitable for this weed (Figure 4).

FIGURE 2 Results of jackknife test of relative importance of predictor variables for *A. adenophora* for the current distribution. (A) Jackknife of regularized training gain. (B) Jackknife of AUC. Predictors used: Bio 3, isothermality; Bio 6, minimum temperature of coldest month; Bio 7, temperature annual range; Bio 14, precipitation of driest month; Bio 15, precipitation seasonality; Bio 18, precipitation of warmest quarter; Bio 19, precipitation of coldest quarter; slope; aspect.



Future invasion risk and change in habitat suitability

The predicted climatically suitable areas for *A. adenophora* would increase under RCP 2.6 for the year 2070 and RCP 4.5 for both the years 2050 and 2070 (Figure 3; Table 3). The highest increase (2%) in area of suitable habitat was predicted for the year 2070 under RCP 2.6 and 4.5. This gain in suitable areas was more prominent in districts like Lamjung, Gorkha, Dhading, Makwanpur, Chitwan, and Tanahun. However, an increase in radiative force (from RCP 4.5 to 8.5) would decrease climatically suitable areas for the weed in both the years 2050 and 2070 (Figure 3; Table 3).

Though the suitable area was predicted to decrease in extreme climate scenarios (RCP 8.5), the upper elevational distribution limit would expand by 31 m and 42 m for the years 2050 and 2070, respectively (Figure 5). Though a small decrease (24 m) in upper elevation limit was predicted under

FIGURE 3 Predicted suitable area for *A. adenophora* in the Chitwan–Annapurna Landscape, Nepal, under (A) current scenario; (B) RCP 2.6 for the year 2050; (C) RCP 2.6 for the year 2070; (D) RCP 4.5 for the year 2050; (E) RCP 4.5 for the year 2070; (F) RCP 8.5 for the year 2050; (G) RCP 8.5 for the year 2070. For future climate scenarios, likely stable, gain, and loss in areas are shown in bar graphs denoted by green, red, and yellow, respectively.

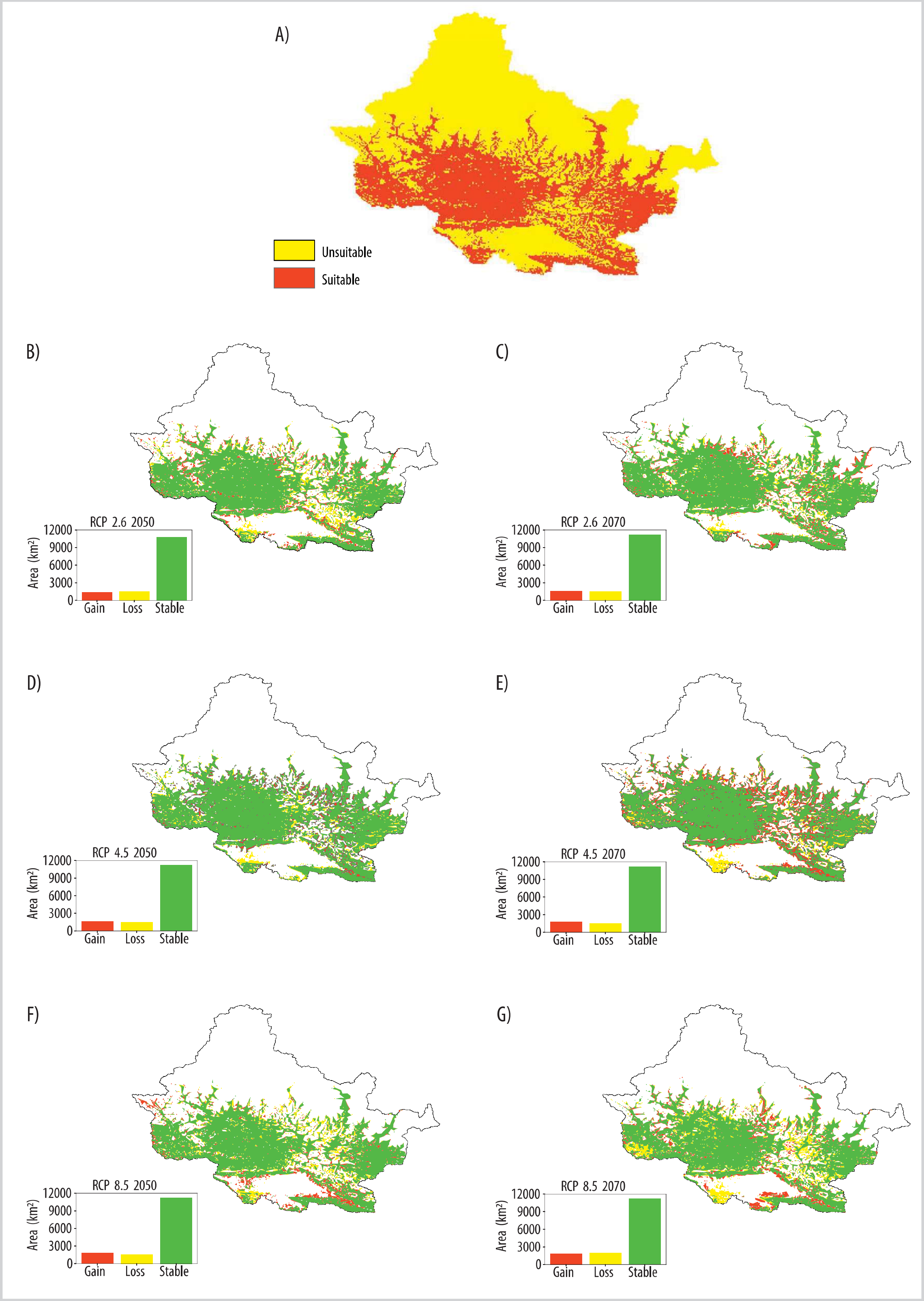
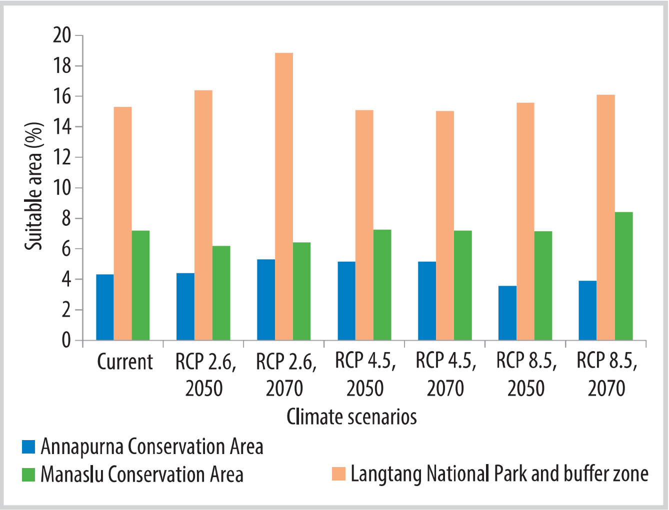


TABLE 3 Predicted climatically suitable area for *A. adenophora* under current and future climate scenarios in the Chitwan–Annapurna Landscape, Nepal.

Scenarios[a)]	Suitable area (km^2)	Suitable area (%)	Change in suitable area (%)
Current	12,215	38	
RCP 2.6, 2050	12,113	38	0
RCP 2.6, 2070	12,758	40	2
RCP 4.5, 2050	12,385	39	1
RCP 4.5, 2070	12,889	40	2
RCP 8.5, 2050	11,836	37	−1
RCP 8.5, 2070	11,823	37	−1

[a)] RCP 2.6 (2050 and 2070), Representative Carbon Pathway 2.6 (lowest emission scenarios) for years 2050 and 2070; RCP 4.5 (2050 and 2070), Representative Carbon Pathway 4.5 (medium emission scenarios) for years 2050 and 2070; RCP 8.5 (2050 and 2070), Representative Carbon Pathway 8.5 (highest emission scenarios) for years 2050 and 2070.

FIGURE 4 Change in suitable areas of *A. adenophora* in different protected areas of the Chitwan–Annapurna Landscape, Nepal, under current and future climate scenarios.



RCP 2.6 in 2050, the highest increase of 48 m was expected under RCP 2.6 for the year 2070 in comparison to current climatic conditions. However, under the medium emission scenario of RCP 4.5, the model predicted a contraction in the upper elevational limit for both years 2050 and 2070. In contrast, the lower elevation limit of *A. adenophora* would either remain stable or contract in future climate scenarios (Figure 5).

With climate change, all physiographic regions except the Middle Mountain region were expected to gain climatically suitable areas. Though a minimal loss in suitable areas was predicted for Middle Mountain, this region will still contain the most suitable areas for the weed, followed by High Mountain, Siwalik, and High Himalaya, in all future climate scenarios (Table 4). In 4 of the future climate scenarios—RCP 2.6 in 2050 and 2070, and RCP 8.5 in 2050 and 2070—Siwalik will gain climatically suitable areas. Except under RCP 8.5 for the year 2050, High Mountain will also gain suitable areas in all future climate scenarios. Furthermore, under RCP 4.5 and 8.5 for the year 2070, the climatically suitable area is also predicted to increase in High Himalaya. Among all physiographic regions, the percentage gain in suitable area was highest (2.4%) in Siwalik under RCP 8.5 for the year 2050 (Table 4).

TABLE 4 Predicted suitable area for *A. adenophora* in different physiographic regions of the Chitwan–Annapurna Landscape, Nepal.

Scenarios[a)]	Suitable area (%)			
	Siwalik	Middle Mountain	High Mountain	High Himalaya
Current	36.56	75.09	29.25	0.09
RCP 2.6, 2050	36.33	73.83	30.14	0.07
RCP 2.6, 2070	37.70	77.90	31.81	0.09
RCP 4.5, 2050	34.06	76.00	31.63	0.10
RCP 4.5, 2070	36.29	78.65	33.38	0.10
RCP 8.5, 2050	43.28	71.13	26.77	0.07
RCP 8.5, 2070	40.15	70.53	29.65	0.15

[a)] RCP 2.6 (2050 and 2070), Representative Carbon Pathway 2.6 (lowest emission scenarios) for the years 2050 and 2070; RCP 4.5 (2050 and 2070), Representative Carbon Pathway 4.5 (medium emission scenarios) for the years 2050 and 2070; RCP 8.5 (2050 and 2070), Representative Carbon Pathway 8.5 (highest emission scenarios) for the years 2050 and 2070.

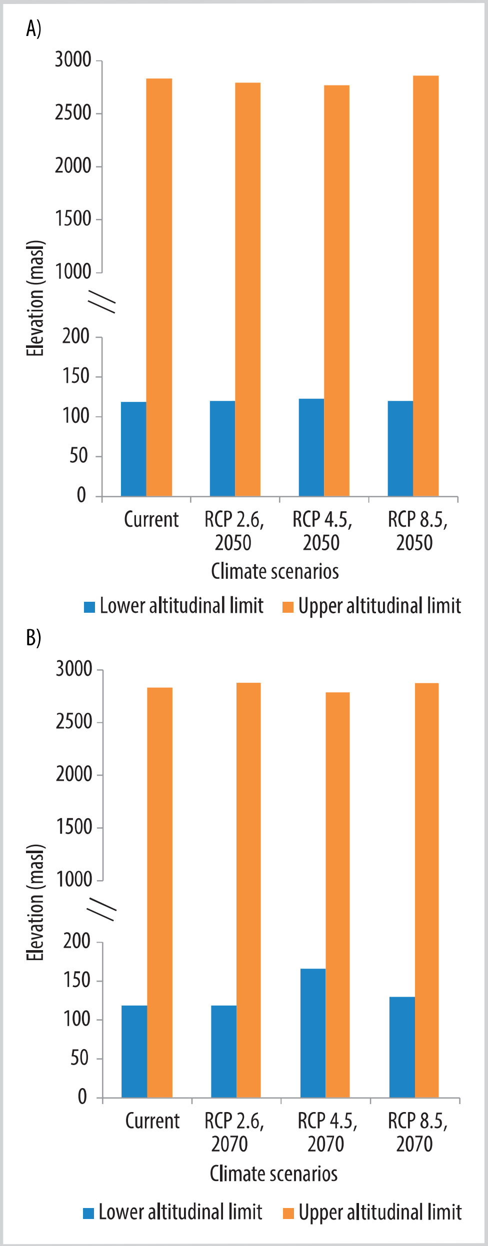
Like under current climatic conditions, in future climate scenarios, Langtang National Park will have more suitable areas than the other 2 protected areas (Figure 4). Under RCP 2.6 and 4.5 for both 2050 and 2070, climatically suitable areas are predicted to increase in Annapurna Conservation Area, whereas under extreme climate scenarios, it will lose some suitable areas. For Langtang National Park, climatically suitable areas will increase under RCP 2.6 and 8.5 for both years, but not under RCP 4.5 (likewise for both years). For Manaslu Conservation Area, a remarkable gain in suitable areas is predicted only under RCP 8.5 for the year 2070.

Discussion

This study is the first to predict current and future suitable habitat for *A. adenophora* in CHAL, Nepal. The model evaluation parameters (AUC and TSS) obtained for our models both lie within a range that confirms the robustness of the models (Table 1): AUC values above 0.8 and TSS values closer to 1 are considered to be acceptable (Swets 1988; Manel 2001; Allouche et al 2006). Though our study did not include a model transferability assessment, this would provide valuable information for model validation as well as model selection (Wenger and Olden 2012).

Climatic factors, such as temperature and precipitation, play a pivotal role in determining the pathways and success of plant invasions (Kathiresan and Gualbert 2016; Wang et al 2017). Our predictions suggest that the minimum temperature in the coldest month is the most influential factor for the distribution of *A. adenophora*. This is in line with the findings of other researchers (Wang and Wang 2006; Zhu et al 2007; Wang et al 2017; Lamsal et al 2018; Thapa et al 2018; Datta et al 2019). According to our model, the minimum temperature in winter (Bio 6) alone contributes

FIGURE 5 Change in upper and lower elevation range of *A. adenophora* in future climate scenarios compared to current conditions. (A) Year 2050. (B) Year 2070.



about 49% to the habitat suitability model (Table 2), and the probability of occurrence of the weed decreased to almost 0 below the minimum winter temperature of 1°C (Appendix S3, *Supplemental material*, https://doi.org/10.1659/MRD-JOURNAL-D-19-00069.1.S1). This indicates that the occurrence of this weed in high mountains is constrained by the lower minimum winter temperature, which is supported by observations in India, where the low temperature in winter limits the uppermost distribution range of *A. adenophora* (Datta et al 2017).

Our ENMs were built based on the occurrence data of invaded regions only, which indicates that the potential niche that we estimated is part of the fundamental niche of the species (Elith and Leathwick 2009; Soberon and Nakamura 2009). Moreover, factors such as biotic interactions and dispersal limitations also restrict the species from occupying its full potential niche (Soberon and Nakamura 2009). Thus, use of occurrence data from both native and invaded ranges would provide a more accurate estimate of the potential niche of the species (Jimenez-Valverde et al 2011).

In current climatic conditions, as well as in future climate scenarios, Middle Mountain is found to have more climatically suitable areas for *A. adenophora* compared to other physiographic regions. The elevation of the Middle Mountain region ranges from 1000 to 2500 masl (DHM 2017), which lies within the suitable range for the distribution of this weed (Wang and Wang 2006; Zhu et al 2007). A recent study that modeled the distribution of 24 invasive alien plants in Nepal also identified the Middle Mountain region as having a particularly large area of invasion hotspots, with suitable areas for the greatest number of species studied (Shrestha and Shrestha 2019). Displacement of native species, such as *Artemisia indica* and *Urtica dioca*, and reduction of the ground vegetation layer of *Digitaria* sp., *Eragostris* sp., and *Imperata cylindrica* by *A. adenophora* have already been observed in the Middle Mountain region (Tiwari et al 2005; Baral et al 2017). Therefore, this region requires the urgent attention of policymakers and land resource managers to implement effective management plans to prevent further spread of this weed. In contrast, the High Himalaya region is unsuitable for the weed because this region has a low minimum winter temperature (−10°C to 5°C) and low annual precipitation (400–1000 mm) (DHM 2017), which limit the distribution of the weed (Datta et al 2017). Most of the current areas predicted to be suitable for *A. adenophora* were found along road verges and river networks, which is in accordance with a study conducted in China (Wang and Wang 2006). These river and road networks enhance the rapid spread of *A. adenophora* (Dong et al 2008; Sang et al 2010). Therefore, control and monitoring efforts for the management of this weed should be focused on areas near rivers and roads.

There is growing evidence that climate change is likely to increase the risk of plant invasions, creating more suitable areas in the future (Bradley, Wilcove, et al 2010). Our projections also showed an increase in climatically suitable areas for *A. adenophora* in the future. The weed will gain suitable areas in 3 future climate scenarios: RCP 2.6 (2050) and RCP 4.5 (2050 and 2070). A recent study also reported that climatically suitable niches for *A. adenophora* would expand by 5.3% under RCP 6.0 in Nepal (Shrestha and Shrestha 2019). Studies in other parts of the world have also predicted the expansion of climatically suitable areas for *A. adenophora* in future climate scenarios (Wang and Wang 2006; Zhu et al 2007; Wang et al 2017; Lamsal et al 2018; Thapa et al 2018). However, a study conducted in Bhutan predicted a contraction of suitable areas for *A. adenophora* by 0.22% in 2050 (Thiney et al 2019). An experimental warming study conducted in China revealed that warming (2°C rise) increased biomass allocation and canopy cover of the weed, making it more stress tolerant (He et al 2012). Similarly, CO_2 enrichment was also reported to increase the relative growth rate and biomass allocation of the weed (Lei et al 2012). Thus, an increase in suitable areas in future climate scenarios with increased temperature and CO_2 concentration can be attributed to the innate and evolutionarily increased ecophysiological tolerances of *A. adenophora* favoring its growth (Blossey and Notzold 1995; He et al 2012; Lei et al 2012). Although, under extreme climate scenarios (RCP 8.5), a loss in total climatically suitable areas is predicted, the weed will still thrive and gain suitable areas in protected habitat and physiographic regions. Districts like Lamjung, Gorkha, Dhading, Makwanpur, Chitwan, and Tanahun are predicted to be vulnerable to further invasion by the weed due to climate change, so it is crucial to implement scientifically informed site-specific management policies, with the participation of local communities.

Despite its apomictic nature, with the associated evolutionary constraints, niche expansion has been observed in *A. adenophora* (Datta et al 2019). In addition, the weed is found to exhibit phenotypic plasticity that helps it to occupy a broader climatic niche (Zhao et al 2012). Our future climate models also demonstrate an expansion of the upper elevational distribution limit of the weed. Our results confirm findings in the Western Himalaya, where the weed was predicted to expand its upper elevational limit by 981 m compared to current climatic conditions (Thapa et al 2018). This indicates that the weed will spread toward cooler and drier regions in future. A similar trend was observed in a study of spatiotemporal patterns in China (Zhu et al 2007). Increased cold tolerance due to epigenetic modifications might help the weed to gain more suitable habitats in cooler and drier places at high elevations (Xie et al 2015). With climate change, all 3 protected areas will gain areas of potential suitable habitat. Though the weed has already been identified as the most problematic weed in Annapurna Conservation Area, impacting native diversity and livelihoods (Thapa and Maharjan 2014), no such studies have been carried out in Langtang National Park and Manaslu Conservation Area. Upward movement and colonization of *A. adenophora* due to recent climate changes have already been observed in Langtang National Park (Lamsal et al 2017). Our model also predicted that Langtang National Park has the highest proportion of climatically suitable areas in current climatic conditions and will continue to do so in future climate scenarios. A range shift of *A. adenophora* might threaten the habitat of 2 endangered animals, *Ailurus fulgens* (red panda) and *Moschus chrysogaster* (Himalayan musk deer), in Langtang National Park (Lamsal et al 2017). Thus, this information should act as a prompt for land managers, the scientific community, conservationists, and policymakers to adopt precautionary measures and formulate effective policies to prevent the further spread of this weed into new regions.

Taking climatic and topographic factors into consideration, our model predicted elevational range expansion, as well as an increase in suitable areas in future climate scenarios. However, other factors, such as biotic interactions, dispersal ability, demography, evolution, adaptation, and land-use change, also play key roles in determining the species range shift with climate change (Sinclair et al 2010; Urban et al 2016). Furthermore, long-term temporal predictions in climate change scenarios are associated with 2 other main errors. The first is the extrapolation of data beyond the training range to nonanalogue environmental conditions (climate change scenarios), which might make predictions unreliable (Fitzpatrick and Hargrove 2009). Another risk is that, as the relationship between climatic variables could change with time, the correlation structure of current and future climate variables might also change, thus reducing the certainty of models (Dormann et al 2013). To overcome these uncertainties and achieve more realistic predictions of species distribution, all factors, abiotic and biotic, that shape the distribution of invasive species should be incorporated in the species distribution models (Gonzalez-Salazar et al 2013; Leach et al 2016). Nevertheless, ENMs provide predictive information on species distribution required by vegetation managers and conservation practitioners for developing effective strategies to prevent further invasion (Peterson 2003). Thus, our study provides useful information about the current distribution of *A. adenophora* and identifies areas that may be at risk in the future on a local scale, demonstrating the urgent need for formulating effective management strategies to mitigate the impact of the weed.

Conclusions and management recommendations

Our study suggests that *A. adenophora* could spread further under future climate scenarios while retaining most of the currently suitable areas. Among the 4 physiographic regions in CHAL, the Middle Mountain region currently has the highest proportion and the High Himalaya region currently has the lowest proportion of climatically suitable areas for *A. adenophora*, and this is projected to continue under future projected climate scenarios. Similarly, Langtang National Park contains a higher percentage of areas suitable for the weed compared to the other 2 protected areas under current and future climate scenarios. All protected areas will gain additional suitable areas in future climate scenarios. Furthermore, it is predicted that the weed will expand its distribution range to higher elevations in future climate scenarios; this will amplify the consequences of climate change, which is already impacting these areas. For physiographic regions (Siwalik and High Himalaya) and protected areas (Manaslu Conservation Area) that have few suitable areas for invasion, regular inspection of habitats is needed to allow effective action to be taken in time to prevent further expansion of the weed. Management strategies for smaller and accessible invaded areas could either be mechanical control, for example, by hand pulling, or chemical control, by using herbicides (Parsons and Cuthbertson 2001; Di Tomaso et al 2013). Local communities in Nepal use *A. adenophora* for cattle bedding, composting, and also for making bio-briquettes (Tiwari et al 2005; Baral et al 2017; Shrestha, Shrestha, et al 2019); these cultural control methods can also be employed to manage the weeds, as long as precautions are taken to prevent seed dispersal. However, for regions like Middle Mountain and protected areas like Langtang National Park that have large areas at risk of invasion, effective management options, such as an integrated weed management approach, must be adopted and implemented along with regular monitoring of suitable habitats. By identifying areas that are potentially at risk in the future, our study constitutes a helpful resource for managers and policymakers to take appropriate and timely action to minimize the risk of invasion by *A. adenophora* associated with climate change.

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Supplemental material

APPENDIX S1 Bioclimatic variables used for modeling suitable habitat of *Ageratina adenophora*.
APPENDIX S2 Correlation matrix of 19 bioclimatic and 3 topographic variables.
APPENDIX S3 Marginal response curves of 2 predictor variables.
APPENDIX S4 Scatter plot of occurrence points and minimum temperature of the coldest month for current climate.

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