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GROWTH PATTERN OF *PINUS ROXBURGHII* UNDER DIFFERENT REGIMES OF INVASIVE SPECIES IN PANCHASE, NEPAL HIMALAYAS

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Abstract

Invasive alien species have taken attention as one of the significant threats to biodiversity worldwide. The ongoing climate change is likely to exacerbate the problem by opening up favorable environments for invasion, putting the ecological, environmental and socio-economic state into stake. The assessment of potential impacts of invasive species on native species is crucial for prevention and mitigation measures. Thus, the current study aimed to assess the impact of invasive species (i.e., *Ageratina adenophora* Sprengel) on the growth of ecologically important native tree species (i.e., *Pinus roxburghii* Sargent) in Panchase, Nepal Himalaya. Furthermore, it explored the dendroecological approach of *Pinus roxburghii* to assess the potential impacts of invasive species on its growth. The growth pattern was focused on regeneration, tree radial growth and inter-nodal growth rate of saplings and seedlings of *Pinus roxburghii* under two different classes of invasion i.e., low and high-density. The study revealed that the density and inter-nodal growth rate of seedlings of *Pinus roxburghii* declined significantly whereas no significant decline occurred in its saplings with the increase in invasion. However, the saplings showed significant increment in its inter-nodal growth rate with the increase in invasion. In the case of its tree, no such significant impact in the radial growth rate was observed. The novel study using dendroecological approach revealed that the invasive species have differential impacts on the growth of nearby native tree species with higher impacts during the seedling stage compared to the saplings and tree stage.

Key words: *Ageratina adenophora*, Dendroecological approach, Inter-nodal growth rate, Radial growth, Regeneration.

Introduction

Alien species are turning into invasive at an alarming rate. One of the major causes of rapid invasion by alien species is ongoing globalization in the form of international trade, tourism, transport, and travel (Sudmeier-Rieux and Ash, 2009) which has been further exacerbated by climate changes (IPCC, 2007; Mooney *et al.*, 2009). As a result, alien species, which had once been isolated, now moved freely and established themselves in a new environment across barriers (IUCN, 2001). The movement of invasive alien species from their native habitat to a new location, either intentionally or accidentally have posed a serious threat to the native species (Holmes *et al.*, 2009), thus taking a serious dimensions in all the seven geographical regions (Sankaran *et al.*, 2005). According to Lowe *et al.*, (2000), there are around 100 worst invasive alien species in the world posing potential threats to different ecosystems and ecological integrity. Invasion in an ecosystem not only affects the distribution, abundance, growth, and reproduction of native species (Sale *et al.*, 1999; Bhardwaj *et al.*, 2014) but also can change or modify the structure and functioning of an ecosystem (Charles & Dukes, 2006). The opportunistic, competitive, and aggressive nature of invasive plant species leads them to rampant growth through acquisition of higher nutrients, water, and other inhibitory benefits that can ultimately outcast the native or indigenous plant species by creating a virtual monoculture (Callaway *et al.*, 2005). Moreover, as biological polluters, they can also modify the genetic make-up of native species (Westbrooks, 1991). In severe case, they can even drive rare and endangered species to extinction (Schmitz & Simberloff, 1997). Because of these potential impacts of invasion on native species, invasive alien species are considered as the second-worst threat to biodiversity (Enserink, 1999; Wilcove *et al.*, 1998). As an emergent call, CBD Article 8(h) (2008), have highlighted the need for prevention, introduction, and mitigation of impacts of alien species such that the impacts can be reduced.

In the context of Nepal, a prominent representation of the Himalayan arch with rich species diversity is not free from the menace. A total of 21 invasive species most notably, *Ageratina adenophora*, *Chromolaena odorata*, *Lantana camara*, etc. have been prioritized for invasiveness character in landscape-level study in Nepal (Tiwari *et al.*, 2005), thus causing a significant harm to the environment, economic systems, and/or human health (Mooney, 1999). Specifically, Panchase, located in western Nepal and an ecologically important mountain ecosystems was also reported being invaded by several alien species namely, *Ageratina adenophora*, *Ageratum conyzoides*, *Chromolaena odorata*, *Eichhornia crissepes*, *Imperata cylindrical*, *Lantana camara*, *Parthenium hysterophorus,* and *Rubus ellipticus* in the study conducted by International Union for

Conservation of Nature (IUCN-Nepal) (IUCN, 2013). Among the invasive plant species in Nepal, *Ageratina adenophora* has been posing high risk in the forest ecosystem as per the study by the Government of Nepal (GoN, 2014). Apart from the invasive species, native species are crucial for the sustainability of forest ecosystem, as they adapted to their natural environment. Specifically, *Pinus roxburghii* Sargent is an ecologically and economically important native pine tree species that cover large geographical areas in the middle and high mountain regions in Nepal. The landscape-level study by the Department of Forest Research and Survey (DFRS) revealed that *Pinus roxburghii* cover almost 8.54% of the total forest cover in Nepal (DFRS, 2015). At the local level, it is widely used for fuelwood and litter collection. Thus, pine tree is an important timber species in Nepal. Apart from Nepal, the species covers the greater landscape of China, India, Pakistan, and Bhutan from 400 to 2300 m a.s.l. (Polunin and Stainton, 1984). Moreover, past studies have revealed that *Pinus roxburghii* trees are suitable for their annual growth assessment and monitoring as they produce clear annual rings and internodes (Aryal *et al.*, 2018; Bhattacharyya *et al.*, 1992; Sigdel *et al.*, 2018). To date, *Pinus roxburghii* has been widely explored for growth response and other climatic studies using dendrochronological approach (Ahmed *et al.*, 2009; Aryal *et al.*, 2018; Brown *et al*., 2011; Sigdel *et al*., 2018). Therefore, the current study further explores the dendroecological approach of *Pinus roxburghii* as it provides a unique opportunity to assess the potential impacts of invasive species in its growth.

As the degree of invasion by alien species are species-specific and site-specific, whereas, the impacts may be random or spatial or temporal (Parker *et al.*, 1999), the impact assessment at the highly diverse ecosystem like in Panchase is of great need in the current scenario. To date, very limited studies have addressed the impact of invasive alien plant species on the growth pattern of native tree species using dendroecological approach (Hartman and McCarthy, 2007). The majority of the existing studies are confined to the impact of invasive alien species on species diversity, allelochemical inhibition, socio-economic impact, etc., (IUCN, 2013). Thus, the comparative study on different degrees of invasion in the same forest area is likely to give the traceable impacts of invasive species on the growth rate of native species in different stages of stand development (i.e., seedlings, saplings, and trees). The study thus aims to assess the impact of invasive species (i.e., *Ageratina adenophora*) on the growth pattern of native tree species (i.e., *Pinus roxburghii*). For this, the objectives mainly covered the impact of invasive species on regeneration, as well as inter-nodal growth rate of saplings and seedlings, and radial growth of trees of native tree species under different regimes of invasive species. For this, three hypotheses was set as; there was a significant impact of invasive species (i.e., *Ageratina adenophora*) on regeneration, inter-nodal growth rate, and radial growth rate of *Pinus roxburghii*. The current study is a novel study for the region and it will advance the existing knowledge on the impact of invasive alien plant species on native plant species in consideration with the growth performance of the native species using dendroecological tools.

Materials and Methods

Study area: The study was conducted in a community forest in Bichari Chautara of Syangja district, Panchase area, western Nepal (latitude of 28° 10' 54.4" N and longitude of 83° 46' 17.2" E) (Fig. 1). It is the natural pine forest nearest to Panchase protected forest, one of the three pilot sites of Ecosystem-based Adaptation (EbA) project of International Union for Conservation of Nature (IUCN) in the mountain ecosystem (IUCN, 2013). According to the Department of Hydrology and Meteorology (DHM), Panchase is one of the high rainfall receiving areas of Nepal dominated by Asian monsoon system where around 80% of the rainfall takes place from June to August. The temperature ranges from 34^{o}C during summer to as low as 3^{o}C in the winter. The current study site represent a natural sub-tropical forest dominated by *Pinus roxburghii*. The field scouting revealed *A. adenophora* as the only invasive alien plant species in the study site. Furthermore, the forest stand boundary was delineated using Global Positioning System (GPS), which was then plotted in ARC GIS 10.0. Thus, the strategy for the selection of forest was purposive.

Study species

***Ageratina adenophora* Sprengel as an invasive species:** *Ageratina adenophora* Sprengel (hereafter, *A. adenophora*) belongs to Asteraceae family. It is a perennial herb (occasionally grows into a sub-shrub), native to Mexico which is known to introduce as an ornamental flower in different parts of the world since the 19th century (Muniappan *et al.*, 2009). Thus, it is also known as Mexican devil and trans-global invader (Evans, 2010), particularly in China and India. Generally, it occurs from 1000 to 2000 m a.s.l. in the hills in Nepal (Sharma and Chhetri, 1977). It is a problematic weed in forest (Morris, 1989) and thus been named '*banmara'* (meaning; killer of the forests) in Nepal (Sharma and Chhetri, 1977). It is a very noxious weed and an extremely aggressive competitor, which can spread by asexual reproduction (Muniappan and Viraktamath, 1993). Each plant can produce more than 30,000─45,000 seeds (Jianghua, 2005) that can even store in soil as dormant, pre-adapted for human dispersal (Rejmanek, 2000). As an adaptive strategy to the non-native area, it can develop pappus hair in its seeds, which enables them to spread efficiently through wind or water in a long way like a parachute (Evans, 2010). Some of its associated traits based on studies on sub-tropical regions of Nepal were highlighted in Table 1. Because of its rampant growth and opportunistic nature, it can outcast native species from their natural habitat thus posing a serious threat to biodiversity.

Regarding the introduction of *A. adenophora* in Nepal, its occurrence was first reported in 1958. It is believed to enter Nepal from India through the eastern border (Banerji, 1958) probably before 1950 (Kunwar, 2003). Recently, it has been reported to expand westward in Nepal (Tiwari *et al.*, 2005). As *A. adenophora* generally flourishes in areas which receive year-round rainfall (Fuller, 1981), Panchase is one of the favorable habitats for its expansion (Tiwari *et al.*, 2005).

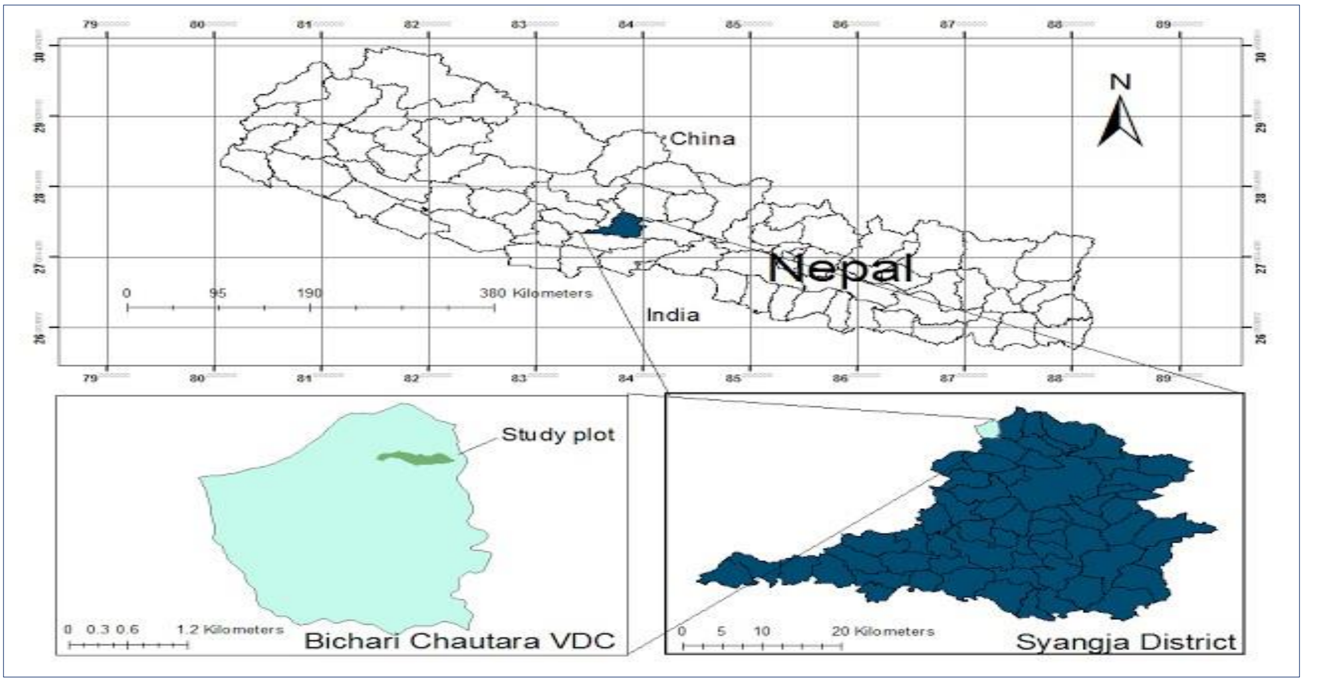

Fig. 1. Map of study area, Syangja district, Bichari Chautara VDC, western Nepal, Central Himalaya.

Table 1. Ranking criteria score for *Ageratina adenophora* (Tiwari *et al.*, 2005).

S. No.	Ranking criteria	Rank
1.	Impact	High
2.	Biology and ecology	High
3.	Distribution and abundance	High
4.	Difficulty to control	High
5.	Invasiveness rank	High

***Pinus roxburghii* Sargent as a native species:** *Pinus* is one of the ecologically significant native trees and the largest conifer genera in the Himalayan region. In Nepal, two types of indigenous species namely *Pinus roxburghii* (chir pine) and *Pinus wallichiana* (blue pine) are found. *Pinus roxburghii* Sargent (hereafter, *P. roxburghii*) is the three-needled pine tree, native to the Himalayan range. It is the major forest type of western Nepal, Himalaya region, generally occurring in the elevation between 1000 to 2000 m a.s.l. (Jackson, 1994) on an exposed south-facing slopes and well-drained area, which is sufficiently dry. Its natural regeneration takes place by means of older pine trees bearing seeds (Duryea, 1992). Regarding its radial growth, it is marked by the presence of alternating light bands (earlywood) and dark bands (latewood) which represents the growth in one growing season. Moreover, they are known to produce a whorl of branches with delineate internodes representing the height increment of trees during one growing season (Wilde, 1965). Therefore, *P. roxburghii* trees as a biological recorder, records and stores the information from the environment which at the same time indicates its adaptive capacity towards the environment (Wilt, 2018). The environmental changes like reduced light, water or nutrients can reflect through reduction in the inter-nodal growth as well as radial growth of *P. roxburghii*. Moreover, seedlings and saplings determine the regeneration and future composition of forest (Henle *et al.*, 2004).

Methods

Experimental design for ecological assessment: The preliminary study was conducted in December 2015 to assess the site characteristics and selection of sampling points. The final field study was carried out in February 2016. The random sampling technique was adopted for the plot selection and ecological assessment. A total of 30 random circular sampling plots were located in the selected forest area as per ARC GIS 10.0. However, homogeneity in the topography was maintained in sample selection as far as possible to control or minimize the influence of difference in edaphic factors on growth patterns. The plot layout given by Lafon (2004) was adopted with plots of 400 m^2 for trees, sub-plots of 200 m^2 for saplings and four nested plots of 2 m^2 for seedlings of *P. roxburghii* (Fig. 2). In the field, different measurements on the growth of *P. roxburghii* were taken along with the counting of the number of *A. adenophora* in all respective plots.

Regeneration survey of *Pinus roxburghii*: The DBH (diameter at breast height i.e., 1.3 m above ground) of each trees and saplings of *P. roxburghii* was measured using Kinglon diameter tape (D-tape). The individuals were categorized into trees, saplings, and seedlings based on the DBH (Sundriyal & Sharma, 1996). Accordingly, the DBH greater than 10 cm was taken as tree and DBH lesser than 10 cm along with height of 30 cm was considered as sapling whereas DBH < 10 cm with height lesser than 30 cm was considered as seedling. Then, the regeneration survey was carried out in all the quadrats by counting the number of saplings and seedlings of *P. roxburghii* along with counting of number of *A. adenophora* in respective plots. The chi-square test (χ^2) was performed to see if there was any significant association between the occurrences of these two species as per Yates's correction for 2×2 contingency table. The test was based on the presence/absence of *A. adenophora* and seedlings of *P. roxburghii* on 120 plots (4 nested plots×30 plots). In the case of significant association, the nature of association i.e., positive or negative was determined based on the observed frequencies of both the species occurrence as per Kent & Coker (1992).

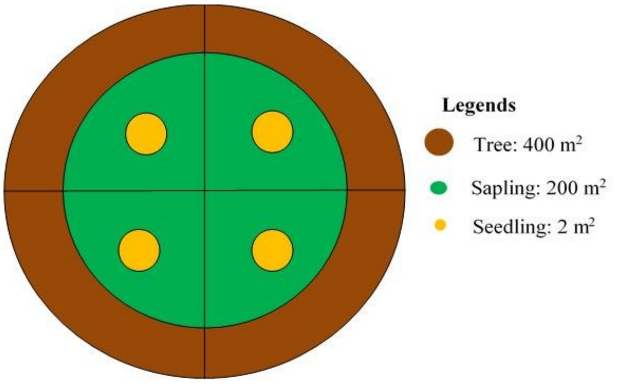

Fig. 2. Overview of the sampling plot. Source: Lafon (2004).

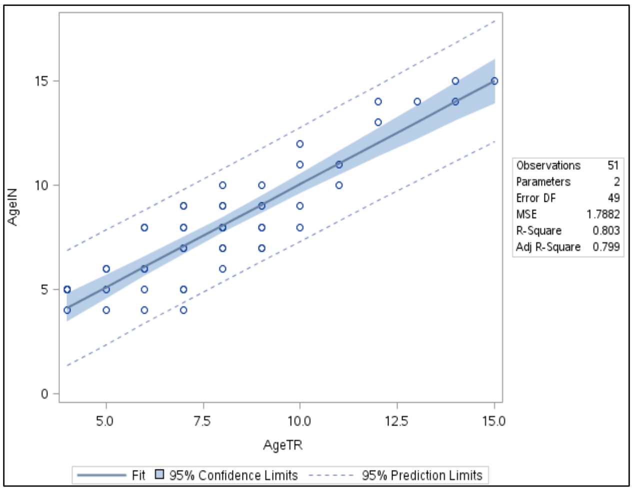

Fig. 3. Relationship between inter-nodal age (AgeIN) and radial age (AgeTR) of saplings of *Pinus roxburghii*.

Ring width measurement: The tree cores were extracted from two of the healthy and dominant trees of *P. roxburghii* occurring in each 400 m^2 plot using a Swedish Increment Borer Haglof following standard dendroecological procedure (Fritts, 1976; Speer, 2010). From each selected trees, two cores were extracted at its diameter at breast height (DBH). A total of 116 (n = 116) cores were collected from the overall sampling plots. Upon returning to the laboratory, the collected tree cores were air-dried and then mounted in the mounting stick with the transverse surface facing up. These were then polished with the sanding paper of grid number 200─800. Thus, the standard methods were followed for processing the cores (Fritts, 1976). After the processing of the collected tree core, each ring was counted under stereo zoom microscope (Leica) and the width of each ring was measured to the nearest 0.01 mm precision in LINTAB measuring system which was connected to the computer having TSAP (Time Series Analysis and Presentation Program) (Rinn, 2013). The ring width measurement of the entire tree cores were done in the dendro-lab of the Department of Forest Research and Survey (DFRS), Kathmandu. The data of each ring width in total length of the core and the number of rings in the tree core was used to calculate the mean annual increment (mm/yr) of *P. roxburghii* tree for each sample plot.

Inter-nodal growth rate: The inter-nodal distance (annual height increment) of saplings and seedlings of each *P. roxburghii* were measured by using a centimeter tape. Then the average inter-nodal growth rate (cm/yr) was calculated as the sum of inter-nodal distance to the number of nodes. To validate the age of saplings based on node counts (AgeIN), cut stumps and increment cores samples from the same individuals were collected, analyzed and cross-validated using the regression model with age based on radial growth (AgeTR) (Fig. 3). Results showed that both techniques can be used to estimate the age of seedling and saplings of *P. roxburghii*.

Data management: The occurrence of *A. adenophora* in all three types of plots was converted to density (individuals per hectare) as per Kent and Coker (1992). The distributions of data on the density of *A. adenophora* in all three types of plots were found asymmetrical i.e., right-tailed with significant skewness (Gerard, 2013). Thus, they were transformed into natural logarithmic form (Tabachnick & Fidell, 2007) to improve the normality of data. The details of descriptive statistical after the normalizations was shown in the Table 2. Then the density of *A. adenophora* was classified into two classes of invasion as low and high using median as their basis (Gerard, 2013) for each plot type. Accordingly, below and above median value was considered as 'low invasion class' and 'high invasion class', respectively. The data analysis was performed in Microsoft Excel 2016 and R-software version 3.5.0 whereas the graphical representation involved R-studio (R Core Team 2018).

Analysis of growth pattern: The linear regression was fitted for tracing the growth pattern of seedlings, saplings, and trees of *P. roxburghii* based on the low and high-density class of *A. adenophora*. The logarithmically transformed data on density of *A. adenophora* were used for the regression analysis. The study dealt with the impact of *A. adenophora* on the growth pattern of *P. roxburghii* thus the density of invasion was considered as an independent variable whereas the growth pattern of *P. roxburghii* mainly radial growth, and inter-nodal growth including their occurrences were considered as dependent variables. Furthermore, the growth patterns of *P. roxburghii* based on the invasion classes were tested for significance using t-test for unequal variances at p = 0.05.

Results and Discussion

Impact of invasion of *Ageratina adenophora* on regeneration of *Pinus roxburghii*: The invasion density was found to have differential impacts on the number of occurrence of both seedlings and saplings of *P. roxburghii*. The seedlings of *P. roxburghii* showed a sharp decline in its occurrence with the increase in invasion by *A. adenophora* (Fig. 4a). The reduction accounted for 45.85% in the average number of seedlings in a low and high-density class of invasion. The result was found to be statistically significant ($p<0.05$). Moreover, the linear regression showed a sharp declining trend of seedlings of *P. roxburghii* with the increase in *A. adenophora* in both the density classes (Figs. 4b and 4c) with even high R-squared value of 0.87 in high invasion density class (Fig. 4c). The chi-square test of association (χ^2) based on the presence/absence of the seedlings and *A. adenophora* showed a calculated χ^2 of 4.56 at the 0.05 level. Further, the exceeded value of expected frequency (33.13) than the observed frequency (27) further supported the significant negative association between the occurrence of *P. roxburghii* seedlings and *A. adenophora.*

The current finding suggested a significant decrease in the occurrences or density (no./ha) of seedlings of *P. roxburghii* with increasing extent of invasion can be the result of greater suppressing tendency of *A. adenophora* through rampant growth to a larger area. Its dense thickets with increased leaf area can intercept sunlight (Parsons and Cuthbertson, 1992). Moreover, as an aggressive competitor, *A. adenophora* can absorb most of the nutrients from the soil (Tiwari *et al.*, 2005). Due to its competitive use of resources for its rampant growth, the seeds and seedlings of *P. roxburghii* have to compete with the invasive plant for space, light, and nutrients for its germination and growth (Wilde, 1965). In addition to this, invasive alien plant species have high tendency for water uptake (Malan and Day, 2002), thus they intensify the evaporation rates (Chamier *et al.*, 2012) in the form of evapotranspiration. This condition is likely to result in water-stress conditions during the germination stage of seed or growth of seedlings of *P. roxburghii*. As sunlight, water and nutrients are needed for the germination; any obstacles in the timely availability of these resources will suppress the germination stage severely, which ultimately hinders the germination or even cause death *in P. roxburghii*. As per Tiwari *et al.* (2005), *A. adenophora* suppresses the regeneration of tree species by blocking sunlight and even releasing inhibitory chemicals in the forestland. A similar result was reported in the study conducted by the Government of Nepal in Chure (GoN, 2011). Accordingly, the occurrence of seedlings was found declining with the increase in coverage of the invasive plant species.

Another reason for the suppression of seedlings of *P. roxburghii* is likely the release of allelochemicals by *A. adenophora*. It has been proved that *A. adenophora* inhibits the growth of seedlings or nearby vegetation through the release of allelochemicals like cadinene sesquiterpenes (Kaul & Bansal, 2002) and volatile compounds which ultimately constrain the growth of native vegetation (Ferguson *et al.*, 2003). These allelochemicals thus create an unsuitable environment for the germination of native tree seed (Inderjit *et al.*, 2008). The study by Orr *et al.*, (2005) also found the effect of allelochemicals from invasive species being stronger during the early stage of growth or establishment of native species, which ultimately reduce the survival, and growth of seed of native species. Similarly, studies by Bhardwaj *et al.* (2014) and Ferguson *et al*., (2003) also found retardation of germination due to allelochemicals released by *A. adenophora*. Furthermore, the invasive species can have anti-microbial activity (Ehrenfeld, 2003) and can alter the nitrogen fixation by releasing allelopathic chemicals (Wardle *et al.*, 1994). These strategies will benefit *A. adenophora* to be an aggressive competitor against the native species.

The invasive plant can even use the previously adapted mechanisms of invasion for the expansion of its territory and form a dominant coverage. In fact, once the invasion is prominent, they can escape the negative effects set up by the native species for suppression of the growth of alien species (Callaway & Ridenour, 2004). Despite the known effect of soil acidification by root exudates by pine forest (Fernandez *et al.*, 2006), *A. adenophora* is likely to escape the impact. Hence, invasive species can even neutralize the negative effects of microorganisms and native species present in an ecosystem, such that the growth of invasive species is not hindered. Furthermore, the chi-square test for the presence/absence of seedlings of *P. roxburghii* and *A. adenophora* also suggested a significant negative association between the occurrences of these two species. This showed that the site with invasion of *A. adenophora* is unlikely to be with the presence of *P. roxburghii*. Hence, under the presence of *A. adenophora*, the regeneration of the pine forest will be critical as the increase in density of *A. adenophora* reduces the seedlings of *P. roxburghii* significantly.

In the case of saplings of *P. roxburghii*, the average number or density of saplings (individuals per hectare) in the low and high-density classes of *A. adenophora* were found to be 3.44 and 3.71 respectively (Fig. 4d). The comparison between the numbers of saplings in two invasion density classes showed an average increment of 7.84% in its occurrence. The linear regression for both the classes also showed the increasing trend of saplings of *P. roxburghii* with increase in the invasion of *A. adenophora*. However, it was found to be statistically insignificant ($p>0.05$) (Figs. 4e and 4f). The slight increase in the saplings of *P. roxburghii* with the increase in invasion of *A. adenophora* is likely due to a comparatively higher buffering capacity and lower mortality rate at the sapling stage than at the seedling stage of *P. roxburghii*. That means, when a *P. roxburghii* reaches its sapling stages, it over compasses the height of *A. adenophora* taking comparative benefits for light resources and can even escape the negative impacts of allelochemicals released by *A. adenophora* and use resources efficiently. The similar result was obtained in the study conducted in Chure range in Nepal by the Ministry of Forests and soil Conservation, Government of Nepal (GoN, 2011) in which the presence of invasive species could not have significant impact on saplings of native tree species as it had in their seedlings with the increase in coverage of invasion. In addition to this, decomposition of high biomass content of invasive alien plant species is likely to enhance the soil organic matter (Hoevers & Mboob, 1996). This enrichment in soil nutrients can enhance the growth of saplings in a particular area.

Table 2. Descriptive statistics of density of *Ageratina adenophora*.

S. No.	Descriptive statistics of density of *Ageratina adenophora*	Values		
		400 m^2	200 m^2	8 m^2
1.	Mean	7.84	7.85	9.00
2.	Median	7.59	7.44	8.74
3.	Standard deviation (SD)	0.96	0.94	1.07

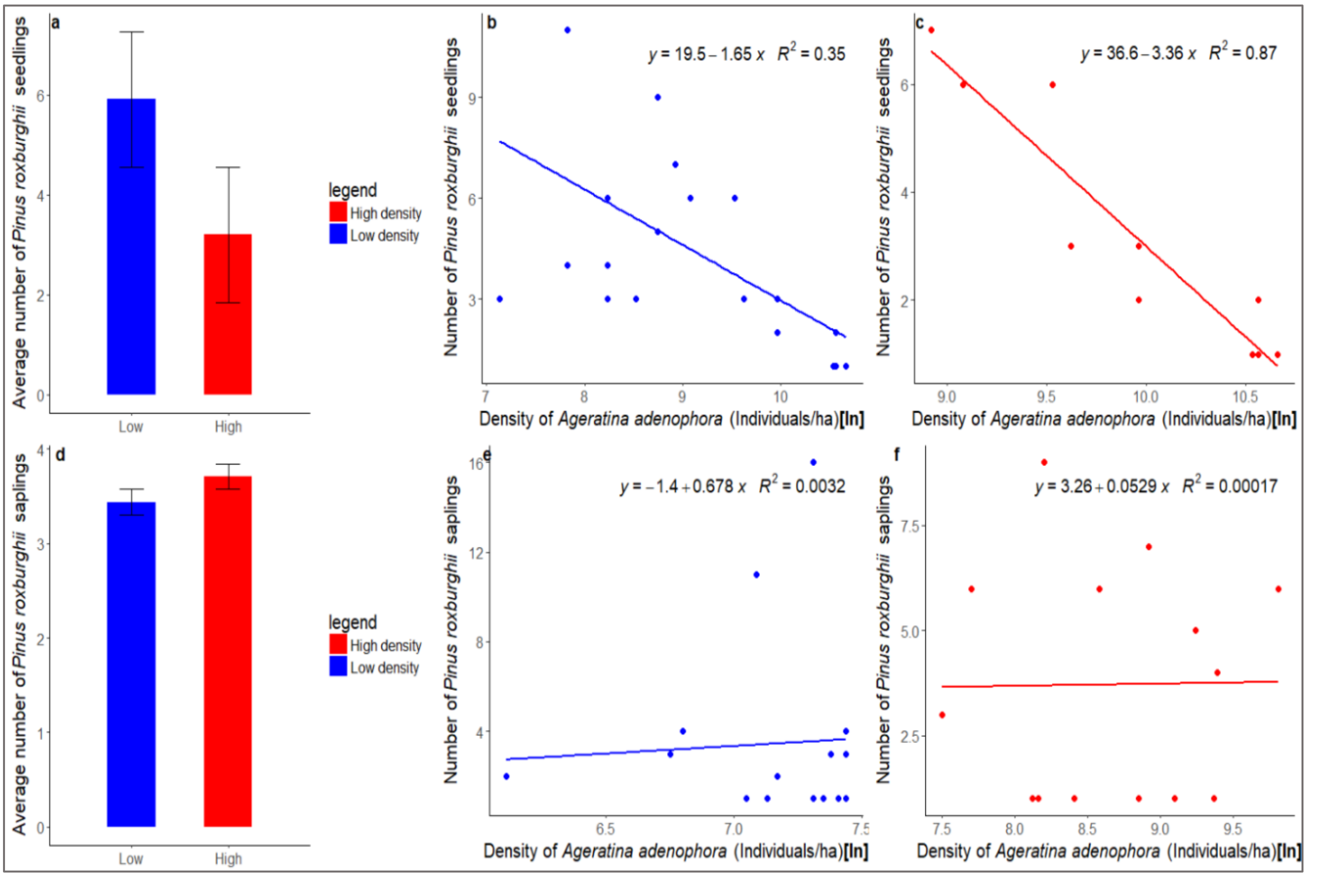

Fig. 4. Average number of seedlings (a) and saplings (d) of *Pinus roxburghii* present in low and high-density classes of *Ageratina adenophora*. The error bar indicates SD ± average value. Right panel presents linear regression of number of seedlings and saplings of *Pinus roxburghii* with low (b and e) and high density class (c and f) of *Ageratina adenophora*. The density of *A. adenophora* (individuals per hectare) was logarithmically transformation for the linear regressions.

Therefore, the strongest inhibition by the invasive alien species was observable or traceable during the germination and emergence stage of seed. If the seedlings are suppressed by the invasive species, the regeneration of an ecosystem will be hindered. In severe cases, the invasion can even reduce the forest to the herbaceous system (Bruce *et al.*, 1995) by preventing the regeneration of trees (Reichard, 1996). The forest will be unsustainable with the reducing of the woody ecosystem into herbaceous ecosystem with almost monocultures of invasive species spread all over the forest. The study by Thapa & Maharjan (2014) also highlighted *A. adenophora* reducing the regeneration of *Alnus nepalensis* through high invasion. This reduced regeneration of tree species as shown by the significant decline in the occurrence of seedlings of *P. roxburghii* with the increase in invasion density is the most prominent impact of invasion. Thus, the ecosystem integrity will be greatly hampered.

Impact of invasion of *Ageratina adenophora* on radial growth of *Pinus roxburghii*: With the advancement of invasion from low to high-density class of *A. adenophora*, there was a slight increase in the mean annual radial growth of *P. roxburghii* from 1.97 to 2.04 mm/yr, which accounted the total increment of 3.55% (Fig. 5a). However, the t-test revealed statistically insignificant differences in increment (p>0.05). According to the linear regression, the average annual radial growth showed increasing trend with the increase in density of *A. adenophora* in low-density class (Fig. 5b). However, after a certain extent of invasion, the radial growth showed decreasing trend with the increase in invasion in high-density class (Fig. 5c). This indicated that the growth rate of *P. roxburghii* was favored by *A. adenophora* only up to a certain degree of invasion but then the further increase in the density of *A. adenophora* retarded the radial growth of *P. roxburghii* as in the high invasion class.

From our study, we found that *A. adenophora* did not have significant impact on the average annual radial growth rate of tree stage individuals of *P. roxburghii* despite a higher average annual growth in high invasion class (Fig. 5a). This is likely because *A. adenophora* as herb (and occasionally shrubs) species could not retard the radial growth of *P. roxburghii* trees. One of the main reasons is that tree being competitive and resistant woody species, the impact of invasive alien plant species can be buffered through different means. Some of the notable developments are greater expansion of canopy coverage, development of advanced root system spreading laterally and vertically as compared to herbaceous species. These peculiar characteristics of the trees makes them comparatively resistant to invasion (Wilde, 1965). Moreover, *P. roxburghii* can withstand weed competition better than the other species (Jackson, 1994) since pine as a tree species can pose adverse effect on the understory vegetation by increasing acidity in the soil through litterfall (Anderson *et al.*, 1969); releasing allelochemicals; and intercepting light and precipitation (Anderson *et al.*, 1969). Similarly, the Novel Weapon Hypothesis (NWH) suggests that the success of invasive species is the outcome of its allelochemicals (Rai & Tripathi, 1982). As both the species i.e., *A. adenophora* and *P. roxburghii* are known to have allelochemical release, their simultaneous occurrence under the same area is likely to result in the partial suppression on occurrence and growth of each other. Moreover, morphologically, *P. roxburghii* shows high tolerance towards the environmental fluctuations in temperature and precipitation, disturbances, poor soil nutrients that might prevail in an area (Knight *et al.*, 1994). These peculiar characteristics of *P. roxburghii* are likely to suppress the impact of understory invasion of *A. adenophora*. Gupta & Dass (2007) also concluded that pine trees can adversely affect the development of understory herbage. Therefore, the invasion by *A. adenophora* is unlikely to have a prominent impact on radial or diameter growth rate *Pinus roxburghii* at tree stage.

The growth rate of trees varies with age, stand density, competition with nearby individuals, etc. (Fritts 1976, Speer 2010). This also indicates the threshold limits in the influence of invasion on the growth of pine trees. One of the surplus in the presence of *A. adenophora* is the enrichment in the soil organic matter and soil pH, which results due to its high biomass production (Hoevers & Mboob, 1996). Moreover, it could be better explained by before and after invasion by *A. adenophora*. That means, in the beginning during the establishment of trees, the site was unlikely to be with the higher number of *A. adenophora* such that the radial growth rate was in increasing trend. Later years, after the establishment of *P. roxburghii* as tree, *A. adenophora* turned out invasive which could have retarded the radial growth rate of the *P. roxburghii*. However, the tree being the advanced vegetation with its canopy coverage, well-adapted root system for nutrient and water uptake, the presence of *A. adenophora* could not retard the radial growth significantly, as it did in the seedlings stages of *P. roxburghii*.

Impact of invasion of *Ageratina adenophora* on inter-nodal growth rate of *Pinus roxburghii*: With the increase in invasion of *A. adenophora*, there was a decline in the average inter-nodal growth rate (height increment) of seedlings of *P. roxburghii* (Fig. 6a). The decline in the inter-nodal growth rate was observed from 19.80 to14.26 cm/yr in low and high-density classes respectively, which accounted for a fall of 27.98%. Moreover, the t-test between growth rates of the seedlings in two density classes of *A. adenophora* also supported the significant difference ($p<0.05$). Similarly, the linear regression revealed a declining trend in the inter-nodal growth rate of seedlings of *P. roxburghii* with the increase in invasion in both low and high-density classes (Figs. 6b and 6c). The significant negative impact on the inter-nodal growth rate of the seedlings reveals the capacity of *A. adenophora* to suppress the growth of *P. roxburghii* in its early growth stage. The retardation in the growth is likely due to allelochemicals released by *A. adenophora* (Ferguson *et al.*, 2003) that inhibit the growth of nearby native species. Besides, the light interception by dense thickets of *A. adenophora* and high uptake of nutrients and water by the invasive plant could have stressed the seedlings to grow. These stresses could have resulted in significant retardation in the inter-nodal growth rate of seedlings in high invasion density class than the low density (Figs. 6b and 6c). A study by Wilde (1965) also concluded pine species showing reduced inter-nodal growth in the presence of weed. The impact is most prominent in the early stage of growth (Orr *et al.*, 2005) i.e., in the seedling stage of *P. roxburghii*. As the seedling is comparatively less competitive and less resistive than saplings and tree stage, the strong effect of resources competition and allelochemicals by *A. adenophora* may have affected in the early stage. In addition to this, Panchase being an area with high rainfall, the interception of precipitation in the aerial parts of *A. adenophora* such as leaves with comparatively more allelochemicals (Zhao *et al.*, 2009) can cause the allelochemicals to leach to the soil. Thus, the intense natural leaching of allelochemicals from the aerial tissues of *A. adenophora* can amplify the inhibitory effect on the growth of nearby vegetation especially in the early stage of growth of native species.

The results on saplings of *P. roxburghii* revealed an increment in inter-nodal growth rate from 13.77 to 16.24 cm/yr in the low and high-density class of *A. adenophora* respectively (Fig. 6d). This difference in the inter-nodal growth rate of the saplings accounted for the total increment of 17.93% which was statistically significantly (t-test, $p<0.05$). This could be due to the enrichment of soil organic matter by the invasive species (Hoevers & Mboob, 1996). Moreover, the buffering capacity of saplings could have contributed to reduce the negative impacts of invasion and take advantage of nutrient enrichment in soil to uplift the inter-nodal growth rate. The linear regression showed increasing trend in the inter-nodal growth rate of the saplings to some extent but then the growth rate retarded afterward. However, very low correlation value were found in the high and low-density classes (Figs. 6e and 6f). The reduced growth in the later period of invasion is likely due to the cumulative effects of resource competition as well as the release of allelochemicals.

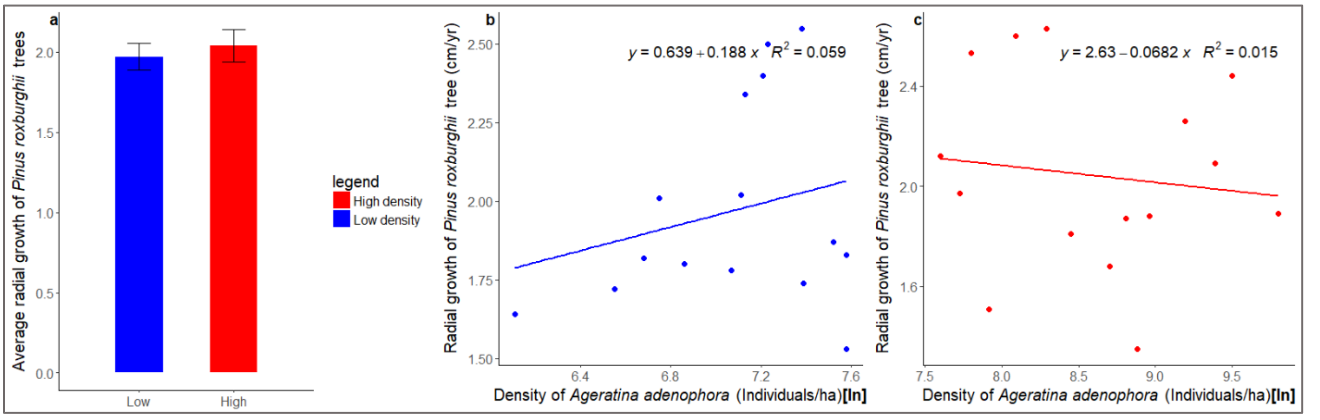

Fig. 5. Average radial growth of *Pinus roxburghii* with invasion class of *Ageratina adenophora* (a), and linear regression of average radial growth of tree of *Pinus roxburghii* in low (b) and high-density classes (c) of *Ageratina adenophora*. The error bar indicates SD ± average value. The density of *A. adenophora* (individuals per hectare) was logarithmically transformation for the linear regressions.

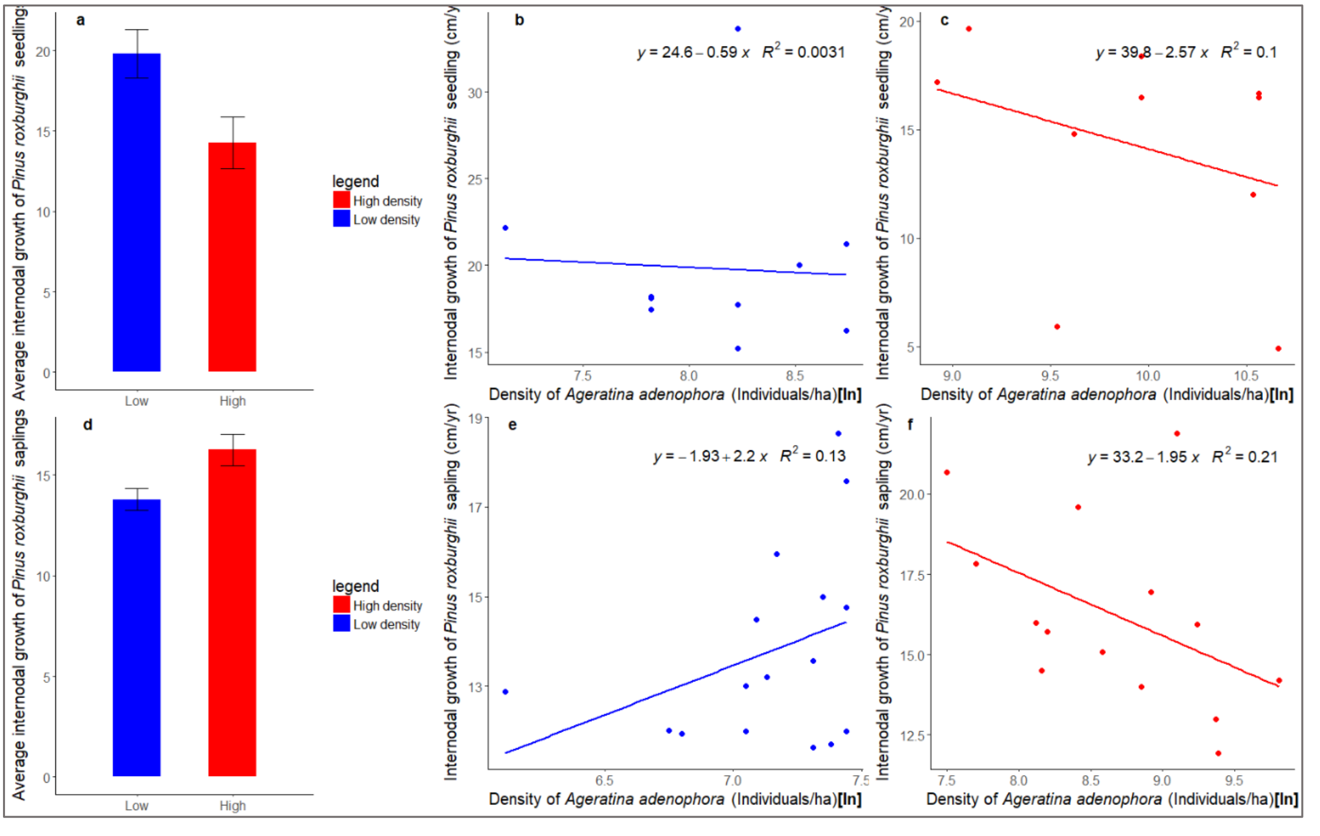

Fig. 6. Average inter-nodal growth rate of seedlings (a) and saplings (d) of *Pinus roxburghii* invasion classes of *Ageratina adenophora*. The error bar indicates SD ± average value. Right panel presents linear regression between number of seedlings and saplings of *Pinus roxburghii* in low (b and e) and high density class (c and f) of *Ageratina adenophora*. The density of *A. adenophora* (individuals per hectare) was logarithmically transformation for the linear regressions.

Conclusions

The current study advances the existing knowledge on dendrochronology as it explored the potential impacts of invasive species (i.e., *Ageratina adenophora*) in the growth pattern of native tree species (i.e., *Pinus roxburghii)* using dendroecological approach. Our study revealed that the invasion by *A. adenophora* has differential effects on three stages of *P. roxburghii* (seedling, sapling, and tree). Among the three stages, the most prominent impacts were observed during its seedling and sapling stages. The sharp decline in occurrences including the inter-nodal growth rate was observed in the seedlings of *P. roxburghii*, being likely from resources competition and inhibitory effect through the release of allelochemicals by *A. adenophora*. However, the increase in the inter-nodal growth rate in saplings of *P. roxburghii* could be the outcome of the enrichment of soil organic matters by invasion and buffering capacities at the sapling stage.

Successful regeneration is a foundation of the establishment of plant species in a forest, which determines the sustainability of a forest ecosystem. Our study revealed that invasion by alien plant species pose a serious threat to the regeneration of ecologically and economically important native pine forest. The impact can further exacerbate in the context of ongoing rapid climate change as it opens up opportunities for expansion of invasive species. Therefore, the early eradication of alien species will be the wisest approach to tackle invasive species and its associated impacts. The advancement of the study incorporating allelopathic effects and nutrient acquisition can further strengthen our understanding on impacts of invasive species on native species.

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