# Impacts of Invasive Alien Species on Native Vegetation in Central Nepal

Lal Bahadur Thapa

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in Biology
Prince of Songkla University
2016

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| **Thesis Title** | Impacts of Invasive Alien Species on Native Vegetation in Central Nepal |
| **Author** | Mr. Lal Bahadur Thapa |
| **Major Program** | Biology |

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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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Candidate

**ชื่อวิทยานิพนธ์** ผลกระทบจากการรุกรานของพืชต่างถิ่นต่อสังคมพืชธรรมชาติในภาคกลางของประเทศเนปาล
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## บทคัดย่อ

สำรวจศึกษาโครงสร้างสังคมพืชบริเวณป่าดิบเขาระดับต่ำของภูเขาจามปาเทวีในเขตเมืองกาฏมาณฑุ และบริเวณป่าสาละ (“Sal” forest) ในเขตเมืองจิตตวัน ภาคกลางของประเทศเนปาล เพื่อศึกษาผลกระทบจากการรุกรานของพืชต่างถิ่นต่อสังคมพืชธรรมชาติ การศึกษานี้ได้เลือกพืชรุกรานต่างถิ่นในเนปาลสองชนิดได้แก่ *Ageratina adenophora* (Spreng.) R.M. King & H. Rob จากป่าในภูเขาจามปาเทวี และ *Chromolaena odorata* (L.) King & Robinson จากป่าสาละ ซึ่งจากการวิเคราะห์สังคมพืชในบริเวณป่าของภูเขาจามปาเทวีโดยพิจารณาจากองค์ประกอบพรรณไม้ โครงสร้าง และถิ่นอาศัย ตลอดจนบริเวณที่พบ พบว่ามีสังคมพืชแตกต่างกัน 5 แบบ โดยที่สังคม *Schima-Alnus* เป็นสังคมพืชที่ถูกรุกรานจาก *A. adenophora* เป็นอย่างมาก ซึ่งสังเกตได้จากการมีความหลากหลายและความหนาแน่นของพืชพื้นเมืองน้อยในสังคมพืชดังกล่าวในบริเวณที่ถูกรุกราน และการศึกษาในป่าสาละในเมืองจิตตวัน พบว่ามีผลกระทบของ *C. odorata* ต่อพืชพื้นเมืองคือต้นสาละ (*Shorea robusta* C.F. Gaertn.) ในทำนองเดียวกัน ซึ่งพบว่าการรุกรานของ *A. adenophora* มีความสัมพันธ์เชิงผกผันกับความสูงของพื้นที่ที่พบสังคมพืชและความสูงของเรือนยอด โดย *A. adenophora* ได้ทำลายสังคมพืชตามธรรมชาติโดยการยับยั้งการเจริญในลักษณะต่างๆกันผลจากการทดลองแสดงให้เห็นว่าดิน ซากอินทรีย์ (litter) รวมถึงผลกระทบที่มาจากสารระเหยจาก *A. adenophora* ยับยั้งการเจริญของต้นกล้า *Schima wallichii* (DC.) Korth. นอกจากนี้ใบและสารสกัดจากใบสดยังยับยั้งการเจริญของต้นกล้า *S. wallichii* และ *Alnus nepalensis* D. Don. ยิ่งกว่านั้นซากอินทรีย์ของ *A. adenohora* ยับยั้งการเจริญของรากต้นกล้า *S. wallichii* ที่ความหนาแน่นต่ำ การศึกษาในครั้งนี้แสดงให้เห็นว่าการยับยั้งการรุกรานของ *A. adenophora* อาจทำโดยการทำให้พืชพื้นเมืองในสังคมพืชธรรมชาติมีความหนาแน่น และการรักษาระดับการปกคลุมของชั้นเรือนยอด

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## ABSTRACT

The vegetation surveys were conducted in a lower montane Champadevi hill forest (Kathmandu district) and a tropical "Sal" forest (Chitwan district) of central Nepal in order to study the impacts of invasive alien species on native vegetation. Due to the present aspect, alien species in Nepal, i.e. *Ageratina adenophora* (Spreng.) R.M. King & H. Rob from Champadevi hill forest and *Chromolaena odorata* (L.) King & Robinson from the tropical Sal forest were selected as they grow invasively in the natural forests. Five types of plant communities identified in the Champadevi hill forest were specific in terms of species composition, structure and location. In this forest, *Schima-Alnus* plant community was highly invaded by *A. adenophora.* Native species richness and density were significantly low at *A. adenophora* invaded sites. Likewise, the species composition, native species richness and *Shorea robusta* C.F. Gaertn. ("Sal" tree) seedlings were severely impacted by *C. odorata* in the tropical Sal forest. *A. adenophora* invasion was negatively correlated with altitude and tree canopy. It threatens natural vegetation by suppressing native species through different mechanisms of inhibition. Experiments showed that *A. adenophora* invaded soil, its litter and the air-born effect of litter inhibited the seedling growth and development of *Schima wallichii* (DC.) Korth. Fresh leaves and leaf extract of *A. adenophora* inhibited the growth of the seedlings of *S. wallichii* and *Alnus nepalensis* D. Don. Moreover, *A. adenohora* litter reduced the root growth of *S. wallichii* at low seedling densities. The results suggest that high native plant density and maintenance of tree canopy could be possible management strategy for *A. adenophora* invasion.

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**Lal Bahadur Thapa** **May 16, 2016**

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# ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| CCA | Canonical correspondence analysis |
| DPR | Department of Plant Resources, Nepal |
| EIH | Evolution of Invasiveness Hypothesis |
| ENH | Empty Niche Hypothesis |
| MFSC | Ministry of Forest and Soil Conservation |
| NBS | Nepal Biodiversity Strategy |
| NEH | Natural Enemy Hypothesis |
| NWH | Novel Weapons Hypothesis |
| USAID | United States Agencies for International Development |

# LIST OF PAPERS

I. **Thapa, L.B.**, Kaewchumnong, K., Sinkkonen, A. and Sridith, K. 2016. Plant Communities and *Ageratina adenophora* Invasion in Lower Montane Vegetation, Central Nepal. International Journal of Ecology & Development 31 (2): 35-49.

II. **Thapa, L.B.**, Kaewchumnong, K., Sinkkonen, A. and Sridith, K. 2016. Impacts of invasive *Chromolaena odorata* on species richness, composition and seedling recruitment of *Shorea robusta* in a tropical Sal forest, Nepal. Songklanakarin Journal of Science and Technology (Accepted for publication in Volume 38, No. 6).

III. **Thapa, L.B.**, Kaewchumnong, K., Sinkkonen, A. and Sridith, K. Plant invasiveness and target plant density: High densities of native *Schima wallichii* seedlings reduce negative effects of invasive *Ageratina adenophora.* Weed Research (Submitted Manuscript, 2016).

IV. **Thapa, L.B.**, Kaewchumnong, K., Sinkkonen, A. and Sridith, K. Volatiles or direct soil contact or both – the inhibitory effects of *Ageratina adenophora* on native species revisited. Acta Physiologiae Plantarum (Submitted Manuscript, 2016).

V. **Thapa, L.B**., Kaewchumnong, K., Sinkkonen, A. and Sridith, K. “Soaked in rainwater” effect of *Ageratina adenophora* on seedling growth and development of native tree species in Nepal (Unsubmitted Manuscript, 2016).

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## Paper I

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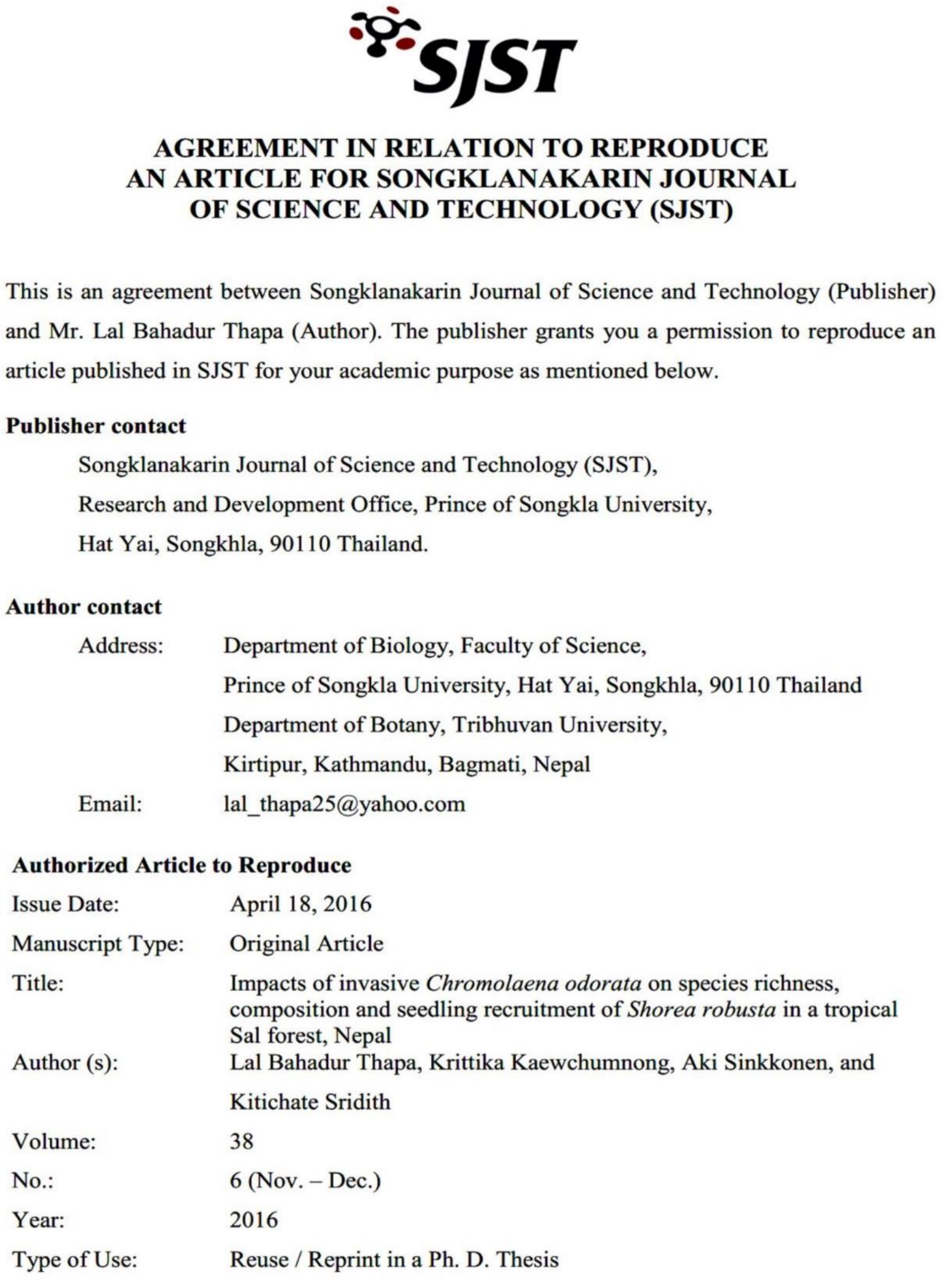

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# Paper III

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21 April 2016

Dear Lal Bahadur Thapa

**Manuscript WRE-2016-0019 "Plant invasiveness and target plant density: High densities of native *Schima wallichii* seedlings reduce negative effects of invasive *Ageratina adenophora*"**

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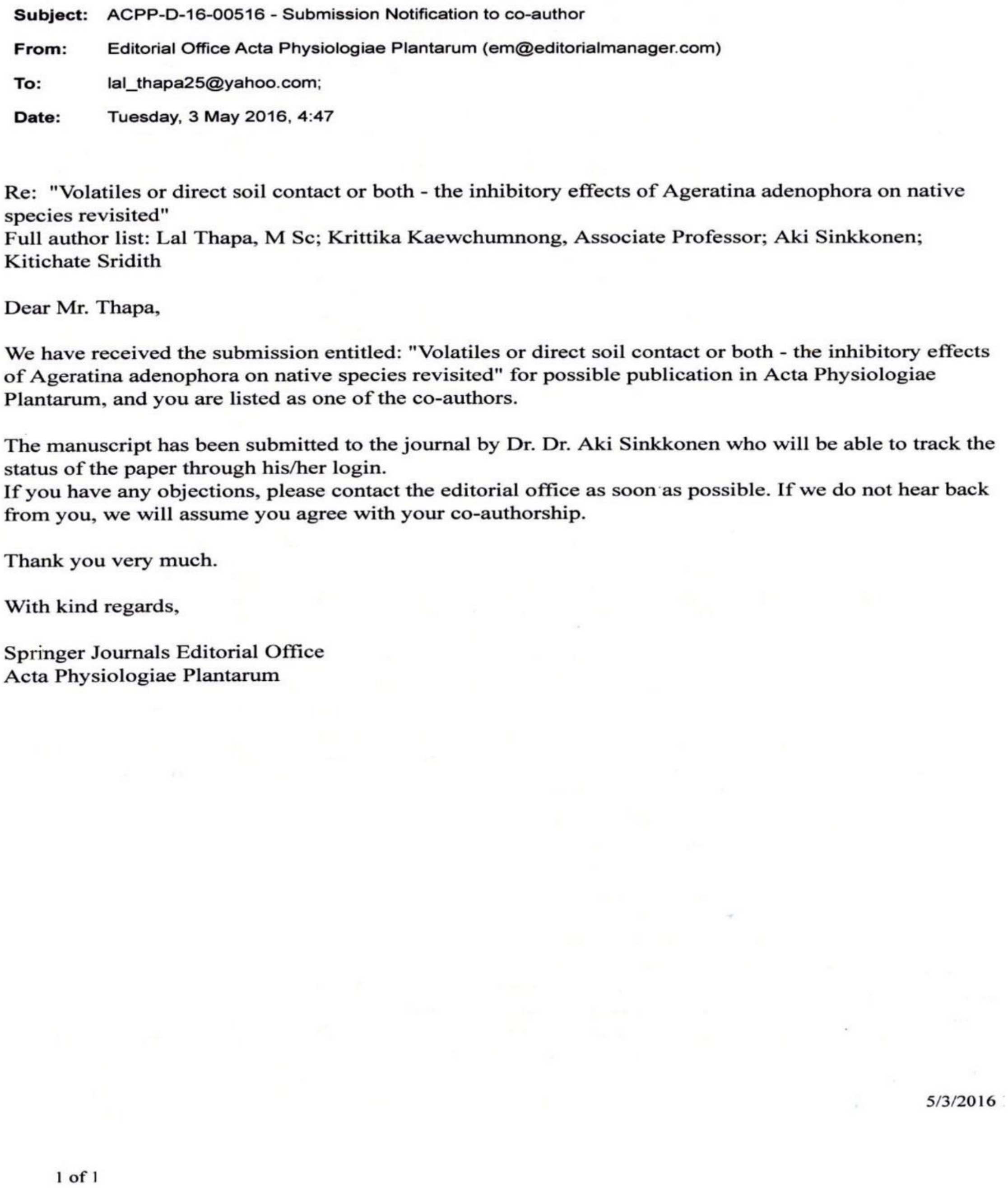

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**Date:** Tuesday, 3 May 2016, 4:47

Re: "Volatiles or direct soil contact or both - the inhibitory effects of Ageratina adenophora on native species revisited"
Full author list: Lal Thapa, M Sc; Krittika Kaewchumnong, Associate Professor; Aki Sinkkonen; Kitichate Sridith

Dear Mr. Thapa,

We have received the submission entitled: "Volatiles or direct soil contact or both - the inhibitory effects of Ageratina adenophora on native species revisited" for possible publication in Acta Physiologiae Plantarum, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Dr. Aki Sinkkonen who will be able to track the status of the paper through his/her login.
If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

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1 of 1

# INTRODUCTION

## Background

Nepal, a Himalayan landlocked country lies between 26°22'-30°27' N and 80°40'-88°12' E. The unique vegetation of Nepal is classified into tropical (below 1000 masl.), subtropical (1000-2000 masl.), temperate (2000-3000 masl.), sub-alpine (3000-4000 masl.) and alpine (above 4000 masl.) zones. Each zone is characterized by distinct climatic and altitudinal variations that make the country one of the richest countries in the world in term of biodiversity. A total of 6,973 species of angiosperm have been reported in Nepal, which represents 3.2% of total world flora (MFSC, 2014). Nepal's position between Palearctic region in the north and Indo-Malayan region in the south has made the country a distinctive hotspot for species from both regions (MFSC, 2014).

Government of Nepal, Ministry of Forest and Soil Conservation, Nepal has enumerated 118 ecosystems including 75 vegetation types in Nepal, ranging from tropical monsoon forests to alpine meadows (MFSC, 2014). According to the same report, the middle mountains of the country are among the richest concerning ecosystem diversity (53 ecosystems) compared with high mountains (38 ecosystems), and Tarai and Siwalik, which contribute 14 and 12 ecosystems, respectively. Moreover, Nepal has 35 natural forest types and about 99% of them are naturally regenerated in the country (USAID/NEPAL, 2010).

Habitat destruction, over manipulation of biological resources, human intentional management as well as climate change effects are becoming current major threats to the native vegetation and plant communities worldwide including Nepal (Chaudhary, 2000; Dirzo and Raven, 2003; Panta et al., 2008). On the other hand, alien species invasion has been taken as a serious and major challenge in conservation and management of natural ecosystems due to having its antagonistically negative impacts on natural habitats, native species, ecosystem functioning and community characteristics (Gordon, 1998; Stohlgren et al., 1999; Ehrenfeld, 2003; Vila et al., 2011).

## Invasive alien species

Any species introduced outside from its natural place of distribution and any part, gametes, seeds, or propagules of the species survive and reproduce magnificently is called alien species (Tiwari et al., 2005). They can be introduced into their non-native ranges as agricultural crops; horticulture, medicinal and ornamental purposes purposefully by people. Others can be introduced inadvertently through various media such as transport, travel, trade or by natural means of dispersal.

Some of alien species introduced into a new area may have rather high dispersion ability and adaptability to colonize a new habitat/ecosystem. In that area they may cause ecological, economic or health related problems. Such species are called invasive alien species (McNeely, 2001). Invasive plant species can be defined as “the naturalized plants species that produce offspring in large numbers, at considerable distances from parent plants which have the prospective to spread over a extensive area” (Richardson et al., 2000).

Common characteristics of invasive alien plant species are strong vegetative growth including abundant seed production ability. They may have high seed germination rate and rapid growth of their seedlings (Grotkopp and Rejmánek, 2007). In several species, their seeds are small and light, which allows seeds to travel a long distance by wind or other media (Sakai et al., 2001). They are also characterized by phenotypic flexibility and have the capability to survive on various types of habitat and environmental condition (Tiwari et al., 2005). They may also compete with native species as they have no natural predator in novel geographic areas and cause ecological havoc (Mack et al., 2000). Changes in morphology, behavior and genetic characteristics may occur in many invasive alien species to develop characteristics beneficial to grow, survive and develop in new area (Suarez and Tsutsui, 2008).

### Invasive alien species in Nepal

Nepal, having extensive variation in climate and geography, has provided suitable environment for the introduction of a number of alien species. There are about 218 alien plant species in Nepal representing 53 families of angiosperms (Tiwari et al., 2005; Siwakoti and Shrestha, 2014). Family Asteraceae has the highest number of alien species (34 species) followed by Solanaceae (23 species) and Fabaceae (20 species) (Siwakoti and Shrestha, 2014). Among them 21 species have been listed as the most problematic invasive alien species in Nepal (Tiwari et al., 2005).

Tiwari et al. (2005) categorized problematic invasive alien species in Nepal into: highly invasive, medium threat, low threat and non-significant threat. The species *Ageratina adenophora* (Spreng.) R.M. King & H. Rob. (Asteraceae), *Chromolaena odorata* (L.) King & Robinson (Asteraceae), *Eichhornia crassipes* Solms (Pontenderiaceae), *Ipomoea carnea ssp. Fistulosa* Jacq. (Convolvulaceae), *Lantana camara* L. (Verbenaceae) and *Mikania micrantha* Kunth (Asteraceae) are considered as highly invasive members. The medium threat members are *Alternanthera philoxeroides* Griseb. (Amaranthaceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Parthenium hysterophorus* L. (Asteraceae) and low threat species are *Ageratum conyzoides* L. (Asteraceae), *Amaranthus spinosus* L. (Amaranthaceae), *Argemone mexicana* L. (Papaveraceae), *Senna tora* (L.) Roxb. (Fabaceae), *Hyptis suaveolens* (L.) Poit. (Lamiaceae), *Leersia hexandra* Sw. (Poaceae) and *Pistia stratiotes* L. (Araceae). Five species: *Bidens pilosa* L. (Asteraceae), *Senna occidentalis* (L.) Roxb. (Fabaceae), *Mimosa pudica* L. (Fabaceae), *Xanthium strumarium* L. (Asteraceae) and *Oxalis latifolia* Humb. (Oxalidaceae) have no significant threat in Nepal (Tiwari et al., 2005)

Recently, additional four alien species *Ageratum houstonianum* Mill. (Asteraceae), *Erigeron karvinskianus* DC. (Asteraceae), *Galinsoga quadriradiata* Ruiz & Pav. (Asteraceae) and *Spermacoce alata* Aubl (Rubiaceae) have also been proposed as invasive members in Nepal (Shrestha, 2016). *C. odorata*, *A. adenophora, M. micrantha*, *L. camara* and *E. karvinskianus* are categorized as invaders of forest ecosystems (Tiwari et al., 2005; Sapkota, 2007; Shrestha, 2016). Out of these five forest invaders, *A. adenophora*, *L. camara* and *C. odorata* have an ability to disrupt natural ecosystem, replace native species and form their pure stands in native vegetation (Tiwari et al., 2005; Shrestha, 2016).

## Impacts of invasive alien species

Invasive alien plant species growing aggressively in different ecosystems are considered major threat to native biodiversity and natural ecosystems (Gurevitch and Padilla, 2004; Vilà et al., 2011). The introduced alien species for food crops have less or no threats to biodiversity and natural ecosystem (Pimentel et al., 2005) but other invasive alien species have mostly negative impacts on habitat, ecology, environment, species diversity and socio-economy of local communities (Pimentel et al., 2005; Thuiller et al., 2006; Pyšek and Richardson, 2010).

Invasive alien species alter invaded habitats and replace native species leading to a change of native diversity into homogenous stand of alien species (Denslow, 2003; Miller and Gorchov, 2004). Habitat alteration may be caused by changes in original soil quality, water regime and nutrition (Ehrenfeld et al., 2001). Replacement of native species by alien invaders after invasion brings changes in community composition. Some examples of invasive species responsible to bring modification to native community composition are *Lantana camara* (Gooden et al., 2009), *Eucalyptus camaldulensis* (Tererai et al., 2013), *Ageratum conyzoides* (Dogra et al., 2009), *Parthenium hysterophorus* (Timsina et al., 2011) and *Mikania micarantha* (Yu et al., 2009) etc. The changes in community composition and native diversity finally affect trophic structure of an ecosystem (Simberloff et al., 2013).

Some aggressive invaders (e.g. *C. odorata*, *A. adenophora, M. micrantha*) have been reported to have an effect on native tree rejuvenation in forest ecosystem (Honu and Dang, 2000; Sapkota, 2007; Yu et al., 2014). One reason behind this is an alteration in mutualistic association between native tree seedlings and belowground community by invasive species (Stinson et al., 2006). Above ground competition between native and invasive species (for example to achieve light) is also an important cause for reduction in native species regeneration (Gorchov and Trisel, 2003). The effect of invasive species on tree regeneration may affect the succession of forest and alter species composition (Flory and Clay, 2010).

In an introduced area chemicals released by invasive species could be unusual or novel for native species and may harm growth and development. Allelopathy (inhibitory impacts of novel chemicals) is an important attribute of invasive species to bring changes to community composition, native seedling recruitment and belowground community (Fisher, 1980; Sharma et al., 2005; Wolfe and Klironomos, 2005; Rudgers and Orr, 2009). A majority of aggressive invaders have impacted native species replacement, growth and diversity by this mechanism.

Many invasive members are responsible for causing fire in the forests (Mooney and Hofgaard, 1998). Increase of fire occurrence may affect regeneration and reclamation of native species. On the other hand, if alien species are closely related to some native species they could interbreed with the native species resulting in changes in genetic make-up by hybridization which eventually affect original gene pool (Huxel, 1999; Bossdorf et al., 2005).

Alien species invasion is also associated with economy, development and livelihood of community people (van Wilgen et al., 2001). Socio-economic impacts by invasive species are caused through their adverse effects on native forest diversity on which people depend for food, medicine and other purposes. Economic impact is related to loss of money to control invasive species and manage their effect such as restoration or recovery of ecological processes (Gordon, 1998; Pimentel et al., 2005). Human health impact of invasive species is another side of socio-economic impacts. An example is *Lantana camara* which provides habitat for tsetse fly which is the vector of sleeping sickness parasite (Syed and Guerin, 2004). Besides human health impact, *L. camara* and *Parthenium hysterophorus* are also poisonous for cattle and cause cattle diseases (Sharma et al., 1988; Timsina et al., 2011).

## Mechanism of alien species invasion

There are several hypotheses proposed for successful invasion of alien species in non-native range. Seven major hypotheses (natural enemies, evolution of invasiveness, empty niche, novel weapons, disturbance, species richness and propagule pressure) have been described by Hierro et al. (2005).

**I. The natural enemies hypothesis (NEH)** is one of the important hypotheses for alien species invasion (Hierro et al., 2005). According to this, the natural enemies play important role to regulate plant population. In a native range the enemies have less impact on alien species introduced than native species of that range (Colautti et al., 2004; Keane and Crawley, 2002). An alien species in an introduced range experiences less number of natural enemies, resulting in its rapid increase in distribution and abundance (Keane and Crawley, 2002).

**II. The evolution of invasiveness hypothesis (EIH)** states that alien species get experience of genetic changes due to the pressure of biotic and abiotic factors in a new environment (Lee, 2002). This mechanism has been considered as an important mechanism for biological invasions (Baker, 1974). Character modifications for adapting to new environment occurs rapidly in alien species and that is a reason they may establish easily, flourish and become invasive (Novak, 2007). Hybridization is an important phenomenon that serves as a stimulus for the evolution of invasiveness (Ellstrand and Schierenbeck, 2000).

**III. The empty niche hypothesis (ENH)** alien species can utilize resources which have not been utilized by native species (Davis et al., 2000; MacDougall, 2009). Empty niche helps alien species to establish and invade into a new environment (Gioria and Osborne, 2014). For example, *Centaurea solstitialis* L. has been reported as a successful invader in California grassland for utilizing unused water which was not utilized by native species (Hierro et al., 2005; Spencer et al., 2011).

**IV. The novel weapons hypothesis (NWH)** has been proposed as an important mechanism for the success of alien invasion. It explains that native species in invaded area are sensitive to novel chemical compounds produced by invasive plant species (Callaway and Ridenour, 2004). Novel chemicals are helpful to protect invasive species from herbivores and the chemicals inhibit native plants (allelopathy) as well as affect microbial association (Barto et al., 2010). This mechanism may contribute to the ability of alien species to become dominant in invaded plant communities (Callaway and Ridenour, 2004).

**V. The disturbance hypothesis** assumes that community composition, structure and functioning of an ecosystem depend on the level of disturbance. Although moderate level of disturbance is considered beneficial for species diversity, it may also increases invasion probability in the community (Hobbs and Huenneke, 1992). One of the determining factor for invasion success and impact of alien species on native community is the type of disturbance (Lake and Leishman, 2004).

**VI. The species richness hypothesis** states that the plant communities are susceptible for alien species invasion if they are poor in species richness. Species-rich communities are stable due to having an intricate system of checks and buffers (Elton, 1958) and a strong interaction of species in such communities reduces possibilities of alien invasion (Case, 1990).

**VII. The propagule pressure hypothesis** explains that the establishment success of species depends on the number of propagules. Higher amount of propagules increases the likelihood of survival, and invasion chance is greater than lower amount of propagules. Therefore, high propagule number of alien species in a new environment increases survival rate whereas few number of propagules are more likely to be extinct (Lockwood, 2005).

## Research concept

Relatively little is known about natural plant communities, alien species invasion and their impacts on native diversity in the tropical to highland vegetation of Nepal. A planned study from natural vegetation to threats of alien invasion in an invaded area is therefore beneficial for understanding the interaction between native vegetation and alien species, potentially invasive alien species. The current status of plant communities, degree of alien plant invasion and threats as well as factors associated with invasion success are indispensable for Nepalese native vegetation. Such investigations are needed in order to maintain successive conservation of natural plant communities that contain many rare and vulnerable plant species.

This study is based on three major concepts (Fig. 1). Identification of plant communities is the first action before carrying out alien species invasion study because different plant communities have unique characteristics in microhabitats in ecosystems. Second, if there is alien species invasion, the measurement of the level of invasion should be taken which can provide valuable information on plant community susceptible to alien invasion. Third, laboratory bioassays could illustrate valuable evidences for the understanding of mechanism of alien species invasion.

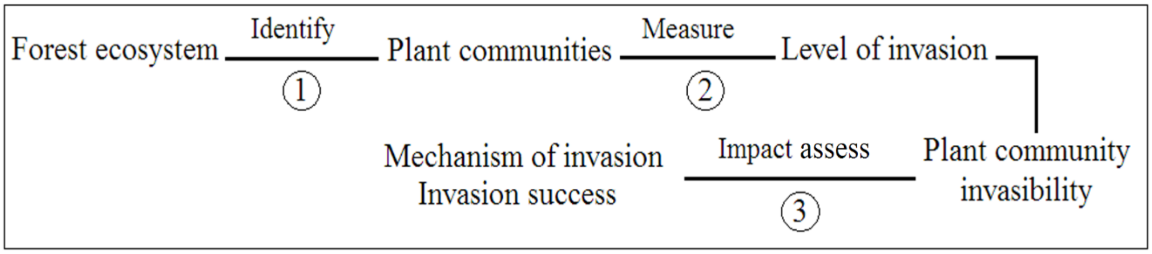

Fig. 1 Concept of study

This study was carried out in the central part of Nepal and selected native plant communities were explored to measure their species richness, composition and alien species invasion. In addition, exploring invasive species of these plant communities, measurement of their impacts in the field and understanding of potential mechanisms behind their negative impacts on native diversity were major themes of the study. A combination of field surveys and laboratory experiments was designed. The field studies explored plant communities and their status including the measurement of alien invasion, and the greenhouse experiments achieved the understanding of different modes of effects of selected invasive species on some native species of central Nepal.

Concerning different modes of invasive species effect, plant parts of them may have contribution to release toxic substances to the soil which ultimately affect native species growth and development. It was hypothesized that the invaded soil, aerial plant parts (mainly fresh leaves) and leaf litter of invasive species have important role to inhibit native seedling growth. Ecologically relevant research and selection of native species which are associated with invasive species are essential points to be considered. Test experiments such as the effects of fresh leaves and extracts from the leaves or roots as well as the litter of selective invasive species on seedling growth of native species can be useful to understand particular mechanisms of invasion on native vegetation. Moreover, as density-dependent growth inhibition is the key characteristic of chemical interference (Weidenhamer et al., 1989; Sinkkonen, 2001, 2003, 2007) it could add additional information to understand native plant responses towards invasion toxicity at different densities and useful to propose whether soil-based phytotoxins from invasive species contribute to invasion or not.

## Methods

### Study sites

Two different study sites that represented different major types of natural vegetation in Nepal were selected for field study: one from the tropical (Tarai) region and the other from sub-tropical (lower montane) region in central Nepal. The study site in the tropical region was located at Panchakanya community forest in Chitwan district (170-216 masl., 27°38′ N and 84°29′ E) where the study on the impacts on native vegetation and seedling recruitments of *Shorea robusta* C.F. Gaertn. (“Sal” tree) by invasive *Chromolaena odorata* (L.) King & Robinson was carried out.

The other site was in the sub-tropical region located at Champadevi hill forest (altitude range between 1300-2400 masl.) in the south-western part of Kathmandu valley (27˚42′ N and 85˚19′ E) where plant communities, alien species invasion, impacts of *A. adenophora* on native vegetation and factors associated with invasion were explored (Fig. 2). The greenhouse experiments were conducted to achieve different modes of impacts on native species by invasive alien species at the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal. All the studies were carried out at the selected sites from April 2014 to September 2015.

**Fig. 2** Nepal map showing study sites

## Test species

Test species were selected on the basis of field observations. Invasive species that aggressively colonizes native vegetation was selected as “donor species”. On the other hand, native dominant species that are supposed to be affected by the invasive colonizer were selected as target species in the physiological studies as follows:

**Invasive species:** *Ageratina adenophora* (Spreng.) R.M. King & H. Rob. (Asteraceae) was selected as an invasive donor species. It is a perennial aggressive shrubby herb in lower montane vegetation in the *Schima-Alnus* plant community (see Paper I). Biological characters of *A. adenophora* and a brief account of chemicals isolated or identified from aerial parts of *A. adenophora*, which are generally considered as allelochemicals, were reviewed in papers III, IV and V.

**Native species:** Native plant species selected for physiological experiments were *Schima wallichii* (DC.) Korth and *Alnus nepalensis* D.Don due to the fact that both species are very common species (medium to large sized trees) and dominant in lower montane Champadevi hill forest (invaded by *A. adenophora*) at the study sites described in Paper I and Paper III. The distribution and important characters such as habitat, flowering, fruiting and seed biology of *S. wallichii* were provided in paper III and paper V while those of *A. nepalensis* were described in paper V.

The details of methods used in the study are given in method section of each paper.

# RESEARCH QUESTIONS AND OBJECTIVES

## Research questions

Main research question of the study was “what are the impacts of invasive alien plant species on the native plant communities in central Nepal?”

The main research question was divided into specific questions as follows:

1. What is the status of some selected plant communities in Nepal?
2. What is the invasion status of aggressive alien species in those plant communities?
3. Which factors influence the alien species invasion in the natural plant communities?
4. What are the potential adverse impacts of invasive alien species on the native plant species?

## Research objectives

1. To identify vulnerable plant communities to alien species invasion.
2. To measure the degree of alien species invasion in the invaded plant communities.
3. To understand the impacts of invasive alien species on species richness, community composition and seedling recruitment of the native species.
4. To understand different inhibition mechanisms by invasive alien species on selected native species.

# RESULTS AND DISCUSSION

## Paper I

### Plant communities, natural recovery of conserved forest, altitude as factors affecting species richness and *Ageratina adenophora* invasion

This paper includes results obtained in a vegetation study conducted in the Champadevi hill forest, central Nepal in 2014. The results incorporated the number of total plant species reported from the study site with information of different plant groups. The information of native, alien and invasive alien species in the forest are provided. *A. adenophora* was reported as one of the most frequent and aggressively growing invasive species. Five types of plant communities, i.e. *Schima*-*Alnus*-mixed community; *Schima*-*Alnus*-herbaceous community; pine-mixed community; transitional community and *Quercus* community are identified by cluster analysis and Sørensen dissimilarity index. They are illustrated and described in detail with information on species richness, composition and structure. The second part of the results reveals associations of species richness and *A. adenophora* cover with altitude by regression and correlation analyses. The regression result and negative association of *A. adenophora* coverage with increasing altitude are illustrated in the paper.

Possible influencing factors determining plant communities, such as altitudinal gradient, physical setting of habitats and anthropogenic disturbance are discussed. Natural recovery of the forest without intended human activities is described. It is discussed utilizing data on *Schima*-*Alnus*-mixed plant community compared with pine-mixed plant community. On the basis of nature and frequency or dominance, the *A. nepalensis* and *S. wallichii* are proposed as important pioneering elements for natural vegetation recovery process in the lower-montane vegetation. The transitional community is explained as an ecotone between *Schima*-*Alnus*-mixed and *Quercus* community. In the lower altitude, two plant communities viz. *Schima*-*Alnus*-mixed and *Schima*-*Alnus*-herbaceous communities were found highly invaded by *A. adenophora* and potential impacts of its invasion in these communities are described in the discussion. Anthropogenic disturbances, tree canopy cover and other environmental factors are anticipated as factors affecting *A. adenophora* cover in the plant communities. The paper can be beneficial for understanding natural recovery of disturbed forests, plant communities and alien species status in the forests in lower montane vegetation region of central Nepal.

## Paper II

### *Chromolaena odorata* changes native species richness, alters community composition and impacts *Shorea robusta* seedling recruitment

This paper documented the impacts of *C. odorata* on species richness, species composition and *S. robusta* seedling recruitment in Panchakanya community forest as a representative of the tropical "Sal" forest of central Nepal. The results of this paper comprise a list of species recorded during the field study and contain information on the native and invasive alien species. Effects of *C. odorata* invasion on species composition, richness (total species count per plot) and *S. robusta* seedling density are illustrated with explanation. The data were analyzed using CCA (Canonical Correspondence Analysis) and the results indicated that the impacts on all parameters studied were significant.

The discussion includes important baseline data on the impacts of *C. odorata* on the species richness, composition and seedling recruitment of the dominant native species *S. robusta* ("Sal" tree) in the Sal forest of central Nepal. It is expected that the paper is useful for future comparisons, establishing and monitoring conservation or restoration efforts in tropical "Sal" forest in Nepal. Moreover, an effort was made in order to relate the current findings with those in previous studies, e.g. how possible the invasion of *C. odorata* affects native vegetation. Future or long-term impacts of *C. odorata* invasion on the dynamics of Sal forest are also extrapolated. Expected future consequences are discussed in this paper. There can be severe changes in soil ecology, eradication of important species, reduced number of *S. robusta* seedlings and the establishment of pure stand of *C. odorata* in the Sal forest if the level of *C. odorata* invasion is higher.

## Paper III

### *Ageratina adenophora* invaded soil and litter inhibit native *Schima wallichii* seedling growth, and higher native plant density reduces *A. adenophora* litter toxicity

This paper includes the results obtained from analyzing the data of two physiological experiments using ANOVA (one-way and two-way ANOVA). The first experiment considers soil and *A. adenophora* litter property and the other studies how native plant density affect toxic effect of *A. adenophora* litter. The first experiment resulted in an observation that soil invaded by *A. adenophora* and its litter were toxic to the seedlings of *S. wallichii*. Invaded soil inhibited root and/or shoot length, root dry weight as well as shoot:root ratio of *S. wallichii* seedlings. *A. adenophora* litter was also toxic to the root of *S. wallichii*. The results of the second experiment, the density-response experiment elucidated that *S. wallichii* seedlings exposed to *A. adenophora* litter had smaller roots than those in the control treatment at low plant densities while the inhibition disappeared at high plant densities. Moreover, the root length and the dry weight of the control treatment decreased gradually at increasing plant densities. This suggested that the toxin effect was diluted at high plant densities, and that plants competed for soil nutrients. The detailed pattern of an effect of the litter thickness (0, 0.5, 1.0 and 1.5 cm) on different plant densities (1, 3, 6 and 12 plants/pot) is explained with illustrations in this paper.

In the discussion part, sensitivity of *S. wallichii* roots to both *A. adenophora* invaded soil and its litter, and promising complications in the natural condition are discussed. The results were compared with the earlier finding (e.g. Inderjit *et al.* 2011).

Possible mechanisms of inhibition by litter are described, i.e. the volatiles produced from the thick litter layer during litter decomposition might be trapped by the seedlings. Moreover, the results are in accordance with the hypothesis that soluble compounds from the litter and non-volatile toxins might inhibit *S. wallichii* growth. The hard survival of *S. wallichii* seedlings at lower densities is expected to occur in nature during the dry season due to the toxic effect of litter to their roots. The recommendation on conservation aspects is that *S. wallichii* seedling density can be managed to overcome the deleterious effects of *A. adenophora*. The low pH in the invaded soil was also expected as one of the factors inhibiting *S. wallichii* seedling growth. As the litter of *A. adenophora* was rich in nutrients (from the soil analysis), it was considered a contributing factor to the fast growth rate of *S. wallichii* seedlings at higher plant density. In any case, an application of density response bioassay in the context of plant invasiveness is highlighted. Possible interactions of several other factors such as moisture, temperature, soil aeration and soil microbe effects on plant growth and development are commented in the discussion.

## Paper IV

### ***Schima wallichii* seed germination is inhibited by the air-borne effect of *Ageratina adenophora* leaf litter, *S. wallichii* seedling growth slows down in invaded soil, and forest tree canopy cover is negatively correlated with *A. adenophora* cover**

Paper IV has combined the results of the field experiments carried out along *A. adenophora* invaded and non-invaded sites at Champadevi hill forest, Kathmandu, Nepal, together with physiological experiment, conducted at the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal. Considering the field experiment, it showed that tree canopy coverage was negatively correlated with *A. adenophora* cover, i.e. higher tree canopy coverage reduced *A. adenophora* cover significantly. Comparing native species richness and total plant density between invaded and non-invaded sites, *A. adenophora* (invaded plots) is associated with lower values of both parameters. The analysis was done using t-test and non-parametric correlation in SPSS program (version 20).

The physiological experiment was conducted to understand an airborne effect of *A. adenophora* litter on *S. wallichii* growth using both invaded and non-invaded soils. The results obtained from this experiment was illustrated as an important finding. The air-borne effect of *A. adenophora* litter was inhibitory to the rate of *S. wallichii* seed germination. Moreover, it showed that the shoots of *S. wallichii* seedlings were shorter in chambers containing leaf litter, while the root growth was reduced in the invaded soil but not by the litter. Interestingly, the accumulation of shoot and root dry weight was inhibited by both the invaded soil and litter.

It is proposed here that maintaining high tree canopy coverage can be an economically feasible practice in order to reduce *A. adenophora* invasion on the basis of the inversed relation of *A. adenophora* to the forest canopy cover. A novel finding from the physiological experiment, i.e. the air-borne inhibitory effect of *A. adenophora* leaf litter on native tree species has been discussed. In addition, the belowground effect of soil has been highlighted for its strong impact on the establishment of *S. wallichii* seedlings. Two important issues from the results, (i) *S. wallichii* germination could be inhibited by the air-borne effect of *A. adenophora* leaf litter and (ii) *S. wallichii* seedling growth could be slowed down in the invaded soil, were focused in the discussion part. Possible causes behind the inhibition, i.e. soil feedback mechanism and the allelopathic inhibition as well as the comparison of the current finding with previous work have also been included in the discussion of this paper.

## Paper V

### Fresh leaves of *Ageratina adenophora* inhibits native species (*Schima wallichii* and *Alnus nepalensis*) growth and development

Paper V included results obtained from a physiological experiment in which the seedling growth of two native tree species (*S. wallichii* and *A. nepalensis*) was altered using *A. adenophora* fresh leaves and fresh leaf and root extracts. The data of native seedling growth parameters were analyzed by one-way ANOVA using the software SPSS program (version 20). *A. adenophora* fresh leaves and fresh leaf extract decreased *S. wallichii* shoot and root length as well as its dry weight. In case of *A. nepalensis,* both the fresh leaves and the leaf extract of *A. adenophora* were inhibitory to the shoot and root length as well as its dry weight. The results have been explained with illustrations in the result part of Paper V.

The inhibitory effect of *A. adenophora* extracts on the seedling growth of both native test species is explained in the discussion part. Sensitivity of native seedlings to *A. adenophora* contaminated soil from the early age of the development and the pattern of inhibition to the native species root and shoot growth with the possible mechanisms are described. Some allelochemicals identified and isolated from the aerial parts of *A. adenophora* are listed with explanation of their possible roles to contaminated natural soil and have effect on the native species seedlings. Moreover, it is also anticipated and discussed that there might be an indirect effect of allelochemicals on the growth of native seedlings through changes in soil quality or soil microbial communities. Discussion of ecological relevance of the bioassay of this experiment such as the use of fresh leaves of invasive species, extraction by soaking invasive species plant parts and the selection of genuine native species were elaborated. The habitat similarities of both test species with the alien species (*A. adenophora*) was also compared and expected future impacts of *A. adenophora* on the survival and growth of the native species were summarized in the discussion of this paper.

# CONCLUDING REMARKS

In conclusion, the subtropical *Schima-Alnus* forest and tropical Sal forest in the central part of Nepal have been greatly invaded and impacted by the invasion of *A. adenophora* and *C. odorata* respectively. The plant communities in lower montane Champadevi forest are characteristic in terms of species richness, composition and structure but *Schima-Alnus* plant community particularly have higher level of *A. adenophora* invasion. These plant communities are diverse as they are naturally recovered without human activities and show good examples of natural recovery of lower montane forest in Nepal after the forest has been kept undisturbed. Considering the forest recovery process, two dominant native elements, i.e. *S. wallichii* and *A. nepalensis* play important roles (Paper I).

*C. odorata* has negative impacts on native species richness, community composition and *S. robusta* seedling recruitment in the tropical Sal forests of Nepal. *C. odorata* replace native species and threats the natural regeneration of the Sal trees. The degree of *C. odorata* invasion could be expected to be more severe in the future, therefore, appropriate measures should be implemented for the control and management of *C. odorata* in the native sal forest ecosystems in Nepal (Paper II).

*A. adenophora* invaded soil and its litter are capable to inhibit native *S. wallichii* seedling growth. The inhibitory effect of *A. adenophora* litter is high at low densities of *S. wallichii* and the primary impact is on their roots, whereas the effect would be disappearing at high plant densities. It is suggested that the increase of native plant seeding density in the *A. adenophora* invaded habitats could reduce deteriorate effect of *A. adenophora* leaf litter and this might be an economically practicable approach of management against alien species invasion (Paper III).

Higher tree canopy coverage reduces *A. adenophora* invasion. Field studies revealed that native species richness and density are reduced by *A. adenophora* invasion in *Schima-Alnus* plant community of Champadevi hill forest, central Nepal. Air-borne effect of *A. adenophora* leaf litter inhibits the germination of *S. wallichii*. In addition, the air-borne effect shortens the shoot of *S. wallichii* seedlings but root length is not affected. The accumulation of shoot and root dry weight is inhibited by both the *A. adenophora* invaded soil and the air-borne effect of its litter (Paper IV). *A. adenophora* extracts from aerial part contribute to natural soil contamination which has an adverse effect on the growth of seedlings of native trees (*S. wallichii* and *A. nepalensis*). Fresh leaves of *A. adenophora* and extracts of fresh leaves are toxic to the seedling growth of both native trees (Paper V). The findings of the field impacts of *A. adenophora* on native species (Paper IV) is supported by the results obtained from physiological experiments (Paper III, IV and V).

Density response bioassay to study the ecological significance of chemical interference in the context of plant invasiveness and air-borne bioassay to show that the simultaneous air-borne as well as the belowground effects on the native species by the alien species were important novel cases in invasion ecology. It is proposed that the air-borne effect of *A. adenophora* litter and soil-based phytotoxins contribute to *A. adenophora* invasion. Field tests to find out seedling performance against invasion toxicity, maintenance of tree canopy and further ecologically relevant study are recommended.

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# Paper I

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# Plant Communities and *Ageratina adenophora* Invasion in Lower Montane Vegetation, Central Nepal

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## ABSTRACT

*Relatively little is known about the current status of existing plant communities and invasive alien species in lower montane vegetation. This paper aims to distinguish and describe plant communities concentrating on Ageratina adenophora invasion and altitude as factor that affects species richness and A. adenophora cover. Twenty plots were established in different locations of study site. Five types of plant communities were identified in the study area from cluster analysis. Higher species richness with higher level of A. adenophora invasion was found in naturally recovered lower altitude plant communities. Two native species; Schima wallichii and Alnus nepalensis were found as important elements in forest recovery process, while pine plantation reduced species richness. In general, A. adenophora threats species rich lower altitude plant communities and therefore regular monitoring of these plant communities is needed for proper conservation and management.*

**Key words:** vegetation, plant communities, invasive species, species richness, tree canopy

**Mathematics Subject Classification Number (MSC):** 62P10, 92F05

**JEL Classification Number:** Q57

## INTRODUCTION

Nepal, lying in the central part of Himalayas with unique geography and climatic variation, provides diversity of habitats for different plant communities. The unique vegetation of Nepal is classified into tropical, sub-tropical, temperate, sub-alpine and alpine zones (Barnekow Lillesø et al., 2005; Bhuju et al., 2007). Each vegetation zone is characterized by typical climatic and altitudinal variations that make the country rich in biodiversity (Ghimire et al., 2008; Ohsawa, 1993). However, studies on different aspects of plant communities within each vegetation type are still deficient and neglected by researchers.

Among various factors affecting natural vegetation alien species invasions are one of the challenging issues for conservation and management activities (Stohlgren et al., 1999). Invasive alien plants are known to alter ecosystem and community because many of them replace native species, change soil structure, function and characteristics (Ehrenfeld, 2003; Gordon, 1998; Timsina et al., 2011; Villa et al., 2011). *Ageratina adenophora* (Spreng.) R.M.King & H.Rob., commonly called 'forest killer', is one among many problematic invasive weeds in Asian continents including Nepal (Tiwari et al., 2005). Hence, studies on recognition of microhabitats, plant communities, *A. adenophora* invasion status in natural communities and its impacts are urgently needed.

This study was carried out with an aim to distinguish plant communities in a Lower montane vegetation (Champadevi hill forest as a case study) lying in Central Nepal. We measured *A. adenophora* cover in these plant communities and additionally analyzed the **effect** of altitude on species richness and *A. adenophora* cover.

## METHODS

### Study area

The study area is Champadevi hill forest lying on south-western part of Kathmandu valley, central region Nepal (27˚42' N and 85˚19' E) (Fig 1). The vegetation type of the area represents subtropical and transition between subtropical and temperate zones. The elevation ranges from 1300 to 2400 masl and the site faces towards north-east. The climate of the area is hot and humid in summer and dry in winter. Annual mean temperature is approximately 18˚C with some variation in summer (24˚C) and winter (9˚C) seasons. Lower belt of the forest was previously disturbed but community people have conserved the forest for the last 20 years by planting pine in huge area as well as without intervening natural regeneration or planting activities. The area without planted activities adjacent to pine planted area has been recovered by *Schima wallichii* (DC.) Korth. and *Alnus nepalensis* D.Don Top of the hill was characteristics with *Quercus* dominated forest. The study was conducted from April to November, 2014.

### Plot sampling

A total of 20 plots were placed within the forest area (Fig. 1). Ten plots were established along a transact throught different vegetations (*Schima-Alnus* dominated site, mixed forest site and *Quercus* dominated site) starting from 1396 masl to the top of the hill (2200 masl). Two plots (PL19 and PL20) were established at both right and left side of the topmost plot (Fig. 1). One set of four plots were established in the *Pine* forest (PL10 to PL13) and another set of three plots were established along horizontal transect across *Schima-Alnus* forest patches (PL7 to PL9). The number of plot distribution was at least 3 in each vegetation type except pure *A. nepalensis* site where there was a single plot (PL1). The plot size was 20×40m$^2$ and geographic coordinates were taken by GPS (Gramin GPSmap 60CSx) at the center of each plot.

*A. adenophora* cover was estimated as a percentage cover (1%, 2%, 5% and then in 5% increments upto 100%) in each plot. Vascular plants encountered in each plot were counted and identified using standard literature (Polunin and Stanton, 1984; Press et al., 2000; Rajbhandari et al., 1986; Stanton, 1988). Voucher specimens collected were deposited at the the National Herbarium and Plant Laboratories, Godawari (KATH), Nepal.

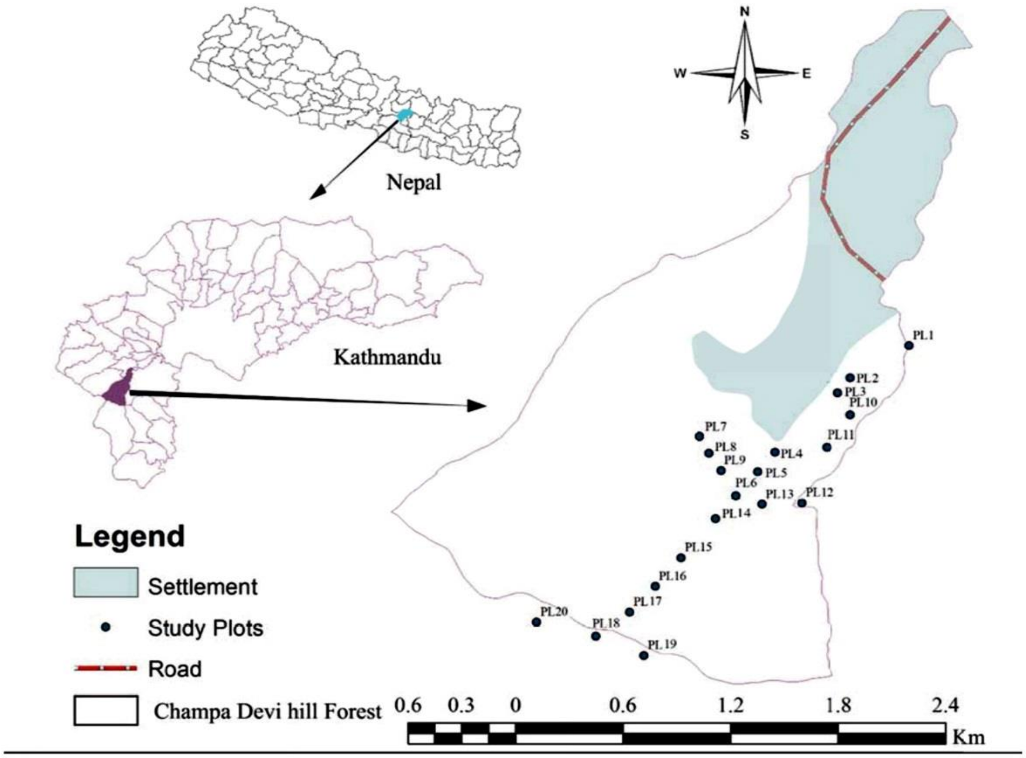

**Figure 1 :** Study area and sampling plots

(The above figures 1 is for academic purpose only and does not represent any accurate political or geographical area of any country).

### Data Analysis

Sørensen dissimilarity coefficient ($D_s$) was calculated between the study plots using formula $D_s = b+c/2a+b+c$, where '*a*' is the number of species found on both compared sites, '*b*' and '*c*' are number of species found on only in one of the site (Kent and Coker, 1992). The dissimilarity values of the index vary from 0-1 (100% similarity to 100% dissimilarity) which were converted into similarity values by subtracting from 1 (1-Ds). Average linkage clustering based on minimum average distance between groups was used to distinguish plant communities. Sørensen dissimilarity index and cluster dendrogram were generated to distinguish plant communities (Oksanen, 2015). Linear regression was used to evaluate strength of the relationship between altitude and species richness. Spearman's rank correlation was used to analyze the relation of altitude to *A. adenophora* cover as the cover data was not normal. **R** Software version 3.0.0 (R Development Core Team, 2013; *package Vegan*) was used for data analysis.

## RESULTS

### Species richness, composition and plant communities

A total of 163 species of vascular plants were recorded from the study plots belonging to 140 genera and 76 families. Among them 11 species were ferns and fern allies, two species were Gymnosperms and 150 species were Angiosperms. Asteraceae was the richest family (13 species) followed by Rosaceae (12 species); Araceae (7 species) and Poaceae (6 species), and 35 families were represented by only single species (Appendix I). The vegetation was represented by 144 native species and 19 alien species. Among the alien species; 7 species (*Ageratum conyzoides* L., *A. houstonianum* Mill., *A. adenophora, Argemone mexicana* L., *Bidens biternata* (Lour.) Merr. & Sherff, *Lantana camara* L., *Parthenium hysterophorus* L.) were recognized as invasive. The *A. adenophora* was one of the most frequent and aggressively growing invasive species found in all plots except PL19.

The cluster analysis and Sørensen similarity matrix on the basis of species composition distinguished five types of plant communities (Fig. 2; Table 1). The analysis also distinguished higher altitude plant communities (PL 16–20) from lower altitude communities (PL 1-15) (Fig. 2). *Quercus* community and Transitional community are located at higher altitude while *Schima-Alnus*-mixed community and *Schima-Alnus*-herbaceous community are located at lower altitude. Similarly, pine planted community (PL 10-13) is separated from naturally growing communities (Fig. 2). The proposed plant communities and species richness are as follows:

**(A) *Schima-Alnus*-mixed community**: A total of 8 plots (plot no. 4-9, 14 and 15) were included in *Schima-Alnus*-mixed community. This community is situated at lower altitude (1500 to 1700 masl). It was highly influenced by anthropogenic disturbances previously. However, it was characterized by the highest species number (109 species) and it had characteristic growing profiles of *Schima wallichii* (DC.) Korth. (Fig. 2 and Fig. 3c, d, e, f, j). Common associated tree species in the community were *Ziziphus incurva* Roxb., *Myrsine capitellata* Wall., *Persea duthiei* (King ex Hook.f.) Kosterm., *Lyonia ovalifolia* hort. and *Zanthoxylum armatum* DC. etc. Common shrubs were *Pyracantha crenulata* (D.Don) M.Roem., *Sarcococca coriacea* Sweet, *Maesa chisia* D.Don, *Camellia kissi* Wall., *Caryopteris foetida* Thell., *Osbeckia stellata* Wall., *Mahonia napaulensis* DC., *Jasminum humile* B.Heyne ex Wall., *Osyris wightiana* Wall. ex Wight and *Eurya acuminata Wall.*

Important climbers in the community were *Cissampelos pareira* L. and *Rubus paniculatus* Roxb., *Hedera nepalensis* K.Koch and *Smilax* sp. The ground floor was covered by *Gonostegia hirta* (Blume) Miq., *Galium palustre* Pourr. ex Willk. & Lange, *Galium aparine* L., *Stellaria* sp. *Rubia manjith* Roxb. ex Flem., *Oplismenus burmannii* (Retz.) P.Beauv. Ferns and fern allies were represented by *Polystichum polyblepharum* Nakai, *Gleichenia gigantea* Wall., *Onychium japonicum* (Thunb.) Kunze, *Pteris vittata* Schkuhr, *Dryopteris* and *Tectaria* sp. This community is divided into two types of sub-communities, one type was characterized by mixed stand of *Schima wallichii* (DC.) Korth. and *Alnus nepalensis* D.Don (Fig. 2, PL 7-9; Fig. 3j). The associated species in this type were mainly *Ziziphus incurva* Roxb. and *Sarcococca coriacea* Sweet. Second type sub-community was highly heterogeneous where *Schima* *wallichii* (DC.) Korth. was intermixed with *Castanopsis indica* A.DC., *Myrica esculenta* Buch.-Ham. ex D.Don, *Lyonia ovalifolia* hort., *Maesa chisia* D.Don and *Alnus nepalensis* D.Don in moist patches with shrubby second layer vegetation (Fig 2, PL 4-6 & 14; Fig. 3e, f).

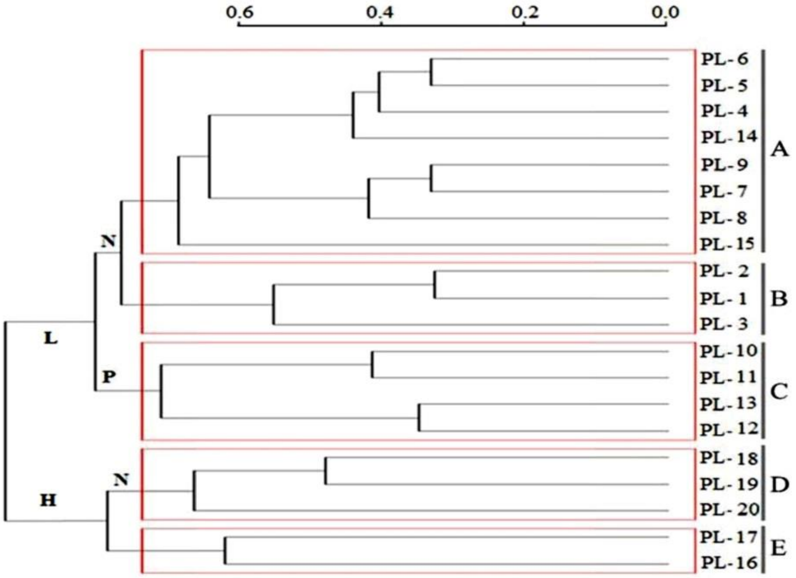

**Fig. 2** Cluster dendrogram based on similarity of species among the plots (Scale indicates dissimilarity among the plots). A to E; Plant communities, H; high altitude community, L; lower altitude community, N; natural community, P; planted pine community

**Table 1.** Sørensen similarity matrix between the plots

| | PL1 | PL2 | PL3 | PL4 | PL5 | PL6 | PL7 | PL8 | PL9 | PL10 | PL11 | PL12 | PL13 | PL14 | PL15 | PL16 | PL17 | PL18 | PL19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PL2 | **0.74** | | | | | | | | | | | | | | | | | | |
| PL3 | 0.49 | **0.62** | | | | | | | | | | | | | | | | | |
| PL4 | 0.51 | 0.47 | 0.53 | | | | | | | | | | | | | | | | |
| PL5 | 0.40 | 0.41 | 0.48 | **0.73** | | | | | | | | | | | | | | | |
| PL6 | 0.37 | 0.36 | 0.43 | **0.62** | **0.73** | | | | | | | | | | | | | | |
| PL7 | 0.47 | 0.39 | 0.47 | 0.56 | **0.62** | **0.61** | | | | | | | | | | | | | |
| PL8 | 0.33 | 0.31 | 0.36 | 0.37 | 0.45 | 0.43 | **0.65** | | | | | | | | | | | | |
| PL9 | 0.42 | 0.44 | 0.45 | 0.48 | 0.59 | 0.48 | **0.73** | **0.68** | | | | | | | | | | | |
| PL10 | 0.27 | 0.11 | 0.32 | 0.38 | 0.33 | 0.32 | 0.49 | 0.38 | 0.36 | | | | | | | | | | |
| PL11 | 0.23 | 0.16 | 0.38 | 0.47 | 0.43 | 0.40 | 0.51 | 0.45 | 0.40 | **0.72** | | | | | | | | | |
| PL12 | 0.26 | 0.27 | 0.42 | 0.34 | 0.42 | 0.36 | 0.51 | 0.44 | 0.42 | 0.36 | 0.40 | | | | | | | | |
| PL13 | 0.28 | 0.31 | 0.43 | 0.34 | 0.46 | 0.37 | 0.51 | 0.38 | 0.45 | 0.46 | 0.49 | **0.67** | | | | | | | |
| PL14 | 0.31 | 0.28 | 0.40 | 0.59 | **0.65** | **0.69** | 0.50 | 0.33 | 0.37 | 0.24 | 0.36 | 0.37 | 0.35 | | | | | | |
| PL15 | 0.14 | 0.17 | 0.29 | 0.41 | 0.48 | 0.46 | 0.46 | 0.36 | 0.40 | 0.17 | 0.22 | 0.29 | 0.19 | **0.53** | | | | | |
| PL16 | 0.17 | 0.18 | 0.26 | 0.32 | 0.36 | 0.34 | 0.41 | 0.31 | 0.40 | 0.22 | 0.17 | 0.29 | 0.24 | 0.35 | 0.41 | | | | |
| PL17 | 0.09 | 0.06 | 0.14 | 0.31 | 0.33 | 0.29 | 0.37 | 0.30 | 0.32 | 0.25 | 0.31 | 0.25 | 0.27 | 0.26 | 0.38 | **0.53** | | | |
| PL18 | 0.27 | 0.28 | 0.30 | 0.42 | 0.38 | 0.34 | 0.45 | 0.28 | 0.42 | 0.27 | 0.29 | 0.24 | 0.25 | 0.28 | 0.32 | 0.50 | 0.49 | | |
| PL19 | 0.25 | 0.26 | 0.23 | 0.36 | 0.33 | 0.31 | 0.30 | 0.15 | 0.25 | 0.25 | 0.23 | 0.28 | 0.26 | 0.29 | 0.15 | 0.28 | 0.27 | **0.61** | |
| PL20 | 0.07 | 0.07 | 0.10 | 0.15 | 0.21 | 0.21 | 0.21 | 0.18 | 0.21 | 0.09 | 0.12 | 0.17 | 0.12 | 0.17 | 0.22 | 0.25 | 0.39 | 0.50 | 0.43 |

**(B) *Schima-Alnus*-herbaceous community:** This community (plot no. 1-3) was found as an isolated patch in the lowermost altitude (1300 to 1500 masl). It was separated from the main forest patch and located along moist stream habitats. It was the second rich community in term of plant species richness (70 species). The community was characterized by canopy of basically *Alnus nepalensis* D.Don or *Schima wallichii* (DC.) Korth. (Fig. 2 and Fig. 3a, b). Below the tree canopy herbaceous layer was present except few shrub species (*Ziziphus incurva* Roxb., *Maesa chisia* D.Don and *Phyllanthus* sp.).

The herbaceous cover was mainly composed of *Centella asiatica* (L.) Urb., *Arisaema erubescens Schott*, *Barleria strigosa* Willd., *Cyanotis vaga* (Lour.) Schult.f., *Viola biflora* L., *Polygonum barbatum* Comm. ex Meisn., *Drymaria diandra* Blume, *Hydrocotyle javanica* Thunb., *Hemiphragma heterophyllum* Wall., *Justicia simplex* D.Don, *Mariscus cyperoides* (L.) Urb., *Persicaria capitata* (Buch.-Ham. ex D.Don) H.Gross.

**(C) Pine-mixed community:** A total of 44 plant species were reported from pine-mixed community (plot no.10-13). *Pinus roxburghii* Sarg. and *Pinus wallichiana* A.B.Jacks. were planted many years ago in previously disturbed area. These two species has been established as pine forest along foothill of eastern site of Champadevi hill (1400 to 1600 masl) (Fig. 2 and Fig. 3i).

Many native shrub species such as *Lyonia ovalifolia* hort., *Schima wallichii* (DC.) Korth. , *Castanopsis indica* A.DC., *Eurya acuminata* DC., *Osbeckia stellata* Wall. etc. were found grown naturally under pine canopy. Herbaceous ground layer was composed of *Coniogramme intermedia* Hieron., *Anaphalis busua* DC., *Remusatia hookeriana* Schott, *Remusatia vivipara* (Roxb.) Schott, *Oplismenus compositus* (L.) P.Beauv., *Arisaema tortuosum* Steud. ex Engl., *Lygodium japonicum* (Thunb.) Sw., *Tetrastigma serrulatum* (Roxb.) Planch. and *Scutellaria discolor* Colebr.

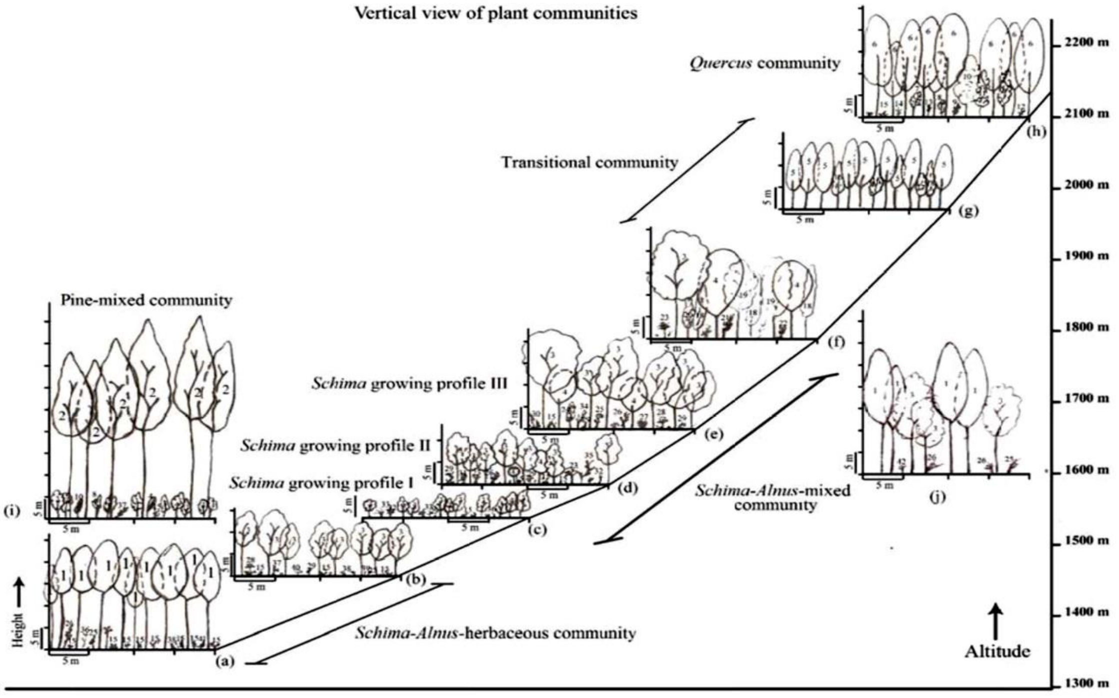

**Fig. 3** Structural profiles of plant communities at Champadevi hill forest, Kathmandu, Central Nepal

(**1**; *Alnus nepalensis* D.Don, **2**; *Pinus roxburghii* Sarg., **3**; *Schima wallichi* (DC.) Korth. , **4**; *Castanopsis indica* (Roxb.) Miq., **5**; *Quercus glauca* Thunb, **6**; *Quercus lanata* Sm, 7; *Myrica esculenta* Buch.-Ham. ex D. Don, **8**; *Lyonia ovalifolia* (Wall.) Drude, **9**; *Berberis aristata* DC., **10**; *Rhododendron arboreum* Sm., **11**; *Quercus semicarpifolia* Sm., **12**; *Gaultheria fragrantissima* Wall., **13**; *Arundinaria* sp., **14**; *Indigofera heterantha* Wall. ex Brandis., **15**; *Ageratina adenophora* (Spreng.) King & H.Rob., **16**; *Castanopsis tribuloides* (Sm.) A.DC., **17**; *Persea duthiei* (King ex Hook. f.) Kosterm., **18**; *Myrsine capitellata* Wall., **19**; *Rhus javanica L.*, **20**; *Myrsine semiserrata* Wall. **21**; *Camellia kissi* Wall., **22**; *Jasminum humile* L., **23**; *Mahonia napaulensis* DC., **24**; *Osyris wightiana* Wall., **25**; *Ziziphus incurva* Roxb., **26**; *Sarcococca coriacea Sweet*, **27**; *Maesa chisia D.Don*, **28**; *Dichroa febrifuga* Lour., **29**; *Phyllanthus* sp., **30**; *Caryopteris foetida* Thell., **31**; *Eurya acuminata* DC., **32**; *Zanthoxylum armatum* DC., **33**; *Pyracantha crenulata* (D.Don) M.Roem., **34**; *Salix* sp., **35**; *Viburnum cylindricum* Buch.-Ham. ex D. Don**, 36**; *Rubus ellipticus* Sm., **37**; *Osbeckia stellata* Buch.-Ham. ex D.Don, **38**; *Gleichenia gigantea* Wall. ex Hook. & Bauer, **39**; *Thomsonia nepalensis* Wallich, **40**; *Arisaema intermedium* Blume, **41**; *Urtica dioeca* L.)

**(D) Transitional community:** This community (plot no. 16-17) is located at an altitudinal range from 1700 to 2000 masl which was poor in plant diversity (36 species). The landscape was rocky with slope having thick layer of litter. It was specialized by single layered dense tree canopy of *Quercus* with association of elements from *Schima-Alnus*-mixed communities. *Quercus glauca* Bürger ex Blume, *Quercus semecarpifolia* Sm., *Castanopsis tribuloides* (Sm.) A.DC., *Rhododendron arboreum* Sm., *Ziziphus incurva* Roxb., *Myrsine semiserrata* Wall., *Persea duthiei* (King ex Hook.f.) Kosterm., *Rhus javanica* L. etc. **were associated components** (Fig. 2 and Fig. 3g).

**(E) *Quercus* community:** This community (plot no. 18-20) is located at the top of the hill at altitude (2000-2400 masl) from where 45 plant species were reported. It was characterized by nicely arranged canopies of *Quercus* (Fig. 2 and Fig. 3h). The dominant species in the community was *Quercus lanata* Sm. and associated species were *Q. glauca* Bürger ex Blume, *Q. semecarpifolia* Sm., *Gaultheria fragrantissima* Wall., *Lyonia ovalifolia* hort., *Rhododendron arboreum* Sm. and *Myrica esculenta* Buch.-Ham. ex D.Don. Natural pine was also characteristic to this community with association of *Quercus* sp.

Common shrubs and herbs in this community were represented by *Berberis aristata* DC., *Arundinaria* sp., *Indigofera heterantha* Wall. ex Brandis *Galium aparine* L., *Herminium* sp., *Malaxis acuminata* D. Don, *Rubia manjith* Roxb. ex Flem., *Potentilla fulgens* Wall. ex Hook., *Anaphalis triplinervis* Sims ex C.B.Clarke, *Roscoea purpurea* Sm. and *Rubus foliolosus* D.Don.

### *A. adenophora* cover and effect of altitude

*A. adenophora* cover in the *Schima-Alnus*-mixed community and *Schima-Alnus*-herbaceous community was higher than other communities (upto 48%). In the pine-mixed community the cover was less than 5%. Invasion of *A. adenophora* was also found in high altitude communities, the transitional and *Quercus* communities, but its cover was less than 5% (Fig 4). Regression analysis showed negative correlation of species richness with increasing altitude. The altitude explains 28% variations in species richness (df = 1; ms = 767.8; F = 6.84; $p = 0.017$) (Fig. 5a). *A. adenophora* is associated with lower altitude i.e. its cover decreased on increasing altitude ($r_s = -0.588$, $p = 0.006$) (Fig. 5b).

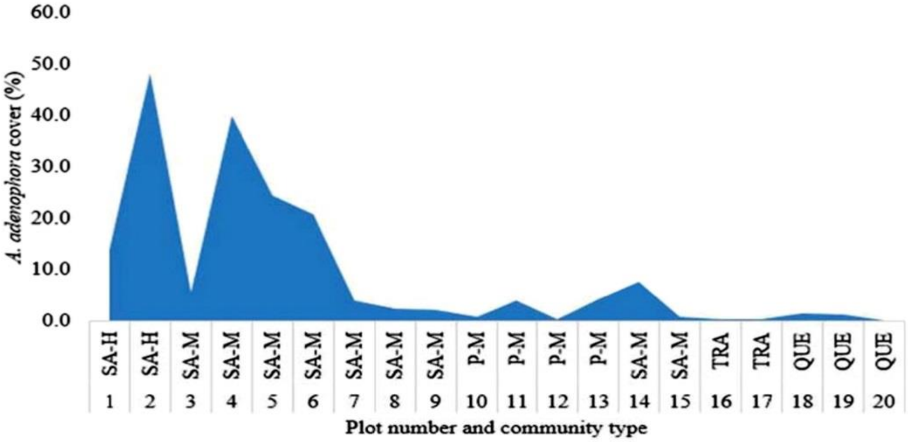

**Fig. 4** *A. adenophora* cover in plant communities (SA-H; *Schima-Alnus* herbaceous community, SA-M; *Schima-Alnus* mixed community, P-M; Pine mixed community, TRA; Transitional community and QUE; *Quercus* community)

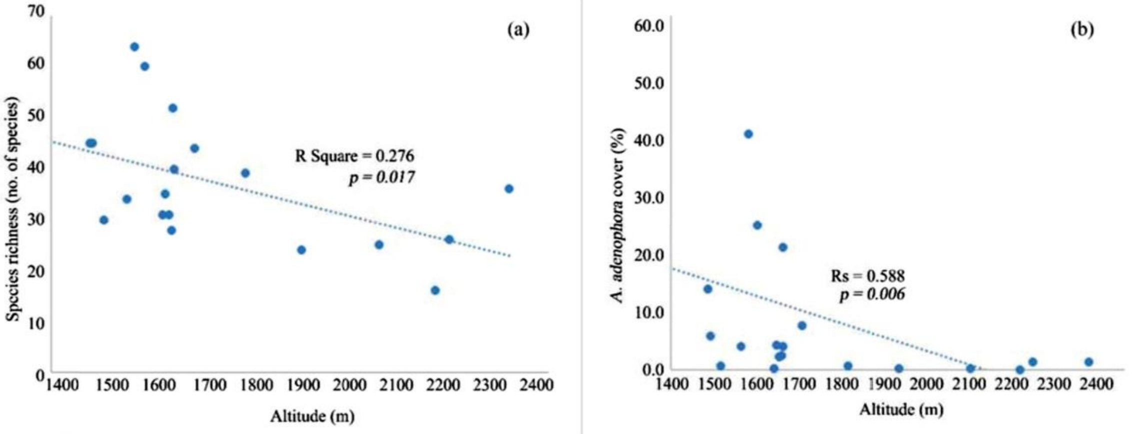

**Fig. 5** Relationship between altitude and species richness (a); altitude and *A. adenophora* cover (b)

## DISCUSSION AND CONCLUSION

In the domicile of native species; five types of plant communities identified by cluster analysis (Fig 2) are different in terms of species richness, composition, structure and habitat location (Fig. 2 and 3) indicating that the altitudinal gradient, physical setting of habitats and human intended regeneration (e.g. pine plantation) affect species distribution in different plant communities.

Distinct growing profiles of *S. wallichii* in the *Schima-Alnus*-mixed community (Fig 3c, d, e) suggest that this community is in succession process. The community is located near human settlement which was severely disturbed in the past and then turned out to be a "conserved" forest patch by the local inhabitants. The area had been left undisturbed without any anthropogenic activity for at least 20 years.

Thereafter, natural recovery has been taken place in the area without any human intended activities such as plantation or sowing any seed or seedlings. It might be due to the left over seed bank in the soil and/or the dispersal of the plant species from neighboring vegetation, which could be an example of how forest recovery takes place if people conserve any disturbed area without plantation.

In addition, the highest species richness in *Schima-Alnus*-mixed community also supports coexistence of diverse species during succession and natural recovery process. The forest history and present level of disturbance exemplify that certain level of disturbance could also increase species richness (Vetaas, 1997). Interestingly, *A. nepalensis* and *S. wallichii* are dominant trees in the community demonstrating that these species could be pioneer for vegetation recovery process in Lower montane vegetation which may also assist other species to be colonized.

Another community, the *Schima-Alnus*-herbaceous community is determined by moist streamside habitat which was also equally impacted by disturbance in the past. Dominance of *S. wallichii* and *A. nepalensis* in this community indicates that these two elements are equally important components of streamside vegetation also.

The history of pine-mixed community is the same as the *Schima-Alnus*-mixed community. The only difference is that: local people planted pine tree several years ago in a huge area as reforestation program because the pine is the most recommended tree for plantation in abandoned areas. Compared to *Schima-Alnus*-mixed and *Schima-Alnus*-herbaceous communities pine plantation has lower species richness (44 species) indicating that the reduction species diversity might be due to the shade effect or pine needle allelopathy. Survival of some herbs (*R. hookeriana*, *R. vivipara*, *O. compositus*, *S. discolor*) shows their resistivity against pine litter impact. It can be expected that if there was no pine plantation this community would be similar to the *Schima-Alnus*-mixed community. On the other hand the *Quercus* Community exists at high altitude and certain species such as *G. fragrantissima*, *L. ovalifolia*, *R. arboreum* and *M. esculenta* demonstrate their flexibility against altitudinal effect as they are also present in lower altitude communities. *Quercus* species and *P. wallichiana* in this community are temperate elements (Ohsawa et al., 1986) which represent this community as connecting link between sub-tropical and temperate zones of the Himalaya.

Vegetation of mountain range shows variation in environmental factors and ecotones may exist in transition zones as transitional community. Some other ecotones between *Schima-Alnus*-mixed and pine-mixed communities, *Schima-Alnus*-mixed and *Schima-Alnus*-herbaceous communities were also observed visually. Such ecotones are difficult to recognize and often neglected (Kent et al., 1997; Kolasa and Zalewski 1995) which have great ecological significance (Gosz, 1992). The transitional community was represented by lower number of species which may be due to higher tree canopies and low nutrients in rocky slopes.

As *A. adenophora* is identified as one of the highly spreading and competitive 'forest killer' species (Muniappan et al., 2009; Tiwari et al. 2005) it was the most frequent and highly colonized invasive species especially at lower altitude communities in the study site showing that *Schima-Alnus*-mixed and *Schima-Alnus*-herbaceous communities are vulnerable to its severe invasion. It might have been introduced into these communities with anthropological disturbances as these communities are located near human settlement. Invasion of *A. adenophora* in lower altitude plant communities even in deep forest patches (field observation) suggests that *A. adenophora* has high potential in developing ability to invade deep inside native vegetation and tolerating shades. It is inhibitory to native species growth and richness (Dogra et al., 2010; Inderjit et al., 2011; Lu and Ma, 2009) and therefore, it is problematic to naturally recovered ecosystems as it may replace native species and change nutrient cycling, productivity and soil microbial community (Niu et al., 2007; Villa et al., 2011).

Presence of *A. adenophora* at higher altitude communities shows that these communities are also prone to its invasion and it has also been developing ability to invade higher altitude plant communities. However, its lower cover indicates that these communities are comparatively less vulnerable to its invasion. Feng et al. (2007) explains that the luxuriant growth of *A. adenophora* even under greater tree canopy is a result of its shade tolerance. However, lower temperature and lower level of disturbance may have contributed to the luxuriant growth of *A. adenophora* at higher altitude plant communities.

Regression analysis showed that the higher altitude communities are associated with lower species richness. This is in accordance with Roy and Behara (2005) and Kharkwal et al. (2005) to conclude a declining trend in species richness with an increase in altitude. The reason might be a variation in abiotic conditions along altitudinal gradient over short distances, e.g. in water availability and temperature (McCain and Grytnes, 2010). Opening of tree canopy in the forest at communities of lower altitude due to disturbances might have enhanced diversity because shade tolerant and light demanding species have a chance to coexist (Bruun et al., 2007; Gao et al., 2009; Shrestha et al., 2012; Vettas, 1997).

In conclusion, the plant communities in the study site are distinctive in species richness, composition and structure. Natural vegetation recovers itself and becomes more diverse when previously disturbed landscapes are kept undisturbed without planting or sowing. Two native trees, *S. wallichii* and *A. nepalensis* are important species in forest recovery process. Species richness decreases with increasing altitude and higher level of *A. adenophora* cover is associated with plant communities at lower altitude.

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## APPENDIX – I

List of plant species with their family and their community (*A = Schima-Alnus-mixed community, B = Schima-Alnus-herbaceous community, C = Pine community, D = Transitional community, E = Quercus community)*

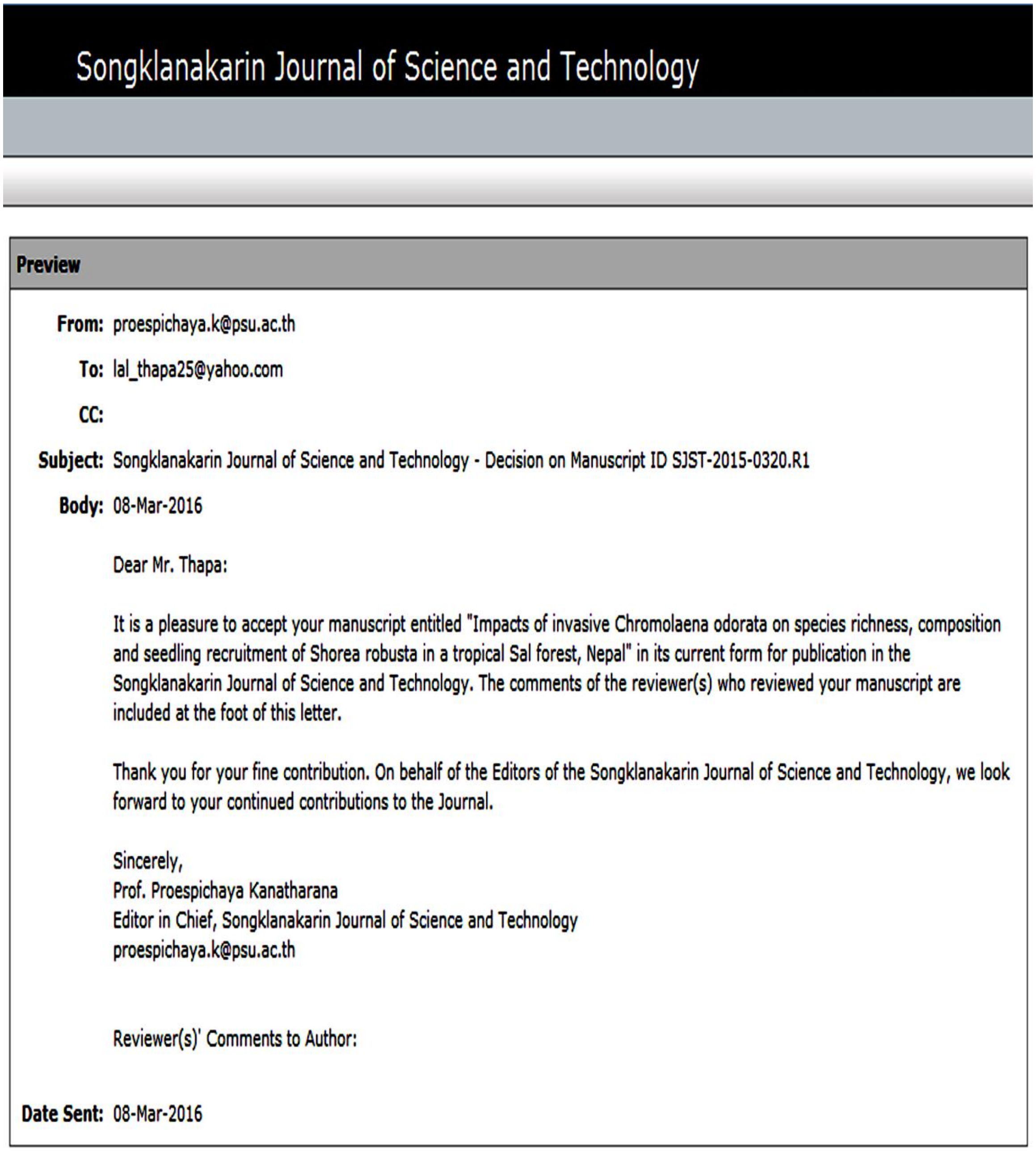
| SN | Species name | Family | Community |
|---|---|---|---|
| 1 | *Barleria strigosa* Willd. | Acanthaceae | B, C |
| 2 | *Justicia simplex* D.Don | | A, B |
| 3 | *Cheilanthes* sp. | Adiantaceae | A, B, C |
| 4 | *Coniogramme intermedia* Hieron. | | C |
| 5 | *Achyranthes aspera* L. | Amaranthaceae | |
| 6 | *Achyranthes bidentata* Blume | | A, B |
| 7 | *Dobinea vulgaris* Buch.-Ham. | Anacardiaceae | A |
| 8 | *Rhus javanica* L. | | A, D, E |
| 9 | *Centella asiatica* (L.) Urb. | Apiaceae | A, B, D |
| 10 | *Hydrocotyle javanica* Thunb. | | B, C |
| 11 | *Ilex dipyrena* Wall. | Aquifoliaceae | D, E |
| 12 | *Amorphophalus nepalensis* | Araceae | B |
| 13 | *Arisaema erubescens* (Wall.) Schott | | A, B, D |
| 14 | *Arisaema intermedium* Blume | | B, C, E |
| 15 | *Arisaema tortuosum* Steud. ex Engl. | | A, B, C |
| 16 | *Remusatia hookeriana* Schott | | C |
| 17 | *Remusatia vivipara* (Roxb.) Schott | | B, C |
| 18 | *Thomsonia nepalensis* Wall. | | B |
| 19 | *Hedera nepalensis* K.Koch | Araliaceae | A, C, D, E |
| 20 | *Ceropegia pubescens* Wall. | Asclepiadaceae | A |
| 21 | *Asparagus racemosus* Willd. | Asparagaceae | A, C |
| 22 | *Ageratina adenophora* (Spreng.) R.M.King & H.Rob. | Asteraceae | ALL |
| 23 | *Ageratum conyzoides* L. | | B |
| 24 | *Ageratum houstonianum* Mill. | | A, B |
| 25 | *Anaphalis busua* DC. | | A, C, D |
| 26 | *Anaphalis margaritacea* (L.) Benth. & Hook.f. | | C |
| 27 | *Anaphalis triplinervis* Sims ex C.B.Clarke | | D |
| 28 | *Artemisia indica* Willd. | | A, B, C |
| 29 | *Bidens biternata* (Lour.) Merr. & Sherff | | A, B, C |
| 30 | *Conyza canadensis* (L.) Cronquist | | A |
| 31 | *Dichrocephala integrifolia* (L.f.) Kuntze | | C |
| 32 | *Elephantopus scaber* L. | | |
| 33 | *Gnaphalium affine* d'Urv. | | A, B |
| 34 | *Parthenium hysterophorus* L. | | B |
| 35 | *Impatiens edgeworthii* Hook.f. | Balsaminaceae | A, B |
| 36 | *Berberis asiatica* Roxb. ex DC. | Berberidaceae | A, B, C |
| 37 | *Berberis aristata* DC. | | D |
| 38 | *Mahonia napaulensis* DC. | | A, D, E |
| 39 | *Alnus nepalensis* D.Don | Betulaceae | A, B |
| 40 | *Salix* sp. | | A, E |
| 41 | *Woodwardia fimbriata* Sm. | Blechnaceae | A |
| 42 | *Sarcococca coriacea* Sweet, | Buxaceae | A, B, C |
| 43 | *Lonicera japonica* Thunb. ex Murray | Caprifoliaceae | A |
| 44 | *Drymaria diandra* Blume | Caryophyllaceae | B, |
| 45 | *Stellaria* sp. | | A, B |
| 46 | *Cyanotis vaga* (Lour.) Schult.f. | Commelinaceae | A, B, D |
| 47 | *Ophiopogon intermedius* D.Don | Convallariaceae | A |
| 48 | *Polygonatum latifolium* Desf. | | C |
| 49 | *Hypericum* sp. | Culsiaceae | |
| 50 | *Kyllinga brevifolia* Boeckeler ex C.B.Clarke. | Cyperaceae | A |
| 51 | *Mariscus cyperoides* (L.) Urb. | | B |
| 52 | *Scleria biflora* Roxb. | | A |
| 53 | *Dioscorea bulbifera* Russ. ex Wall. | Dioscoreaceae | A, C, E |
| 54 | *Dioscorea deltoidea* Wall. ex Griseb. | | A, C |
| 55 | *Dryopteris* sp | Dryopteridaceae | A, B, C |
| 56 | *Polystichum polyblepharum* Nakai | | A, B, C |
| 57 | *Equisetum ramosissimum* Desf. | Equisetaceae | B, |
| 58 | *Gaultheria fragrantissima* Wall. | Ericaceae | ALL |

| | | | |
|---|---|---|---|
| 59 | *Lyonia ovalifolia* hort. | | A, C, D, E |
| 60 | *Rhododendron arboreum* Sm. | | A, C, D, E |
| 61 | *Euphorbia hirta* L. | Euphorbiaceae | |
| 62 | *Mallotus* sp. | | C |
| 63 | *Caesalpinia decapetala* (Roth) Alston | Fabaceae | A |
| 64 | *Desmodium elegans* Schltdl. | | A, B, C |
| 65 | *Indigofera heterantha* Wall. ex Brandis | | D |
| 66 | *Trifolium repens* Walter | | A, B |
| 67 | *Castanopsis indica* A.DC. | Fagaceae | A, B, C, E |
| 68 | *Castanopsis tribuloides* (Sm.) A. DC. | | A, D, E |
| 69 | *Quercus glauca* Bürger ex Blume | | D, E |
| 70 | *Quercus lanata* Sm. | | D |
| 71 | *Quercus semecarpifolia* Sm. | | D, E |
| 72 | *Gentiana pedicellata* Wall. | Gentianaceae | A, D |
| 73 | *Chirita pumila* D.Don | Gesneriaceae | A |
| 74 | *Dicranopteris linearis* (Burm.f.) Underw. | Gleicheniaceae | A, B, C |
| 75 | *Gleichenia gigantea* Wall. ex Hook. & Bauer | | A, B, C, D |
| 76 | *Dichroa febrifuga* Lour. | Hydrangeaceae | A, B, C |
| 77 | *Curculigo orchioides* Gaertn. | Hypoxidaceae | A |
| 78 | *Colebrookea oppositifolia* Sm. | Lamiaceae | |
| 79 | *Elsholtzia fruticosa* (D. Don) Rehder | | |
| 80 | *Leucas lanata* Baker | | C |
| 81 | *Salvia* sp. | | A, B, C |
| 82 | *Scutellaria discolor* Colebr. | | C, |
| 83 | *Holboellia latifolia* Wall. | Lardizabalaceae | A |
| 84 | *Cinnamomum tamala* (Buch.-Ham.) T.Nees & C.H.Eberm. | Lauraceae | A, D, E |
| 85 | *Persea duthiei* (King ex Hook.f.) Kosterm. | | A, E |
| 86 | *Sphenomeris chinensis* (L.) Maxon | Dennstaedtiaceae | A |
| 87 | *Lygodium japonicum* (Thunb.) Sw. | Schizaeaceae | C |
| 88 | *Osbeckia stellata* Wall. | Melastomataceae | ALL |
| 89 | *Cissampelos pareira* L. | Menispermaceae | A, E |
| 90 | *Myrica esculenta* Buch.-Ham. ex D.Don | Myricaceae | A, D |
| 91 | *Maesa chisia* Buch.-Ham. ex D. Don | Myrsinaceae | A, B, |
| 92 | *Myrsine capitellata* Wall. | | A, C, D, E |
| 93 | *Myrsine semiserrata* Wall. | | A, D, E |
| 94 | *Nephrolepis cordifolia* (L.) C.Presl | Nephrolepidaceae | A, C |
| 95 | *Fraxinus floribunda* hort. ex C.K.Schneid. | Oleaceae | A |
| 96 | *Jasminum humile* B.Heyne ex Wall.. | | A, B, D, E |
| 97 | *Jasminum officinale* L. | | A |
| 98 | *Herminium* sp. | Orchidaceae | D |
| 99 | *Malaxis acuminate* D.Don | | D, E |
| 100 | *Pleione* sp. | | A |
| 101 | *Oxalis corniculata* L. | Oxalidaceae | |
| 102 | *Argemone mexicana* L. | Papaveraceae | |
| 103 | *Phyllanthus* sp. | Phyllanthaceae | A, B, C, E |
| 104 | *Pinus roxburghii* Sarg. | Pinaceae | A, C |
| 105 | *Pinus wallichiana* A.B.Jacks. | | C, D |
| 106 | *Piper mullesua* Buch.-Ham. ex D.Don | Piperaceae | A |
| 107 | *Plantago major* Bert. ex Barnéoud | Plantaginaceae | A, B |
| 108 | *Arundinaria* sp. | Poaceae | D, E |
| 109 | *Capillipedium assimile* (Steud.) A.Camus | | A, B, C |
| 110 | *Imperata cylindrica* (L.) P.Beauv. | | A, B, C |
| 111 | *Oplismenus burmannii* (Retz.) P.Beauv. | | A, B, D, E |
| 112 | *Oplismenus compositus* (L.) P.Beauv. | | C |
| 113 | *Setaria incrassata* (Hochst.) Hack. | | C |
| 114 | *Persicaria capitata* (Buch.-Ham. ex D.Don) H.Gross | Polygonaceae | B |
| 115 | *Polygonum barbatum* Comm. ex Meisn. | | B |
| 116 | *Onychium sp.* | Pteridaceae | A, B, C |
| 117 | *Pteris vittata* Schkuhr | | A, B, C |
| 118 | *Ranunculus* sp. | Ranunculaceae | A, B |
| 119 | *Ziziphus incurve* Roxb. | Rhamnaceae | A, B, E |
| 120 | *Eriobotrya dubia* Decne. | Rosaceae | A, C |
| 121 | *Neillia rubiflora* D.Don | | A |

| | | | |
|---|---|---|---|
| 122 | *Potentilla fulgens* Wall. ex Hook. | | A, D |
| 123 | *Potentilla lineate* Trevir. | | A |
| 124 | *Prunus cerasoides* D.Don | | A, E |
| 125 | *Pyracantha crenulata* (D.Don) M.Roem. | | A, B, C |
| 126 | *Pyrus pashia* Buch.-Ham. ex D.Don | | A, C |
| 127 | *Rosa multiflora* Buch.-Ham. exHook.f. | | A |
| 128 | *Rubus acuminatus* Genev. | | A |
| 129 | *Rubus ellipticus* Sm. | | A, B, C, D |
| 130 | *Rubus foliolosus* D.Don | | D |
| 131 | *Rubus paniculatus* Roxb. | | ALL |
| 132 | *Galium elegans* Błocki | Rubiaceae | C, |
| 133 | *Galium palustre* Pourr. ex Willk. & Lange | | A, B, D |
| 134 | *Galium aparine* L. | | A, B, D |
| 135 | *Rubia manjith* Roxb. ex Flem. | | ALL |
| 136 | *Boenninghausenia albiflora* (Hook.) Meisn. | Rutaceae | A |
| 137 | *Zanthoxylum armatum* DC. | | A, B, C, D |
| 138 | *Viburnum cylindricum* Buch.-Ham. ex D.Don | Sambucaceae | A |
| 139 | *Viburnum mullaha* Buch.-Ham. ex D.Don | | A, B |
| 140 | *Osyris wightiana* Wall. ex Wight | Santalaceae | A, E |
| 141 | *Hemiphragm heterophyllum* Wall. | Scrophulriaceae | A, B |
| 142 | *Mecardonia procumbens* (Mill.) Small | | A, B |
| 143 | *Smilax aspera* L. | Smilacaceae | A, C, D, E |
| 144 | *Smilax* sp. | | A, C, D, E |
| 145 | *Solanum aculeatissimum* Moench | Solanaceae | B |
| 146 | *Solanum nigrum* Tausch ex Dunal | | B |
| 147 | *Tectaria* sp. | Tectariaceae | A, B |
| 148 | *Camellia kissi* Wall. | Theaceae | A, C |
| 149 | *Eurya acuminata* Wall. | | A, C, E, R |
| 150 | *Schima wallichii* (DC.) Korth. | | A, B, C |
| 151 | *Daphne papyracea* Wall. ex G.Don | Thymelaeaceae | A |
| 152 | *Grewia* sp. | Tiliaceae | A |
| 153 | *Gonostegia hirta* (Blume) Miq. | Urticaceae | ALL |
| 154 | *Pilea scripta* (Buch.-Ham. ex D.Don) Wedd. | | A |
| 155 | *Urtica dioica* Vell. | | B |
| 156 | *Valeriana jatamansi* Jones | Valerianaceae | A |
| 157 | *Caryopteris foetida* (D.Don) Thell. | Verbenaceae | A, B, C |
| 158 | *Lantana camara* L. | | |
| 159 | *Viola biflora* L. | Violaceae | A, B, D |
| 160 | *Tetrastigma serrulatum* (Roxb.) Planch. | Vitaceae | A, C |
| 161 | *Vitis lanata* Roxb. ex Wall. | | A |
| 162 | *Hedychium sp.* | Zingiberaceae | A, B, C, E |
| 163 | *Roscoea purpurea* Sm. | | D |

# Paper II

**Thapa, L.B.**, Kaewchumnong, K., Sinkkonen, A. and Sridith, K. 2016. Impacts of invasive *Chromolaena odorata* on species richness, composition and seedling recruitment of *Shorea robusta* in a tropical Sal forest, Nepal. Songklanakarin Journal of Science and Technology (Accepted for publication in Volume 38, No. 6, Year 2016).



Songklanakarin Journal of Science and Technology

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Dear Mr. Thapa:

It is a pleasure to accept your manuscript entitled "Impacts of invasive Chromolaena odorata on species richness, composition and seedling recruitment of Shorea robusta in a tropical Sal forest, Nepal" in its current form for publication in the Songklanakarin Journal of Science and Technology. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Thank you for your fine contribution. On behalf of the Editors of the Songklanakarin Journal of Science and Technology, we look forward to your continued contributions to the Journal.

Sincerely,
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Editor in Chief, Songklanakarin Journal of Science and Technology
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Reviewer(s)' Comments to Author:

**Date Sent:** 08-Mar-2016

# Impacts of invasive Chromolaena odorata on species richness, composition and seedling recruitment of Shorea robusta in a tropical Sal forest, Nepal

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| Keyword: | Chromolaena odorata, Shorea robusta, invasive species, species richness, species composition |
| | |

# Impacts of invasive *Chromolaena odorata* on species richness, composition and seedling recruitment of *Shorea robusta* in a tropical Sal forest, Nepal

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## Abstract

*Chromolaena odorata* is an invasive species **known** to have its adverse impacts on native diversity. We studied its impacts on plant species richness, composition and *Shorea robusta* seedlings in a tropical Sal forest of Nepal. We conducted field experiment along transects established in invaded and uninvaded understory vegetation. Our results show differences in native species richness and *S. robusta* seedling density between invaded and uninvaded plots. The invaded plots are associated with fewer species than uninvaded plots. Plot type (invaded and uninvaded), *C. odorata* density and cover show effect on vegetation composition. Moreover, some of the **native species are found replaced from *C. odorata* invaded sites. Overall, the Sal forest protects native diversity and Sal seedling recruitment besides having its high economic value and beneficial impacts on people's livelihood. Therefore, c**ontrol and proper management of *C. odorata* is needed for conserving native vegetation and preventing future problems associated with invasion.

Keywords: *Chromolaena odorata*, *Shorea robusta*, invasive species, species richness, species composition

## Introduction

Many of invasive alien species have caused various ecological, environmental, economic and human health problems (D'Antonio *et al.,* 2002; Pimentel *et al.,* 2001*,* 2005; Vilà *et al.,* 2011). They have been considered as one of the greatest threats to native diversity, ecosystem structure and functioning (Ehrenfeld, 2003; Gurevitch and Padilla, 2004; Higgins *et al*., 1999). Common characteristics of invasive alien species include capacity for high seed production, high germination rate, strong vegetative growth and phenotypic plasticity (Davidson *et al.,* 2011; Tiwari *et al.,* 2005). Beside these characteristics they bring changes in soil properties such as pH, moisture, organic matter content, nutrient concentrations (Ehrenfeld, 2003; Kourtev *et al.,* 1998; Timsina *et al.,* 2011) and microbial community (Chacon *et al.,* 2009; Klironomos, 2002; Mangla and Callaway, 2008).

*Chromolaena odorata* (L.) King & Robinson (Asteraceae), native to tropical America, is one of the serious weeds growing rapidly and invading a wide range of natural vegetation in Asia, Africa and Europe (McFadyen and Skarratt, 1996; Olaoye and Moody, 1986; Zachariades *et al.,* 2009). It had become prevalent in south Asia (India, Nepal and Sri Lanka) and south-east Asia (Myanmar, Thailand, Vietnam and Laos) by early twentieth century (Zachariades *et al.,* 2009). It has invaded tropical ranges in Asia up to 1000 meters above sea level (m.a.s.l.) (McFadyen, 1989). **In Nepal, *C. odorata* is called "Seto**

**banmara" which is becoming aggressive and invasive for four decades from eastern to central part of the country (Tiwari *et al.,* 2005).** Disturbed areas, fallow land, road side, degraded forest margins and tree canopy gaps in the forests are the sites of its colonization (Tiwari *et al.,* 2005; Zachariades *et al.,* 2009). Native vegetation is found to be impacted by this species through altering soil microbial community (Mangla and Callaway 2008), changing soil quality (Mandal and Joshi, 2014; Mboukou-Kimbatsa *et al.,* 2007) and allelopathy (LiJun *et al.,* 2010; Hu and Zhang, 2013).

Tropical **forests** (<1000 m.a.s.l.) in Nepal is widely dominated by *Shorea robusta* C.F. Gaertn. **commonly called 'Sal forest'. Native distribution of *S. robusta* (Sal) is found in Nepal, India and Myanmar (Orwa *et al.*, 2009). The Sal forest is extended into subtropical region or foothill of the Himalayas upto 1500 m.a.s.l. (Gautam, 1990; Tewari, 1995). The understory vegetation in Sal forest is rich in species diversity (Webb and Sah, 2003) and plant communities in the forests represent unique entities in terms of their structure and composition as well (Timilsina *et al.,* 2007). Concerning conservation of forests, community forestry has becoming one of the best practices and successful methods in Nepal but** anthropogenic **disturbances and human intended management efforts have caused negative impacts on them (Shrestha *et al.,* 2010). As an example, alien species invasion has become one of the serious problems in the natural ecosystems (Dogra *et al.*, 2010).**

**The Sal forest habitat in Nepal is facing problem with the detrimental invader,** *C. odorata* (Tiwari *et al.,* 2005). **Luxuriant growth of *S. robusta* seedlings associated with**

**number of native species is found in uninvaded sites and contrasting to this aggressively growing *C. odorata* has dominated over native understory vegetation along the invaded sites of the study area. We anticipated that *C. odorata* might have created severe ecological problems on species richness, composition and *S. robusta* seedling recruitment in the invaded Sal forests.** Previous studies have focused on the effect of *C. odorata* on agroforestry and agricultural systems (eg. Ngobo *et al*., 2004; Roder *et al.,* 1995). **We compared species richness and density of *S. robusta* seedlings in *C. odorata* invaded and uninvaded plots as well as analyzed the effect of plot types, *C. odorata* density and cover on species composition in a Sal forest in Nepal to understand the potential impacts of *C. odorata* in field.**

## Methods

### *Study sites*

The study was carried out in Panchakanya community forest located at Chitwan district of central Nepal. The community forest lies in tropical zone (170-216 m.a.s.l.) having dry-wet climate in winter and hot-humid in summer. Two study sites were selected in the community forest for the investigation. Site 1 was located at the north-west part (27°38′35″N 84°28′53″E) of the forest and site 2 was located at the south-east part (27°38′19″N 84°29′01″E). Each site was characterized by the dominance of *S. robusta* and both of them were homogenous in topography, soil type and vegetation.

### *Vegetation sampling*

Vegetation sampling was done using belt transect method (Timsina *et al.,* 2011) from August to September 2014. Fifty quadrats (plots), each of size 1×1 $m^2$, were placed along 10 transects (100 m long) made at both study sites. A total of five quadrats were placed in each transect and the distance between two quadrats was at least 10 m. Each transect included at least two quadrats in *C. odorata* invaded and two quadrats in uninvaded parts. A quadrat was considered as uninvaded if the cover of *C. odorata* was less than 10%. Growing tree species with height <1 m were considered as seedlings and counted in each plot. All species encountered in each quadrat were recorded and identified using standard literature (Hara *et al.*, 1982; Polunin and Stainton, 1984; Press *et al.*, 2000). Herbarium specimens were deposited at National Herbarium and Plant Laboratory (KATH), Godawari, Lalitpur, Nepal.

### *Data analysis*

Canonical correspondence analysis (CCA) was used to analyze the effect of *C. odorata* on species composition in the study area as the gradient length in the data yielded by **Detrended** correspondence analysis (DCA) was 3.129, **and thus unimodal technique of analysis was applied**. Effects of *C. odorata* density (number of *C. odorata* ramets), *C. odorata* cover and plot type (invaded and uninvaded) were tested on species composition. Species less than four occurrences and *C. odorata* were excluded from the species composition data. The data were down weighted to reduce the effect of the occurrences of rare species in the results. We used permutational multivariate analysis of variance (PERMANOVA) to test the significance of the relationships.

Independent sample t-tests and Mann-Whitney U tests were used to compare the means of total species richness, native species richness and number of *S. robusta* seedlings between invaded and uninvaded plots. Data on number of species at site 2 were square root transformed before analysis to meet the assumptions of the analyses. **All tests were carried out in R Software, version 3.0.0 (R Development Core Team, 2013).**

## Results

### *Species richness*

Thirty species were recorded at site 1 and 36 species at site 2 from both invaded and uninvaded plots. They are included in 31 genera and 17 families. The species at site 1 were also common at site 2. Among them, 26 species were native and 10 species were non-native (Table 1 and 2). Out of the non-native species 4 species were invasive (*Senna occidentalis* **(L.) Link***, Senna tora* **(L.) Roxb.***, C. odorata* and *Mikenia micrantha* Kunth).

Number of native species (native species richness) at both sites was significantly different between invaded and uninvaded plots. The invaded plots were associated with fewer native species. At site 1, numbers of native species recorded were 3.25±0.27 in invaded plots and 4.15±0.31 in uninvaded plots (mean/m$^2$±SE; t-test, t = 2.15, df = 48, $p = 0.036$). At site 2, numbers of native species were 3.92±0.3 in invaded plots and 4.85±0.34 in uninvaded plots (mean/$m^2$±SE; t-test, t = 2.22, df = 48, *p = 0.030*). **Total species (native and non-native) per plot was approximately 5-6 which was not significantly different between invaded and uninvaded plots for both sites.**

### *Species composition*

*C. odorata* invaded and uninvaded plots showed different species composition. The plot types (invaded and uninvaded) explained 2.42% of the total variation in CCA analysis in the species composition data set which is 7.44% of the variation which could be explained by one ordination axis (*p = 0.003*) **(Fig 1).**

Similarly, the factors (both *C. odorata* cover and density) explained 3.12% of the total variation in the species composition data set that is 9.58% of the total variation which could be explained by two ordination axis (*p = 0.038*) (**Fig. 2**). The *C. odorata* cover and density separately explained 2.01% (*p = 0.011*) and 2.21% (*p = 0.006*) of the total variation respectively in the data set. The variations are 6.19% and 6.79% respectively of the total variation that could be explained by one ordination axis (not shown).

### *Shorea robusta seedling recruitment*

Numbers of *S. robusta* seedlings were significantly different between invaded and uninvaded plots. Uninvaded plots are found to be associated with greater number of seedlings. At site 1, numbers of seedlings were 2.73±0.50 in invaded plots and 9.0±1.03 in uninvaded plots (mean/$m^2$±SE; Mann-Whitney U test, $p = 0.036$). Similarly, at site 2, the numbers of seedlings were 5.08±0.95 and 10±1.33 in invaded and uninvaded plots, respectively (mean/$m^2$±SE; t-test, t = 3.04, df = 48, $p = 0.003$).

## Discussion

The results show that *C. odorata* has effects on species richness and composition in a tropical Sal forest. The vegetation composition is different between invaded and uninvaded plots. CCA analysis indicates that non-native herbaceous plants such as *Evolvulus nummularius* **(L.) L.**, *Phyllanthus niruri* **L.** and *Ageratum houstonianum* **Mill.** and native shrub *Clerodendrum viscosum* **Vent.** are associated with *C. odorata* while certain monocots *Oplismenus burmannii* **(Retz.) P. Beauv.**, *Cyanotis* sp., *Costus specious* **(Koenig)**, *Dioscorea bulbifera* **L.,** and dicot shrub species *Helicteres isora* **L.** and *Desmodium* sp. are replaced by *C. odorata*. CCA analysis suggests that *C. odorata* density and cover affect vegetation composition in the invaded plots. This result is similar to the results of Norgrove *et al.* (2008) who found reduction of certain monocots (*Aframomum* sp. and *Murdannia simplex*) and increase of abundance of some alien dicot weeds (*Stachytarpheta cayennensis* and *Ageratum conyzoides*) with the invasion of *C. odorata* in the study of plant community composition in invaded and uninvaded savannah sites in north-west Cameroon. This lends further support to the hypothesis that *C. odorata* invasion causes a shift in native species abundance with an increase of non-native species.

Our results also demonstrate that native plant species richness is negatively affected by *C. odorata* invasion. As a significant difference was found in native species richness between invaded and uninvaded plots but no significant differences in total species richness, which suggests that non-native plant species replace native species in invaded areas or *C. odorata* invasion supports introduction of non-native species during replacement of native plant species. Moreover, significantly lower *S. robusta* seedling density in invaded plots is an evidence of inhibitory impact of *C. odorata* on *S. robusta* seedlings in nature. These findings are in accordance with previous findings by De Rouw (1991), Hu and Zhang (2013), Goodall and Erasmus (1996), Mangla and Callaway (2008), and Norgrove *et al.* (2008).

Negative feedback mechanism by soil microbial community in invaded areas might have essential role in the replacement of native species. An important regulator of plant community structure is the ability of plants to change soil microbial communities (Klironomos, 2002). Mangla and Callaway (2008) had reported that *C. odorata* is able to accumulate high concentration of the generalist soil born fungal pathogen (*Fusarium* spp.) responsible for creating a negative feedback for native plant species and concluded that the impact of *C. odorata* is due to biotic interactions among native plants and soil biotic community.

Allelochemicals released from *C. odorata* could also play an inhibitory role for native species growth in invaded areas. Novel weapons hypothesis proposes that the invasive species can produce novel biochemical weapons as powerful allelopathic agents in exotic range to harm associated vegetation (Callaway and Ridenour, 2004). Allelopathic property of this species has been described previously (eg. Ivens, 1974; Sahid and Sugau, 1993). Hu and Zhang (2013) observed more inhibitory effects of *C. odorata* leaf extract on native species (*Rottboellia exaltata*, *Digitaria sanguinalis, Hemistepta lyrata, Youngia japonica and Dicliptera chinensis*) than non-native species (*Bidens pilosa, Ageratum conyzoides, Amaranthus spinosus, Conyza sumatrensis* and *Chenopodium ambrosioides*). These indicate that the native plant species are more susceptible to allelopathic compounds released by *C. odorata* which is further supported by our findings.

*C. odorata* is also found suppressing the growth of tree species, pine and eucalypt (Matthews and Brand, 2004) similar to our study, i.e. there is significantly lower number of *S. robusta* seedlings in invaded plots than uninvaded plots. *C. odorata* produces masses of rapidly germinating seeds and grows aggressively forming a thick cover over neighboring plants which is another mechanism to suppress native plants (Awanyo *et al.*, 2011; De Rouw, 1991; Goodall and Erasmus, 1996). The *S. robusta* seedlings and other native species might be more susceptible to any above mentioned mechanisms developed by *C. odorata* rather than non-native species such as *Evolvulus nummularius*, *Phyllanthus niruri* and *Ageratum houstonianum*.

Interestingly, it is suggested that *C. odorata* increases soil nutrients (NPK, magnesium) and soil organic content, which is likely to support the growth of several non-native species in invaded areas (Mandal and Joshi, 2014). As *C. odorata* prefers open canopy of forest in non-native ranges (Kluge, 1991; Malahlela*et al.,* 2015; Tiwari *et al.,* 2005), it would be interesting to assess its invasion success in deep Sal forests. Joshi *et al.* (2013) has described an important mechanism of its successful invasion through its underground stem (corm) which is responsible for vegetative growth. The corm cannot be affected by forest fire while upper parts of this plant and native vegetation get destroyed. Later on the corm regenerates shoots in rainy season and dominates over other species in the next growing season (Joshi *et al.*, 2013).

**Currently, the controlling and management activities of *C. odorata* have not been taken seriously by community people and managers which indicates that the level of *C. odorata* invasion would be more severe in future. As long term impact of *C. odorata,* native plant diversity would be negatively affected with eradication of important elements from understory vegetation of Sal forest. The soil ecology and dynamics would be altered and only a pure stand of *C. odorata* could be expected in Sal forests in future. Results of this study could be helpful to raise some questions about certain mechanisms adopted by *C. odorata* for its invasion in the Sal forest which would replace native species and having negative impacts on *S. robusta* seedling establishment.**

In conclusion, ***C. odorata* has great impact on native species diversity, change community composition and create problems with *S. robusta* seedling recruitment** in tropical Sal forests in Nepal. It is capable of replacing native species by supporting non-native species in invaded areas. Importantly, its impact on *S. robusta* seedlings indicates threats to natural regeneration of high valuable tree. **It is expected that the level of *C.***

***odorata* invasion would be increased more severely as there is no more activities of controlling and management of this weed.** Therefore, **managers, responsible authorities and community people should be aware to implement** appropriate measures for controlling and management of *C. odorata* to conserve native ecosystem and diversity.

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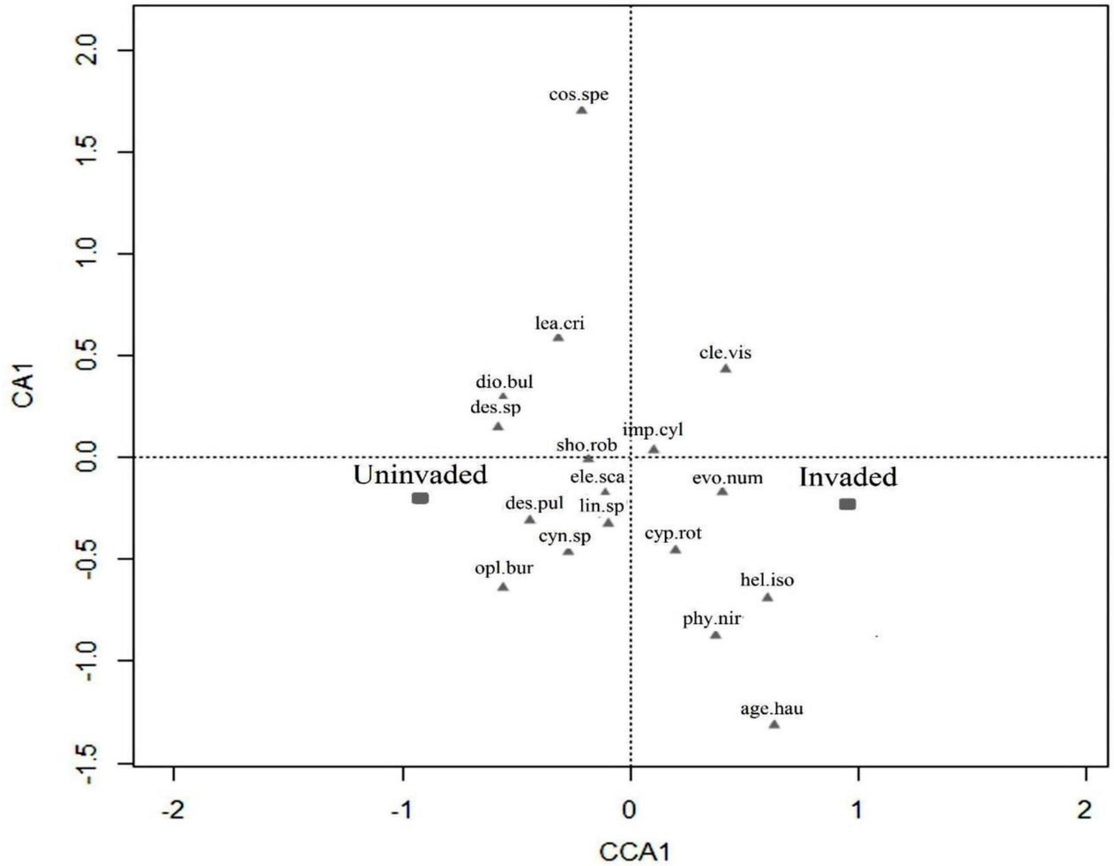

Fig. 1 Results of CCA analysis showing the effect of plot type on species composition in ordination space. Full names and authors of each species are given in Table 2.

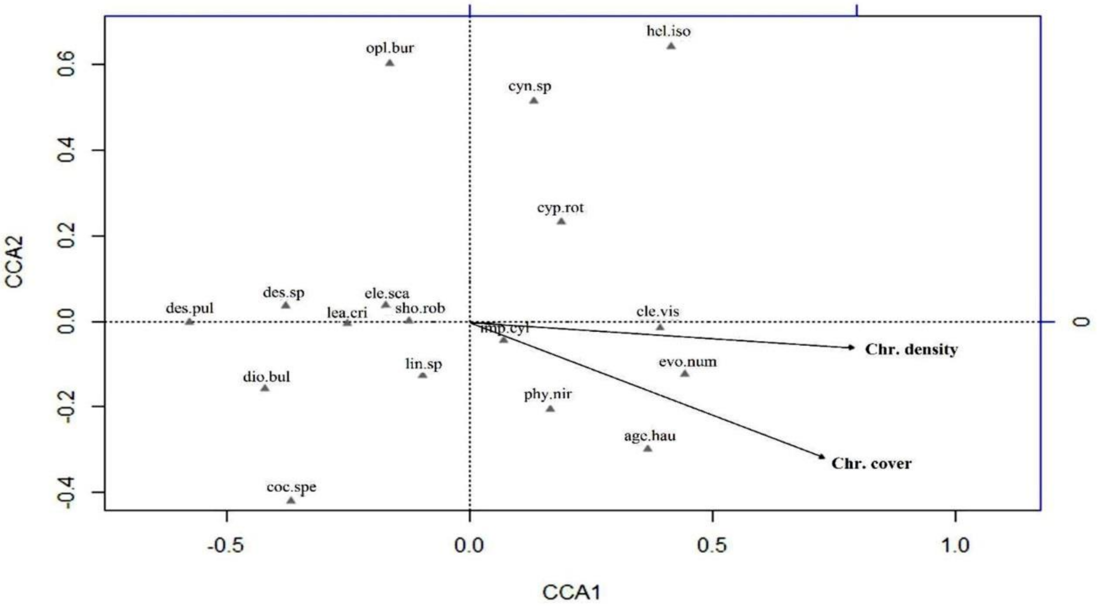

Fig.2 Results of CCA analysis showing the effect of *C. odorata* density and cover on species composition. (Chr.: *Chromolaena odorata*; Full names and authors of each species are given in Table 2)

**Table 1** Numbers of native and non-native species found at both study sites

| Type of species | Site 1 | Site 2 |
|---|---|---|
| Native species | 21 | 26 |
| Non-native species | 9 | 10 |
| Total | 30 | 36 |

**Table 2** Plant species reported from the study plots, their abbreviation, family, growth habits and status

| Species | Abbreviation | Family | Growth habits | Status |
|---|---|---|---|---|
| *Ageratum houstonianum* Mill. | age.hau | Asteraceae | Perennial | Non-native |
| *Cassia fistula* L. | cas.fis | Fabaceae | Perennial | Native |
| ***Senna** occidentalis* **(L.) Link** | cas.occ | Fabaceae | Perennial | Non-native |
| ***Senna** tora* **(L.) Roxb**. | cas.tor | Fabaceae | Perennial | Non-native |
| *Chromolaena odorata* (L.) King & Robinson | chr.odo | Asteraceae | Perennial | Non-native |
| *Clerodendrum viscosum* Vent. | cle.vis | Verbenaceae | Perennial | Native |
| *Colacasia* sp. | col.sp | Araceae | Perennial | Native |
| *Commelina* sp. | com.sp | Commelinaceae | Annual | Native |
| *Costus speciosus* (Koenig) | coc.spe | Zingiberaceae | Perennial | Native |

| | | | | |
|---|---|---|---|---|
| Sm. | | | | |
| *Crotalaria* sp. | cor.sp | Fabaceae | Perennial | Native |
| *Cyanotis vaga* (Lour.) J. A. & J. H. Schult. | cyn.vag | Commelinaceae | Annual | Native |
| *Cyanotis* sp. | cyn.sp | Commelinaceae | Annual | Native |
| *Cyperus rotundus* L. | cyp.rot | Cyperaceae | Perennial | Native |
| *Dalbergia sissoo* Roxb. ex DC. | dal.sis | Fabaceae | Perennial | Native |
| *Desmodium caudatum* DC. | des.cau | Fabaceae | Annual | Native |
| *Desmodium* sp. | des.sp | Fabaceae | Annual | Native |
| *Desmodium pulchellum* **(L.)** Benth. | des.pul | Fabaceae | Perennial | Native |
| *Dioscorea bulbifera* **L.** | dio.bul | Dioscoreaceae | Perennial | Native |
| *Elephantopus scaber* **L.** | ele.sca | Asteraceae | Perennial | Non-native |
| *Evolvulus nummularius* (L.) L. | evo.num | Convolvulaceae | Perennial | Non-native |
| *Galinsoga parviflora* Cav. | gal.par | Asteraceae | Annual | Non-native |
| *Helicteres isora* L. | hel.iso | Sterculiaceae | Perennial | Native |
| *Imperata cylindrica* (L.) P. Beauv. | imp.cyl | Poaceae | Perennial | Native |
| *Kyllinga* sp. | kyl.sp | Cyperaceae | Perennial | Non-native |
| *Leea crispa* Royen ex L. | lee.cri | Leeaceae | Perennial | Native |
| *Lindernia* sp. | lin.sp | Scrophulariaceae | Annual | Native |

| | | | | |
|---|---|---|---|---|
| Sm. | | | | |
| *Crotalaria* sp. | cor.sp | Fabaceae | Perennial | Native |
| *Cyanotis vaga* (Lour.) J. A. & J. H. Schult. | cyn.vag | Commelinaceae | Annual | Native |
| *Cyanotis* sp. | cyn.sp | Commelinaceae | Annual | Native |
| *Cyperus rotundus* L. | cyp.rot | Cyperaceae | Perennial | Native |
| *Dalbergia sissoo* Roxb. ex DC. | dal.sis | Fabaceae | Perennial | Native |
| *Desmodium caudatum* DC. | des.cau | Fabaceae | Annual | Native |
| *Desmodium* sp. | des.sp | Fabaceae | Annual | Native |
| *Desmodium pulchellum* **(L.)** Benth. | des.pul | Fabaceae | Perennial | Native |
| *Dioscorea bulbifera* **L.** | dio.bul | Dioscoreaceae | Perennial | Native |
| *Elephantopus scaber* **L.** | ele.sca | Asteraceae | Perennial | Non-native |
| *Evolvulus nummularius* (L.) L. | evo.num | Convolvulaceae | Perennial | Non-native |
| *Galinsoga parviflora* Cav. | gal.par | Asteraceae | Annual | Non-native |
| *Helicteres isora* L. | hel.iso | Sterculiaceae | Perennial | Native |
| *Imperata cylindrica* (L.) P. Beauv. | imp.cyl | Poaceae | Perennial | Native |
| *Kyllinga* sp. | kyl.sp | Cyperaceae | Perennial | Non-native |
| *Leea crispa* Royen ex L. | lee.cri | Leeaceae | Perennial | Native |
| *Lindernia* sp. | lin.sp | Scrophulariaceae | Annual | Native |

## Paper III

**Thapa, L.B.**, Kaewchumnong, K., Sinkkonen, A. and Sridith, K. Plant invasiveness and target plant density: High densities of native *Schima wallichii* seedlings reduce negative effects of invasive *Ageratina adenophora.* Weed Research (Submitted Manuscript, 27 January 2016).

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# Plant invasiveness and target plant density: High densities of native Schima wallichii seedlings reduce negative effects of invasive Ageratina adenophora

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| Keywords: | allelopathy, density-dependent processes, dose-response, weed control |
| | |

# Plant invasiveness and target plant density: High densities of native *Schima wallichii* seedlings reduce negative effects of invasive *Ageratina adenophora*

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## Summary

Economically feasible strategies to cope with invasive species are urgently needed. Plant density can be increased to reduce toxin effects on target plants. Here we tested the effect of invasive *Ageratina adenophora* on Nepalese native *Schima wallichii* at different plant densities because *A. adenophora* leachates and volatiles are known to reduce growth of several plant species, and field observations support the view that *S. wallichii* is replaced by *A. adenophora* in forests. *S. wallichii* seedlings were grown in pots containing natural or invaded soil with or without *A. adenophora* leaf litter on soil surface. *S. wallichii* seedlings were also grown at four densities under four levels of *A. adenophora* leaf litter. Root and shoot biomass and length were measured as response parameters in both bioassays. *S. wallichii* growth was inhibited by *A. adenophora* leaf litter and invaded soil. High litter levels reduced *S. wallichii* root length and dry weight at low plant densities. The inhibition disappeared at high plant densities. As *A. adenophora* did not inhibit *S. wallichii* growth at high plant densities, adjustments of seedling density should be studied as a possible management strategy for invasion by *A. adenophora* and potentially by other exotic plant species. As density-dependent growth inhibition is the key characteristic of chemical interference, we propose that soil-based phytotoxins contribute to *A. adenophora* invasion.

**Keywords:** invasive species, *Ageratina adenophora*, litter, density-response, chemical interference

## Introduction

Invasive alien species are causing severe changes in natural ecosystems. They typically change species composition, biodiversity and community structure (Dogra *et al.*, 2010; Vila *et al.*, 2011; Caffrey *et al.,* 2014). Several invasion mechanisms have been identified in novel geographic ranges (Catford *et al.*, 2009). The Novel Weapon Hypothesis assumes that compounds released by an invading plant species are ineffective within native range but can be inhibitory against co-occurring plant species within exotic range (Callaway & Ridenour, 2004; Hierro *et al.*, 2005; Kim & Lee, 2011). Another important mechanism, or a combination of several separate mechanisms, is negative soil feedback caused by invasive species within the exotic range to inhibit native species growth (Callaway *et al.*, 2004; Wolfe & Klironomos, 2005; Niu *et al.*, 2007; Meisner *et al.*, 2014). Then native plant species grow less and are less abundant in invaded soil as compared to non-invaded soil (Inderjit *et al.*, 2011).

In case of many invasive species, the relative importance of coexisting invasion strategies is difficult to determine, and methods to distinguish the ecological significance of any specific mechanism are urgently needed. Support for chemical interference caused by phytotoxins, i.e. allelochemicals can be searched using density-response experiments (Weidenhamer *et al.*, 1989). Combined with identification of the active compounds, density response bioassays provide strong support that chemical interference is ecologically significant. Without additional tests, the support is indicative and forms a good basis for further investigation. In density response experiments, plants are grown at several plant densities, and the level of toxic exposure, e.g. dose or even leaf litter thickness, remains constant per unit soil volume (Yoda *et al.,* 1963; Weidenhamer *et al.,* 1989; Sinkkonen, 2001, 2003, 2006, 2007). As the total dose per area or soil volume is constant at all plant densities, the mean dose received by a target plant is high at low plant densities, and low at high plant densities (Weidenhamer *et al.,* 1989; Hansi *et al.,* 2014). The lower the plant density, the more plant response is affected, supposing that plants grow under toxin exposure. Control plant growth is not inhibited by toxins. Instead, it is limited solely by resource competition in most soils and growth conditions.

The first to distinguish the phenomenon were Weidenhamer *et al.* (1987, 1989) who developed a graphic model where dose per plant strongly affected the outcome of a density-response experiment (Thijs *et al.,* 1994; Weidenhamer, 2008). They predicted that control plants have greater biomass than exposed plants at low plant densities, and that at high plant densities the toxic effect may disappear. At high plant densities, resource competition is intense at all soil toxin levels, and the dose per plant remains insignificant as many plants are taking up and sharing the toxins. Importantly, the density-dependent toxin effect contrasts the typical effects of resource competition as the latter leads to maximum plant size at lower plant density. Noteworthily, the phenomenon described by Weidenhamer *et al.* (1989) has been distinguished using radio-labeled herbicides, elemental contaminants and the allelochemical juglone (Hoffman & Lavy, 1978; Weidenhamer *et al.,* 1989; Hansi *et al.,* 2014) but density-response experiments have never been used to evaluate the mechanism behind plant invasion.

*Ageratina adenophora* (Sprengel) R.M. King and H. Rob. is native to central Mexico. It is one of the most devastating invasive plants in many parts of Europe, Australia, Africa and Asia (Cronk & Fuller, 1995; Wang & Wang, 2006; Heystek *et al.,* 2011). In Asia, exotic *A. adenophora* causes negative soil feedback and releases compounds that have been found to reduce the growth of native Asian plant species (Inderjit *et al.,* 2011; Niu *et al.,* 2007). In Nepal, it is called 'forest killer' and has invaded various ecosystems including native forests (Tiwari *et al.,* 2005; Muniappan *et al.,* 2009; Subba & Kandel, 2013). This plant has been invading forests at tropical to subtropical regions of Nepal (Tiwari *et al.,* 2005) and replaces native tree species such as *Schima wallichii* (DC.) Korth. And *Alnus nepalensis* D.Don. in the hill forests of central Nepal. Plant growth inhibition has been observed in studies made with *A. adenophora* leaf, root and litter extracts or compounds in them (Ming-Zhi *et al.,* 2011; Ma *et al.,* 2012; Kundu, 2013; Bhardwaj *et al.,* 2014). *A. adenophora* volatiles also inhibit native species growth (Inderjit *et al.,* 2011). As several independent studies have identified the phytotoxic potential of *A. adenophora*, and as phytoactive compounds have already been identified, we considered *A. adenophora* as a suitable candidate species for our study.

Importantly, the ecological significance of phytotoxic effects in stands of native seedlings is unexplored, even in the laboratory conditions.

We performed two experiments and specifically tested *A. adenophora* effects on a common, native plant species that grows within the invaded habitats in Nepal. We hypothesized that *A. adenophora* invasions have at least two characteristics that can be distinguished: (i) Seedlings growing in soil collected from *A. adenophora* invaded habitats grow less vigorously than seedlings growing in soil that was collected from non-invaded habitats and (ii) *A. adenophora* impacts on the native seedlings are density-dependent so that *S. wallichii* growth is inhibited at low plant densities, while at high plant densities competitive effects dominate both in the presence and absence of *A. adenophora* leaf litter. After investigating these characteristics we propose research directions to control and manipulate *A. adenophora* invasions.

## Methods

### Study species

One of the most problematic invasive alien species, *A. adenophora* of family Asteraceae was selected as a donor species in this study. It is a perennial sub-shrub introduced to Nepal via India and widely distributed in Nepal's forest ecosystem (Tiwari *et al.,* 2005; Ministry of Forest and Soil Conservation, 2013). Luxuriant growth of this species is found in open areas, forest margins, wastelands, and surroundings of agricultural areas. It is frequently found in moist sub-humid climate, although it is not restricted to specific habitats (Wang *et al.,* 2011), altitudes or soil types in Nepal (field observation). It can produce numerous light seeds which are dispersed by wind, water, vehicles or by adhering to clothes, footwear and animal hair (Parsons, 1992). Seed germination requires light but the seedlings are shade tolerant and can grow rapidly (Auld & Martin, 1975).

Zheng *et al.* (2012) found that *A. adenophora* is rich in a phenolic compound, *o*-coumaric acid, which acts as a potent phytotoxin. Zhang *et al.* (2013) isolated a quinic acid derivative, 5-*O*-*trans*-*o*-coumaroylquinic acid methyl ester together with chlorogenic acid methyl ester, macranthoin F and macranthoin G from the aerial parts of *A. adenophora.* Other toxic compounds identified and isolated from the aerial parts of this species are α-phellandrene, camphene, ρ-cymene, 2-carene, α-pinene, limonene, and (z)-3-hexen-1-ol (Zhang *et al.,* 2012) and monoterpenes such as (1*α*,6*α*,7*α*)-8-hydroxy-2-carene-10-oic acid and (1*α*,6*α*)-10-hydroxy-3-carene-2-one (Xu *et al.,* 2014).

The native plant species selected for this study was *S. wallichii*. It is a very common, evergreen, medium to large sized tree species dominant in lower montane forests of eastern to central Nepal. It is found between 900 and 2100 m above sea level alongside both northern and southern slopes. *S. wallichii* is moderately shade tolerant and tolerates a wide temperature range (0-45°C), various habitats and soil types (Orwa *et al.,* 2009). Its natural regeneration occurs through seeds. The winged seeds are dispersed after releasing from woody globose fruit in April/May (field observation). In laboratory, water soaked seeds germinate in 10-30 days. In commercial plantation, the seeds are sown in April so that slowly growing seedlings are suitable for planting during next monsoon season.

*A. adenophora* dry leaves are hanging on branches and wet leaves easily fall at the start of rainy season to the ground and form certain level of litter thickness on soil surface (field observation). Seeds of *S. wallichii* disperse in nature just before rainy season and natural germination may occur when the rainy season starts. This coincidence causes the *S. wallichii* seedlings to come in contact with *A. adenophora* litter or components released from litter during decomposition.

### Sample collection

Seeds of *S. wallichii*, litter of *A. adenophora*, natural and invaded soils were collected from Champadevi hill forest, Kathmandu, central Nepal. The seeds of *S. wallichii* were collected in April 2014. The collected seeds were visually examined to discard damaged seeds and stored at 5˚C in a plastic bag until use after at least 24 h of air drying at room temperature. The natural soil was collected from a native forest vegetation site (27º39'19"N, 85º14'55"E) and the invaded soil was collected from *A. adenophora* invaded site of the same forest (27º39'23"N, 85º14'51"E) in June 2014.

As we used natural and invaded soil in the experiments, soils from the same locations were tested for physiochemical properties (soil nutrients: N, P, K; total organic content; soil pH; moisture and texture (Table 1). Three separately collected replicates per soil type were used and the distance between the collection plots was at least 10 m away from each other at each site. Soil was stored for one week at room temperature until use. Fresh leaf litter was collected from the invaded site and litter nutrients (N, P, K) were also estimated using three replicates of freshly collected litter (Table 1).

### Seedling preparation

Collected seeds were soaked in normal water for 24 h and spread on double layered moist filter paper in a tray. The tray was kept moist and put in dark at room temperature (26±2˚C). The seeds started germination after one week of soaking. The seedlings (30 mm long) were selected and transferred to experimental pots after three weeks of soaking.

### Soil type experiment

The experiment was conducted in a greenhouse of Central Department of Botany, Tribhuvan University, Kathmandu, Nepal from July to September, 2014. Three *S. wallichii* seedlings were grown in each polythene pot (8 cm diameter, 10 cm height with holes at bottom). The pots were filled with 450 g fresh weight of natural or invaded soil. Four treatments were made: (i) Natural soil (ii) Natural soil + leaf litter (iii) Invaded soil (iv) Invaded soil + leaf litter. Three seedlings were grown at each pot and there were 5 replicates per treatment. The litter was placed on top of the soil immediately after planting the seedlings. Litter thickness was 1 cm on the starting day and was allowed to decompose naturally. The pots were randomized and watered with 20 ml tap water every second day. The plants did not experience flooding or water deficiency. Daily temperature variation in the greenhouse was 21-26˚C (minimum) and 31-37˚C (maximum). Normal daylight was 12 h and humidity varied between 52-85%. The experiment was harvested at 48 days after planting. Shoot and root length was measured; shoot and root were separated, dried at 70˚C for 24 hand dry weight (DW) was measured.

### Density-response experiment

This experiment consisted of four levels of *A. adenophora* litter thickness (0, 0.5, 1.0 and 1.5 cm) and four *S. wallichii* densities (1, 3, 6 and 12 plants per pot). Only natural soil (400 g fresh weight per pot) was used. There were 10 replicates per treatment at density 1 and 6 replicates at other densities. The pots were randomized and watered (10-30 ml) every second day depending on evaporation. The plants were harvested at 56 days after planting. Other experimental conditions and measurement of plant characteristics were identical to the above experiment.

### Data analysis

In the soil type experiment, main factors (soil type and litter) and their interaction effect were tested using two-way ANOVA in SPSS version 16. In the density-response experiment, significant differences between control (no litter) and litter treatments at each plant density were tested with one-way ANOVA using a linear contrast for the litter treatment and Tukey's test. As shoot:root ratio data were not normally distributed in density response experiment, they were $\log_{10}$ transformed before the analysis. Other assumptions of the analyses were met.

## Results

### Soil type experiment

*S. wallichii* plants were taller in natural than in *A. adenophora* invaded soil (Fig. 1a; Table 2). *S. wallichii* roots were shorter and lighter when grown in soil collected from *A. adenophora* invaded sites, and *A. adenophora* litter reduced the length of *S. wallichii* roots regardless of soil type (Fig. 1b & c; Table 2). Interestingly, *A. adenophora* litter decreased *S. wallichii* root dry weight in natural soil, but the effect was not clear in invaded soil (Fig. 1c; Table 2). The reason may be that invaded soil *per se* reduced *S. wallichii* growth. However, *S. wallichii* shoot dry weight was similar in all treatments (Table 2). Shoot:root ratio was higher in *A. adenophora* invaded soil and in the presence of *A. adenophora* litter, as compared to *S. wallichii* seedlings that were grown in natural soil without *A. adenophora* leaf litter (Fig. 1d; Table 2).

### Density-response experiment

In the density-response experiment, no significant differences were found in shoot length and dry weight between control (no litter) and litter exposed treatments at1 and 3 plants per pot (Fig. 2a & b; Table 3). Compared to lower densities, the shoots of control plants were smaller at 6 and 12 plants per pot showing the predicted effect of intense resource competition. At higher plant densities, the shoots of litter exposed plants were longer than control plants at litter levels 1 and 1.5 cm (Table 3). At the same time, the sole difference in shoot dry weight was observed at density 6 where the control plants were lighter than litter-treated plants (Fig. 2b; Table 3).

Both mean root length and root dry weight decreased as litter thickness increased at 1 and 3 plants per pot (Fig. 2c & d; Table 3). Root length and dry weight in the control decreased gradually at increasing plant densities showing density effect and competition for soil nutrients. At 6 plants per pot, litter exposed plants had heavier roots than control plants (Fig. 2d; Table 3), while litter exposed plants did not differ from control plants in root length at this density. At 6 plants per pot, however, roots under the thickest litter layer were shorter than those under the thinnest litter layer (Fig. 2c; Table 3). At the highest plant density (12 plants per pot), there were no differences in root characteristics (Fig. 2c & d; Table 3)

To summarize, litter-exposed plants had smaller roots than control at low plant densities, while at the highest density litter increased plant height and did not affect plant dry weight. Further, at 6 plants per pot, a stimulatory effect existed in shoot length and both shoot and root dry weight, while root length was similar with and without litter.

## Discussion

The inhibitory effects of leaf litter and invaded soil indicate that *A. adenophora* invasion is detrimental to native *S. wallichii*. The results of the soil type experiment particularly indicate that *S. wallichii* root growth is sensitive to both invaded soil and litter. The lack of shoot growth inhibition caused the shoot:root ratio of *S. wallichii* to be exceptionally high under the effect of *A. adenophora* (litter or soil or both; Fig 1d), which may complicate water economy outside the monsoon season. In the field, *S. wallichii* seeds germinate during the pre-monsoon, and they must be deep-rooted before the beginning of the dry season. Our study thus indicates that *S. wallichii* seedlings growing in *A. adenophora* invaded soil or under its litter may be more likely to deteriorate during the dry season than those growing in non-invaded soil. Earlier finding highlighted the role of *A. adenophora* volatiles in the inhibition of native Asian vegetation (Inderjit *et al.,* 2011). In addition to volatile effect of *A. adenophora* litter, our experiments indicate that *S. wallichii* growth is inhibited through direct contact in invaded soil or under *A. adenophora* litter. Volatiles produced from thick litter layer and during litter decomposition could be trapped by live *S. wallichii* seedlings. Exposure to volatiles, soluble compounds from the litter and non-volatile toxins already present in invaded soil might have a combined inhibitory effect on *S. wallichii*.

In the density-response experiment, litter thickness affected root growth at low plant densities, while shoot growth was unaffected. This is likely to complicate the survival of *S. wallichii* seedlings during the dry season, assuming that *S. wallichii* seedling density is low. In contrast, at higher *S. wallichii* densities, particularly at 6 plants per pot, *S. wallichii* growth was stimulated by *A. adenophora* litter, and the shoot:root ratio was not affected (Table 3). As a result, there tends to be a plant density at which *S. wallichii* growth is faster in the presence of *A. adenophora* litter than without the litter. If *S. wallichii* density was very high (12 plants per pot), no differences were found between treatments, but shoot length seemed to increase with increasing litter thickness. Our finding, the disappearance of the inhibition of *S. wallichii* seedlings at high plant densities should be tested in field conditions. If the results are similar, seedling density may be adjusted to overcome the deleterious effects of *A. adenophora* on *S. wallichii*. The method should be tested at early stages of invasion when *A. adenophora* litter covers soil but the soil *per se* is unaffected.

Based on our experiment, it is not possible to judge whether low-dose hormesis (Belz & Piepho, 2012; Sinkkonen, 2003) or nutrient release from decaying litter is the reason for *S. wallichii* growth stimulation at 6 plants per pot. Invasive species often cause changes in soil nutrient composition (Ashton *et al.,* 2005; Sinkkonen *et al.,* 2014). In our study, there was no differences in nitrogen, phosphorus or potassium levels between the invaded and natural soils. The pH value in invaded soil was low 4.8 (Table 1) whereas *S. wallichii* prefers soil pH between 5 - 6.5 (Schmidt & Lieu, 2004). Therefore, the low pH in invaded soil might be one of the factors inhibiting *S. wallichii* seedling growth. Interestingly, the leaf litter of *A. adenophora* does not seem to increase soil nutrient levels, even though the *A. adenophora* litter was rich in nutrients N, P and K (Table 1).

The litter nutrients may have contributed to the fast growth at higher densities in the density-response experiment (Fig. 2), but they cannot be used to explain why *S. wallichii* growth inhibition was inversely related to plant density.

Decaying leaf litter often releases phytotoxic compounds that affect seedling growth and development (Facelli & Picket, 1991; Sinkkonen, 2003; San Emeterio *et al.,* 2007). In our study, we did not determine whether the toxins are released from the litter, or whether they were metabolized by micro-organisms during litter degradation. Further, plants may secrete compounds that detoxify or bind toxins (Hansi *et al.,* 2014), which may depend on root density in soil. In any case, the release of toxins in soil is independent of the density of target plants. We do not know any other explanation for these density-dependent differences. Therefore, our study supports the hypothesis (Lodhi, 1978; Inderjit & Callaway, 2003; Inderjit *et al.,* 2011; Ming-Zhi *et al.,* 2011; Soltys *et al.,* 2013) that chemical interference plays an ecologically significant role in the effect of *A. adenophora* on the growth of native plants. As earlier studies have already identified *A. adenophora* phytotoxins (Inderjit *et al.,* 2011; Kundu, 2013), our study is the first case where density-response bioassays are utilized to study the ecological significance of chemical interference in the context of plant invasiveness. It should be kept in mind, however, that density-response experiments cannot be used to prove that the effect of an alien species on native vegetation is alleopathic. This requires a carefully designed combination of long-term field surveys coupled with the identification of the active toxins, and preferably also understanding on the mode of action within plant tissues. As it is not realistic to perform those economically demanding tasks in case of all invading plant species, particularly in species-rich plant communities, we recommend setting up density-response experiments to survey whether any indication of chemical interference can be found in natural-like laboratory or field bioassays.

As far as we are aware of, chemical interference is the only relevant explanation of the density-dependent inhibition of plant growth in the litter experiments. According to our knowledge, neither soil microbes nor root recognition are known to cause a similar pattern. Obviously, soil microbes always metabolize compounds released from decaying litter, and we specifically targeted to study the density-dependence of toxin interactions in study systems were microbes are present and cope with phytochemicals. Our argument is that soil microbes or root recognition are not known to produce inhibition at low but not at high densities. Differences in soil moisture under litter is a classic alternative explanation for density-dependent chemical interference. Nilsson and collaborators have shown that it is an unlikely explanation (Nilsson, 1994), if a plant species is known to show toxic effects on competing plants. We paid particular attention to watering, and it is hard to imagine that low-density plants suffered from dryness with litter, but grew normally in the absence of protecting litter. Further, the pots had holes on the bottom to release excess water.

Variation in soil temperature is another unlikely explanation; plants were grown in one liter pots in a greenhouse where each side and even the bottom of the pots were equally exposed to air temperature. In addition, the pots were randomized every second day, which must have ceased possible differences in soil temperature between pots. One could also speculate that litter layer affected oxygen levels in soil. If this was the case, the inhibition should not have been inversely related to plant density. Our study does not exclude the potential effects of other mechanisms that influence native plants during *A. adenophora* invasion. Those mechanisms include negative soil feedback, such as the manipulation of soil biota (root symbionts) (Inderjit & van der Putten, 2010; Sinkkonen *et al.,* 2014). In addition, *S. wallichii* may be affected positively by native soil microbial communities at the early stage of *A. adenophora* invasion, and after *A. adenophora* establishment the soil communities may be permanently altered and inhibit the growth of the native plant species (Wolfe & Klironomos, 2005; Niu *et al.*, 2007; Mangla & Callaway, 2008; HuiNa *et al.,* 2009; Xu *et al.,* 2012). These mechanisms, however, are not known to cause a density-dependent growth inhibition as observed in this study.

In conclusion, *A. adenophora* invaded soil and its litter inhibit *S. wallichii* seedling growth. The inhibitory effects are pronounced at low *S. wallichii* densities and they affect primarily roots. Importantly, the inhibitory effect of *A. adenophora* litter disappeared at high *S. wallichii* seedling densities. Therefore, field tests should be performed to find out if seedling performance is imporved at high target plant densities or in clusters. We propose to increase seeding densities of native plant species, such as *S. wallichii*, in *A. adenophora* invaded habitats. If field tests are successful, the method can become an economically feasible management strategy against plant invasion. The inhibitory effect of *A. adenophora* on *S. wallichii* may be minimized either by removing litter or maintaining high seedling densities.

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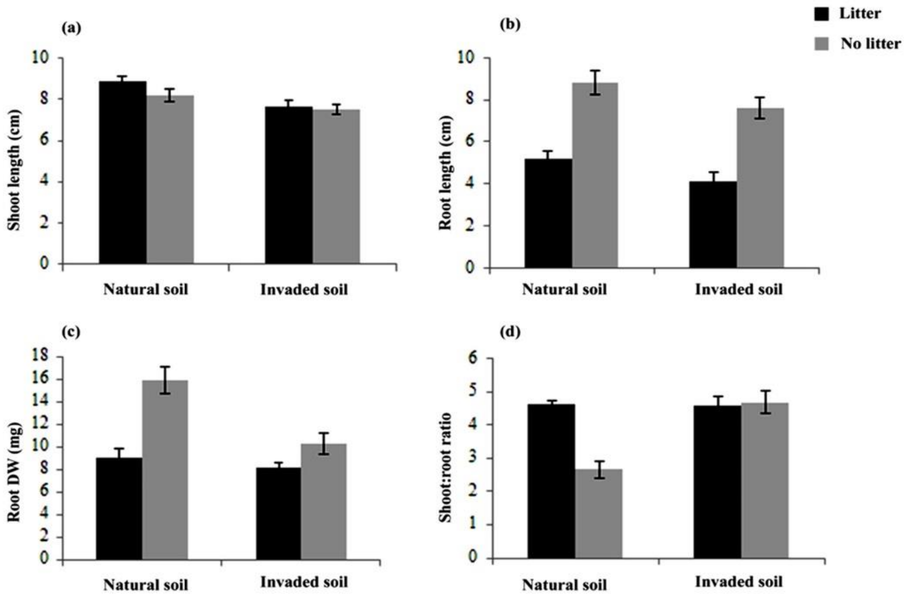


**Fig.1** *Schima wallichii* seedling growth (per plant) in natural and invaded soil in the presence and absence of *A. adenophora* litter: (**a**) Shoot length, (**b**) Root length, (**c**) Root dry weight, (**d**) Shoot:root ratio. Data are means±SE

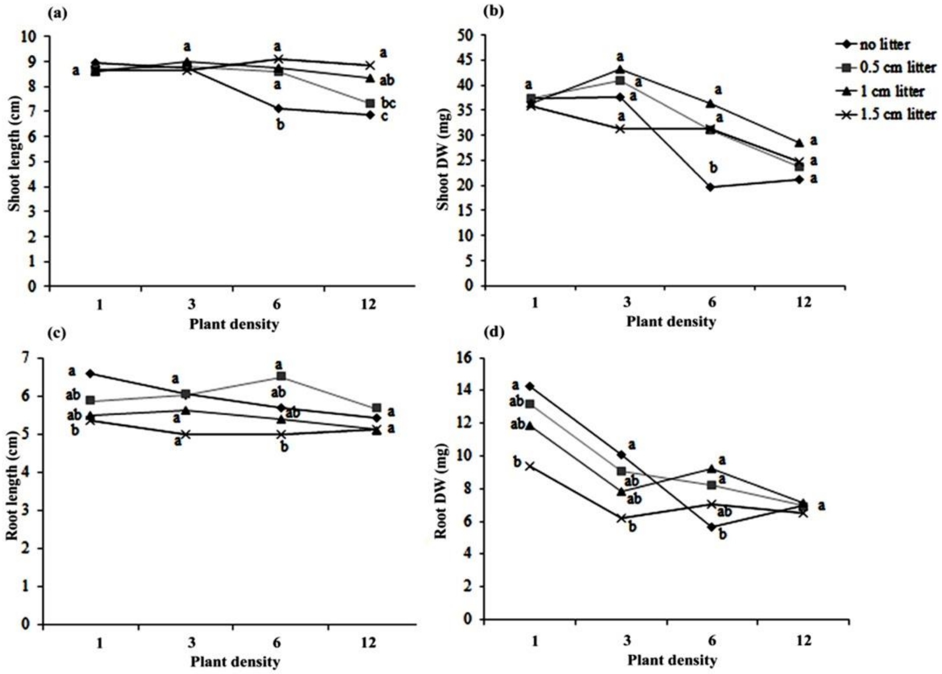


**Fig. 2** *Schima wallichii* seedling growth (per plant) at each density and *A. adenophora* litter level: (**a**) Shoot length, (**b**) Shoot dry weight, (**c**) Root length, (**d**) Root dry weight. Letters indicate homogenous subgroups (p>0.05) at each density in Tukey's test.

**Table 1** Physiochemical properties of soil and litter nutrients (mean±SD, n = 3)

| Soil parameters | Natural soil | Invaded soil | Litter nutrients (inorganic N, P, K %) | |
|---|---|---|---|---|
| pH (1:2.5% $H_20$) | 5.70±0.70* | 4.77±0.15* | Nitrogen | 2.99±0.09 |
| Total Nitrogen (%) | 0.31±0.01 | 0.30±0.01 | Phosphorus | 0.62±0.05 |
| Available Phosphorus (kg/ha) | 16.55±8.60 | 21.21±6.05 | Potassium | 2.90±0.10 |
| Available Potassium (kg/ha) | 513.70±60.40 | 515.73±63.75 | | |
| Organic Matter (%) | 6.05±0.26 | 6.22±0.33 | | |
| Moisture (%) | 26.10±0.63* | 24.31±0.05* | | |
| Sand (%) | 38.53±11.50 | 33.87±9.01 | | |
| Silt (%) | 52.57±8.50 | 53.40±6.00 | | |
| Clay (%) | 8.90±3.04 | 12.73±3.05 | | |

* $p < 0.05$ in Mann-Whitney U-test

**Table 2** Two-way ANOVA results of the effects of soil type (natural/invaded soil) and *A. adenophora* litter (presence/absence) on shoot and root length, dry weight and shoot:root ratio of *S. wallichii*. Detailed ANOVA test statistics are provided in Supplementary table 1

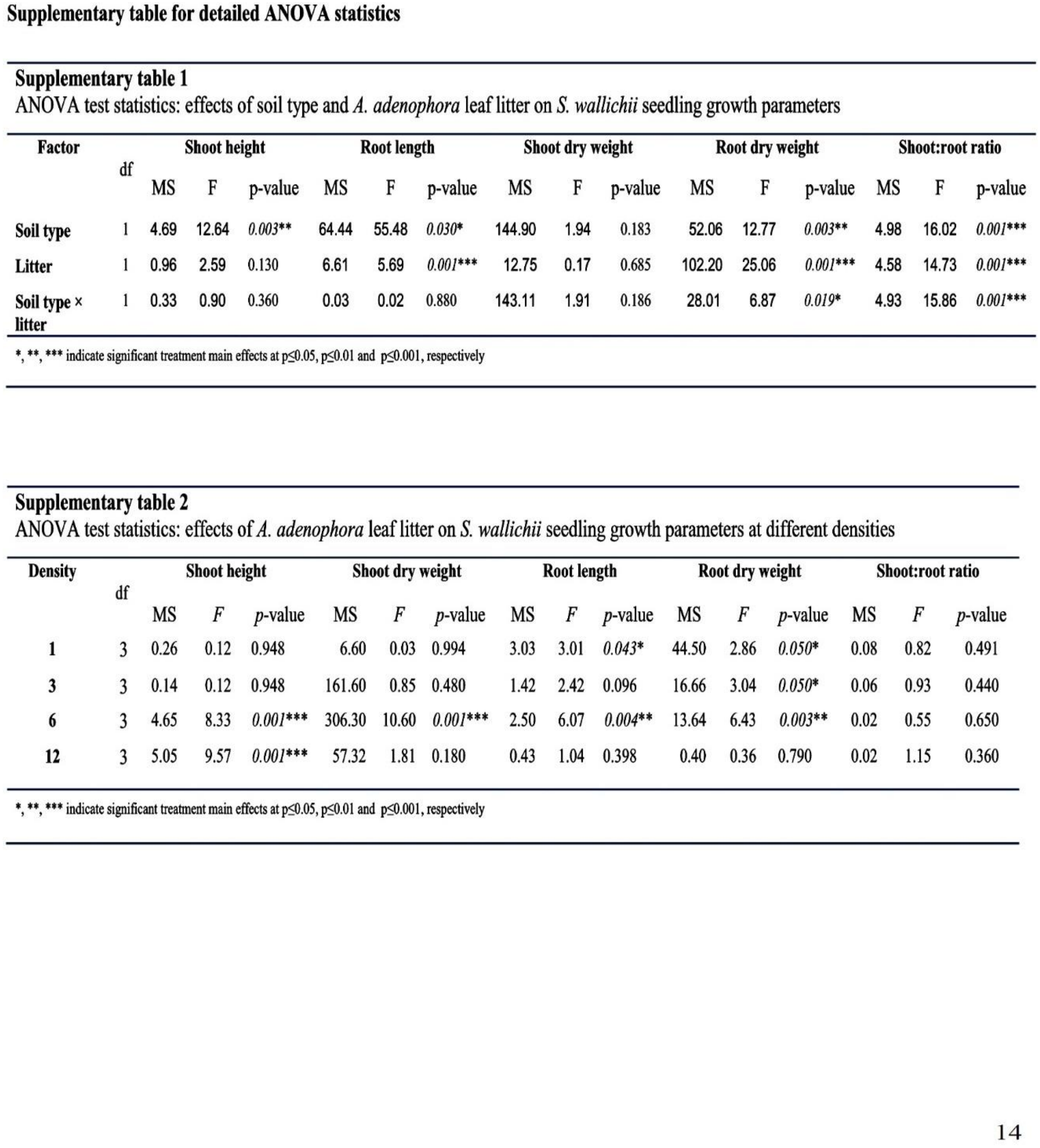
| Factor | df | p-value | | | | |
|---|---|---|---|---|---|---|
| | | Shoot length | Root length | Shoot DW | Root DW | Shoot:rootratio |
| Soil type | 1 | 0.003** | 0.030* | 0.180 | 0.003** | 0.001*** |
| Litter type | 1 | 0.130 | <0.001*** | 0.690 | <0.001*** | 0.001*** |
| Soil type × litter | 1 | 0.360 | 0.880 | 0.190 | 0.019* | 0.001*** |

*, **, *** indicate significant treatment main effects at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively

**Table 3** One-way ANOVA results of the effects of plant densities and *A. adenophora* litter on shoot and root length, dry weight and shoot:root ratio of *S. wallichii*. Detailed ANOVA test statistics are provided in Supplementary table 2

| Density | Df | p-value | | | | |
|---|---|---|---|---|---|---|
| | | Shoot length | Shoot DW | Root length | Root DW | Shoot:root ratio |
| 1 | 3 | 0.950 | 0.990 | 0.043* | 0.050* | 0.490 |
| 3 | 3 | 0.950 | 0.480 | 0.090 | 0.050* | 0.440 |
| 6 | 3 | 0.001*** | <0.001*** | 0.004** | 0.003** | 0.650 |
| 12 | 3 | <0.001*** | 0.180 | 0.390 | 0.790 | 0.360 |

*, **, *** indicate significant treatment main effects at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively



**Supplementary table for detailed ANOVA statistics**

**Supplementary table 1**

ANOVA test statistics: effects of soil type and *A. adenophora* leaf litter on *S. wallichii* seedling growth parameters

| **Factor** | df | **Shoot height** | | | **Root length** | | | **Shoot dry weight** | | | **Root dry weight** | | | **Shoot:root ratio** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | F | p-value | MS | F | p-value | MS | F | p-value | MS | F | p-value | MS | F | p-value |
| **Soil type** | 1 | 4.69 | 12.64 | *0.003*** | 64.44 | 55.48 | *0.030** | 144.90 | 1.94 | 0.183 | 52.06 | 12.77 | *0.003*** | 4.98 | 16.02 | *0.001**** |
| **Litter** | 1 | 0.96 | 2.59 | 0.130 | 6.61 | 5.69 | *0.001**** | 12.75 | 0.17 | 0.685 | 102.20 | 25.06 | *0.001**** | 4.58 | 14.73 | *0.001**** |
| **Soil type × litter** | 1 | 0.33 | 0.90 | 0.360 | 0.03 | 0.02 | 0.880 | 143.11 | 1.91 | 0.186 | 28.01 | 6.87 | *0.019** | 4.93 | 15.86 | *0.001**** |

*, **, *** indicate significant treatment main effects at p≤0.05, p≤0.01 and p≤0.001, respectively

**Supplementary table 2**

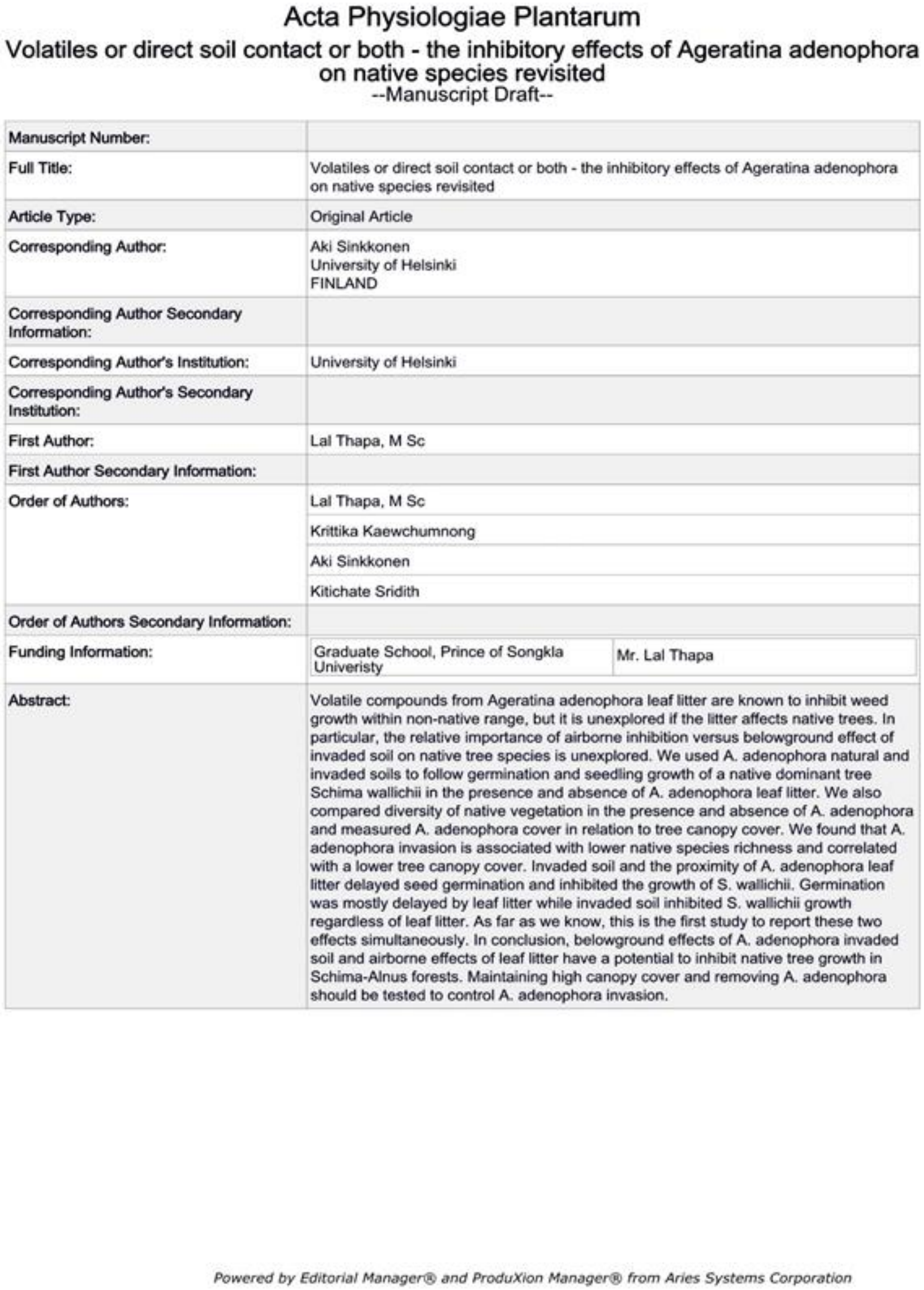
ANOVA test statistics: effects of *A. adenophora* leaf litter on *S. wallichii* seedling growth parameters at different densities

| **Density** | df | **Shoot height** | | | **Shoot dry weight** | | | **Root length** | | | **Root dry weight** | | | **Shoot:root ratio** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | *p*-value | MS | *F* | *p*-value | MS | *F* | *p*-value | MS | *F* | *p*-value | MS | *F* | *p*-value |
| **1** | 3 | 0.26 | 0.12 | 0.948 | 6.60 | 0.03 | 0.994 | 3.03 | 3.01 | *0.043** | 44.50 | 2.86 | *0.050** | 0.08 | 0.82 | 0.491 |
| **3** | 3 | 0.14 | 0.12 | 0.948 | 161.60 | 0.85 | 0.480 | 1.42 | 2.42 | 0.096 | 16.66 | 3.04 | *0.050** | 0.06 | 0.93 | 0.440 |
| **6** | 3 | 4.65 | 8.33 | *0.001**** | 306.30 | 10.60 | *0.001**** | 2.50 | 6.07 | *0.004*** | 13.64 | 6.43 | *0.003*** | 0.02 | 0.55 | 0.650 |
| **12** | 3 | 5.05 | 9.57 | *0.001**** | 57.32 | 1.81 | 0.180 | 0.43 | 1.04 | 0.398 | 0.40 | 0.36 | 0.790 | 0.02 | 1.15 | 0.360 |

*, **, *** indicate significant treatment main effects at p≤0.05, p≤0.01 and p≤0.001, respectively

# Paper IV

**Thapa, L.B.**, Kaewchumnong, K., Sinkkonen, A. and Sridith, K. Volatiles or direct soil contact or both – the inhibitory effects of *Ageratina adenophora* on native species revisited. Acta Physiologiae Plantarum (Submitted Manuscript, 2016)



## Acta Physiologiae Plantarum

# Volatiles or direct soil contact or both - the inhibitory effects of Ageratina adenophora on native species revisited

--Manuscript Draft--

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| **Abstract:** | Volatile compounds from Ageratina adenophora leaf litter are known to inhibit weed growth within non-native range, but it is unexplored if the litter affects native trees. In particular, the relative importance of airborne inhibition versus belowground effect of invaded soil on native tree species is unexplored. We used A. adenophora natural and invaded soils to follow germination and seedling growth of a native dominant tree Schima wallichii in the presence and absence of A. adenophora leaf litter. We also compared diversity of native vegetation in the presence and absence of A. adenophora and measured A. adenophora cover in relation to tree canopy cover. We found that A. adenophora invasion is associated with lower native species richness and correlated with a lower tree canopy cover. Invaded soil and the proximity of A. adenophora leaf litter delayed seed germination and inhibited the growth of S. wallichii. Germination was mostly delayed by leaf litter while invaded soil inhibited S. wallichii growth regardless of leaf litter. As far as we know, this is the first study to report these two effects simultaneously. In conclusion, belowground effects of A. adenophora invaded soil and airborne effects of leaf litter have a potential to inhibit native tree growth in Schima-Alnus forests. Maintaining high canopy cover and removing A. adenophora should be tested to control A. adenophora invasion. |

# Volatiles or direct soil contact or both – the inhibitory effects of *Ageratina adenophora* on native species revisited

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## Abstract

Volatile compounds from *Ageratina adenophora* leaf litter are known to inhibit weed growth within non-native range, but it is unexplored if the litter affects native trees. In particular, the relative importance of airborne inhibition versus belowground effect of invaded soil on native tree species is unexplored. We used *A. adenophora* natural and invaded soils to follow germination and seedling growth of a native dominant tree *Schima wallichii* in the presence and absence of *A. adenophora* leaf litter. We also compared diversity of native vegetation in the presence and absence of *A. adenophora* and measured *A. adenophora* cover in relation to tree canopy cover. We found that *A. adenophora* invasion is associated with lower native species richness and correlated with a lower tree canopy cover. Invaded soil and the proximity of *A. adenophora* leaf litter delayed seed germination and inhibited the growth of *S. wallichii*. Germination was mostly delayed by leaf litter while invaded soil inhibited *S. wallichii* growth regardless of leaf litter. As far as we know, this is the first study to report these two effects simultaneously. In conclusion, belowground effects of *A. adenophora* invaded soil and airborne effects of leaf litter have a potential to inhibit native tree growth in *Schima-Alnus* forests. Maintaining high canopy cover and removing *A. adenophora* should be tested to control *A. adenophora* invasion.

**Key words:** *Ageratina adenophora, Schima wallichii*, invasion, leaf litter, seedling growth

## Introduction

Invasive alien species are known to change soil characteristics and native ecosystems (Ehrenfeld 2003; Vila et al. 2011), e.g. novel root exudates, decomposing litter and symbiotic bacteria can cause cascading effects in native ecosystems (Weidenhamer and Callaway 2010; Sinkkonen et al. 2014). Defense or antimicrobial chemicals produced by invaders act against native species growth (Callaway and Ridenour 2004; He et al. 2009; Sinkkonen 2006) or change the fitness of soil micro fauna and thus aid the invading species (Sinkkonen et al. 2014). Adverse effects of invasive species on biogeochemical processes and resource availability as well as the release of novel chemicals within invaded range can reduce the abundance of native plants (Wolfe and Klironomos 2005). Volatile and soil-based non-volatile compounds have been investigated in many separate studies (Barney et al. 2005; Chen et al. 2013; Inderjit et al. 2011), the relative importance of volatile versus soil-based processes against the growth of a native species has not been investigated in a single study.

Another interesting tendency in invasion research is that researchers have often chosen herbaceous plants, weeds or crops as target species. Study on impacts of forest invaders on native dominant tree species is necessary if the goal is to understand the invasion success of an invader in natural forest ecosystems. Native tree species are usually neglected, perhaps due to their slow growth, uneven germination, seed size and other factors that slow down investigations. However, some previous studies have documented potential growth reduction of native trees in the presence of invasive species (Gorchov and Trisel 2003; Orr et al. 2005; Rudgers and Orr 2009; Stinson et al. 2006). Based on these studies it looks obvious that growth and development comparisons of tree species in non-invaded (natural) and invaded areas and soils will add to the knowledge on invasion success.

One of the most problematic invasive species in Europe and Asia, *Ageratina adenophora* (Spreng.) R.M. King & H. Rob., which is commonly called 'forest killer' is an interesting candidate for forest-oriented research as the species is already known to replace native species and to modify plant communities (Tiwari et al.2005; Wan et al. 2010). The species is aggressively invading forest ecosystems (Tiwari et al. 2005; Wan et al. 2010; Wang and Wang 2006) and it can produce large, ecologically significant volumes of volatile organic compounds-VOCs (Inderjit et al. 2011; Kundu

2013; Ren et al. 2010; Zhang et al. 2012). Early studies have recognized that the extracts and VOCs from *A. adenophora* inhibit seed germination and seedling growth of certain crops, weeds and herbs (Inderjit et al. 2011; Wan et al. 2011; Zhang et al. 2008; Zhang et al. 2012). *A. adenophora* effect on dominant native tree species is virtually unexplored, and the relative importance of the airborne effect of leaf litter VOCs and belowground effects of leaf litter has not been surveyed.

In this study, we documented the effect of *A. adenophora* on native species in the field and performed laboratory investigations. We measured *A. adenophora* cover and tested whether *A. adenophora* invasion reduces native species richness and density in a natural forest. We correlated *A. adenophora* cover with tree canopy cover to observe the relationship between tree canopy and the level of invasion inside the forest. Finally, we tested the relative importance of two invasion mechanisms, namely the belowground effect of invaded soil versus the airborne effect of *A. adenophora* leaf litter. Our hypotheses were (1) *A. adenophora* abundance is inversely related to forest canopy cover, (2) *A. adenophora* reduces native species richness in *Schima-Alnus* forests, and (3) both airborne effects of leaf litter and the belowground effects of invaded soil inhibit the germination and seedling growth of the dominant native tree *Schima wallichii.*

## Methods

### *Study species*

*A. adenophora* (Asteraceae) is a perennial subshrub native to Mexico and one of the worst invasive species in different ecosystems worldwide (Tiwari et al. 2005, Wang and Wang 2006). The plant has simple, ovate, dark green, opposite and serrate leaves. Small white flowers are borne on terminal clusters. It produces numerous minute seeds and reproduces vegetatively in addition to sexual reproduction by seeds (Tiwari et al. 2005). The seeds require light for germination, but after germinated the seedlings can endure shade (Auld 1975; Muniappan et al. 2009).

*A. adenophora* can produce a number of VOCs having phytotoxic effects (Inderjit et al. 2011; Ren et al. 2010; Zhang et al. 2012). In particular, the litter of *A. adenophora* contains higher concentrations of 2-carene and α-phellandrene (volatile monoterpenes) in China and India than in Mexico which is its place of origin. This has been associated with reduced germination of *Bidens biternata* and slow root and shoot growth of *Bambusa arundinacea* (Inderjit et al. 2011). Zhang et al. (2012) identified volatiles such as α-phellandrene, camphene, ρ-cymene, 2-carene, α-pinene, limonene, and (z)-3-hexen-1-ol from leaf tissues having a phytotoxic effect on rice seedlings. Among them α-phellandrene inhibited shoot growth and (z)-3-hexene-1-ol and ρ-cymene inhibited both shoot and root growth. Five types of sesquiterpenes were isolated from leaves of *A. adenophora* by Kundu (2013) and among them 5,6-dihydroxycadinan-3-ene-2,7-dione was identified as one of the most active compounds that inhibit both shoot and root growth of *Phalaris minor* and *Polygonum plebejum.*

We selected *Schima wallichii* (DC.) Korth. as the native target species in our greenhouse experiments. The species is a perennial evergreen tree native to Nepal. It is one of the dominant tree species in *Schima-Alnus* and *Schima-Castanopsis* vegetation. In Nepal, it is distributed along subtropical range from eastern part to mid-western part of the country. The plant produces spherical woody capsule fruits which split when dry in March to April and release small seeds (pers. obs.). It regenerates naturally from seeds, grows on varying soil types and tolerates temperature ranges between 0 to 45°C (Orwa et al. 2009).

### *Study site*

The field study was conducted in Champadevi forest (27˚42′N and 85˚19′ E) located in southwestern part of Kathmandu valley, central Nepal. The elevation of the forest ranges from 1300 to 2400 masl facing towards northeastern direction. Current study was confined to the lower belt of the forest below 1700 masl. where there was a high level of *A. adenophora* invasion and the dominant native trees in the locality are *S. wallichii* and *Alnus nepalensis* D.Don (Thapa et al. 2016). The climate of the area is hot and humid in summer and dry in winter.

### *Field survey*

We placed 40 quadrats of size 10×10m$^2$ along transects made at the study site. The transects were distributed each in pure stand of *Schima wallichii* or *Alnus nepalensis* patches, *Schima-Alnus* mix patches and pine forest. Ten quadrats were laid in each transect except *Schima-Alnus* mix forest where 20 quadrats were laid and the distance between two quadrats was at least 100 m. Canopy cover of the trees and *A. adenophora* was estimated in each 10×10 m$^2$ quadrat in percent cover classes of 1%, 2%, 5% and then in 5% increments upto 100%.

Quadrats (plots) of size 1×1 m$^2$ were placed in *Schima-Alnus* mixed forest randomly at highly invaded sites by *A. adenophora* and non-invaded sites, distance between two quadrats was at least 20 m. In detail, quadrat positioning started from a point in the forest. The transect was first towards west the edge of the forest and after 50 m far from the first transect another transect was made towards east. Transect length was variable according to the accessibility (some very dense thorny plants and some difficult landscape). The quadrats were laid along each transect. Quadrat number per transect was variable but a total of 60 quadrats were established. Both types of quadrats (invaded or non-invaded) occurred along each transect. A. *adenophora* cover was measured in invaded ones. This resulted in a total of 31 invaded and 29 non-invaded quadrats. To ensure randomization, 18 quadrats from each transect were selected randomly (randomly generated numbers from the numbers of invaded or non-invaded plots). Soil type, moisture and altitude were similar between invaded and non-invaded areas in the forest, so there is no reason to suspect bias in the randomized selection of quadrats. Vascular plants encountered in each quadrat were counted for species richness (number of species) and total plant density (number of individuals) was calculated. The plants were identified with the help of standard taxonomic literatures and voucher specimens were deposited at the National Herbarium and Plant Laboratories, Godawari (KATH), Nepal. The study was conducted from June 2014 to September 2015.

### *Soil and airborne effects of A. adenophora on S. wallichii growth*

We tested the belowground effect of invaded versus native soil and the air borne effect of *A. adenophora* leaf litter on seed germination and seedling growth of *S.wallichii* in a greenhouse at Central Department of Botany, Tribhuvan University, Kathmandu, Nepal. Seeds of *S.wallichii* were collected from a medium sized tree at the study site in April 2015 and stored in a plastic bag in a refrigerator until used. Natural soil, invaded soil and *A. adenophora* litter were collected in June 2015. Seeds of *S. wallichii* were soaked in water for 24 h and 10 healthy homogeneous seeds were sown evenly in each pot (6cm diameter and 8cm height) containing 120g soil (fresh weight). The seeds were placed under soil surface in such a way that the micropylar region of the seeds and germinating radicles could experience the outer environment as soon as it emerged out. The pots were placed inside polythene chambers (length 30 cm, width 18 cm and height 11.5cm). An opening of size 3×6 cm was made on either side of each chamber for ventilation. Throughout the experiment, *A. adenophora* leaf litter (20 g) was stored in each chamber outside the pots.

The treatments were as follows: (a) pots filled with natural soil (b) pots filled with natural soil + litter outside the pots (c) pots filled with invaded soil (d) pots filled with invaded soil + litter outside the pots. There were four replicated chambers for each treatment and each chamber contained three pots. Germinated seeds were counted daily for one week after sowing. After completion of germination the plants were thinned to six seedlings per pot. Every second day the chambers and the pots inside each chamber were randomized and each pot was watered with 10 ml tap water. Inside the greenhouse, temperature ranged from 21-38°C with 12/12 h light and dark cycle. The humidity varied between 52-85%. Root and shoot length of *S. wallichii* seedlings was measured 30 days after sowing. Dry weight of root and shoot was measured separately after drying at 70°C for 24h.

### *Data Analysis*

Independent sample t-test was used to evaluate the differences in *A. adenophora* cover, native species richness and plant density in the field study plots. Alien species reported from study plots were excluded in the analysis. Spearman's correlation was used to analyze the correlation between *A. adenophora* cover and tree canopy cover. Two-way analysis of variance (two-way ANOVA) was used to evaluate the effects of *A. adenophora* leaf litter and soil type (natural vs invaded soil) on seed germination and seedling growth parameters of *S. wallichii*. The analyses were carried out using SPSS version 20. The accepted significance level was $p \leq 0.05$. The assumptions of the analyses were met.

## **Results**

### *Tree canopy and A. adenophora cover*

Tree canopy correlated negatively with *A. adenophora* cover ($r_s$ = - 0.63, *p<0.01*). *A. adenophora* cover was higher under sparse (<50%) than dense (>50%) tree canopy cover in 10×10m$^2$ quadrats (t = 3.86; df = 1, 38; *p < 0.001*).

*Effect of A. adenophora on native species richness and plant density*

Regardless of the effects of tree canopy cover, native species richness in 1×1m$^2$ quadrats was lower with than without *A. adenophora* (t = 2.40; df = 1, 34; *p = 0.022*). Among 60 native plant species recorded from the 1x1m$^2$ quadrats, 39 species were found in the quadrats placed under *A. adenophora* canopy (invaded plots) and 59 plant species in the quadrats of non-invaded sites. The mean species number per quadrat was 8.9±0.7 in non-invaded plots and 6.9±0.5 m$^{-2}$ under *A. adenophora* canopy (t = 2.40; df = 1, 34; *p = 0.022*). Furthermore, total native plant density was 35% lower in the plots with *A. adenophora* than the plots without *A. Adenophora* (59.0±8.4 vs. 91.0±11.5, t = 2.24; df = 1, 34; *p =0.032*).

*Effect of A. adenophora on S. wallichii seed germination and seedling growth*

The seeds of *S. wallichii* started germinating at third day after sowing in the greenhouse inside chambers. *A. adenophora* litter in the chambers inhibited seed germination at day 3 (*p<0.001*), 4 (*p=0.028*), 5 (*p=0.016*) and 6 (*p=0.050*) (Fig. 1, Table 1). At days 3 and 4, the percentage of seed germination was higher in the natural soil than in the other treatments, while differences were not as clear thereafter.

Shoots of *S. wallichii* seedlings were shorter in chambers containing leaf litter, while root growth was reduced with invaded soil but not by the litter (Fig. 2, Table 2). Interestingly, the accumulation of shoot and root dry weight was inhibited by both soil type and litter inside the chamber (Fig. 2C, D; Table 2).

## Discussion

The first and second hypotheses in our study were supported, i.e. *A. adenophora* abundance was inversely related to forest canopy cover and *A. adenophora* reduced native species richness. These findings add to the earlier observations about a negative effect of *A. adenophora* on species richness (Dogra et al. 2010; Lu and Ma 2009; Inderjit et al. 2011), and they verify the decreased abundance and diversity of native plant species in the presence of *A. adenophora* in the *Schima-Alnus* forests studied by us. The inverse association increases the ecological relevance of the results in our laboratory experiments. Similarly, in accordance with previously found associations between the level of disturbance and plant invasions (Daehler 2003; Eschtruth and Battles 2009; Lake and Leishman 2004), the negative correlation between tree canopy cover and *A. adenophora* cover indicates a lower level of invasion under high tree canopy cover in natural *Schima-Alnus* forests. As the study area is situated in the vicinity of a human settlement, it is likely that anthropogenic disturbances such as looping and cutting reduce tree canopy. As *A. adenophora* prefers open areas such as road sides and forest margins with high soil moisture (Feng et al. 2007; Tripathi et al. 2012), open forest patches formed by local people aid *A. adenophora* invasion. Even though *A. adenophora* can establish under high tree canopy cover (pers. obs.), we propose that maintaining native tree canopy cover can become an economically feasible semi-natural method to reduce and delay *A. adenophora* invasion in forest habitats.

Ecologically the most interesting hypothesis in our study was the third hypothesis where we separated the airborne and the belowground interference by *A. adenophora* in laboratory conditions. As expected, the presence of leaf litter in the growth chambers inhibited both germination and seedling growth of *S. wallichii* (Fig. 2-3). While Inderjit et al. (2011) were the first to observe the airborne effect, the novelty of our finding is that never before the airborne inhibitory effect of *A. adenophora* leaf litter has been associated with a native dominant tree species. Inderjit et al. (2011) attributed the effect on a Eurasian ruderal plant and on a native bush that is not crucial in the regeneration of native forests. Unlike in the study by Inderjit et al. (2011), the chemical compounds emitted by the litter were not monitored by us and therefore it is not possible to underline the role of leaf litter volatiles as in Inderjit et al. (2011). However, the method used to test the effect of volatiles by Inderjit et al. (2011) was the same with the method used by us, except that the volume of our growth chambers was larger, and that Inderjit et al. (2011) did not write how they ventilated the chambers. For these reasons, we assume that the airborne effect of *A. adenophora* leaf litter is attributable to leaf litter volatiles in our study.

Two of the key findings of the current study are that *S. wallichii* root length and shoot and root biomass were lower in invaded soil, compared with the natural soil, and that the airborne leaf litter effect was weaker than soil effect when shoot and root biomass was analyzed (Fig. 3B-D, Table 2). This indicates that belowground effects can have a strong impact on the establishment of new *S. wallichii* seedlings. In addition, as shoot biomass was lower in invaded soil (Fig. 3C) but shoot length in both soil types was similar (Fig 3A), *S. wallichii* shoots became thinner or their leaf area decreased in invaded soil. Thus, our study indicates that *S. wallichii* germination is mainly inhibited by the airborne effect of *A. adenophora* leaf litter, while *S. wallichii* seedling growth is slowed down in invaded soil no matter if *A. adenophora* leaf litter is present or not. Based on the laboratory results, we speculate that if *A. adenophora* leaf litter and thus the volatiles were removed from invaded habitats, *S. wallichii* seeds would germinate faster and develop healthy shoot and root system which might increase the survival of *S. wallichii* seedlings and contribute to forest regeneration in *Schima-Alnus* forests. Importantly, as litter accumulation was found in dense stands of *A. adenophora* (pers. obs.), removal of decaying litter together with the plants themselves may be a good option to control *A. adenophora* in invaded patches in the natural forests (Hansi et al. 2014).

In our study, basic soil characteristics could not be used to explain differences in plant growth or community composition. In an earlier study, Yu et al. (2005) analyzed pH, organic matter and total NPK in heavily invaded and slightly invaded soils by *A. adenophora* and reported slight differences in these parameters. However, they found decreased native plant biomass in the soil from heavily invaded sites in comparison to the soil from the slightly invaded sites. Analogous to this finding, our soil analysis did not show significant differences in soil nutrients (NPK) and total organic matter between *A. adenophora* invaded and non-invaded plots but the pH in invaded soil was lower than that in the soil sampled from non-invaded plots. Therefore, we cannot rule out the possibility that a low soil pH promotes *A. adenophora* invasion, even though it is unlikely that the whole native plant community suffers by changes in soil pH. Earlier studies have reported a correlation between altered pH and soil microbial communities, e.g. that bacteria are positively related to pH (Rousk et al. 2010, Yu et al. 2005). Our results do not exclude the possibility that a change in soil microbial communities (i.e. negative soil feedback) contributes to the reduced native species richness as well as the inhibitory effects on *S. wallichii* seed germination and seedling growth. Despite the exact mechanism, our study indicates that *S. wallichii* germination delayed mainly by the airborne effect of *A. adenophora* leaf litter. At the same time, however, belowground effect of *A. adenophora* invaded soil contributes to the reduction of biomass of *S. wallichii* seedlings. Importantly, the diversity analyses performed in the field are in accordance with the results of the laboratory findings. We conclude that our study is the first to show that belowground and airborne effects act simultaneously, which pushes forward the understanding of plant invasions.

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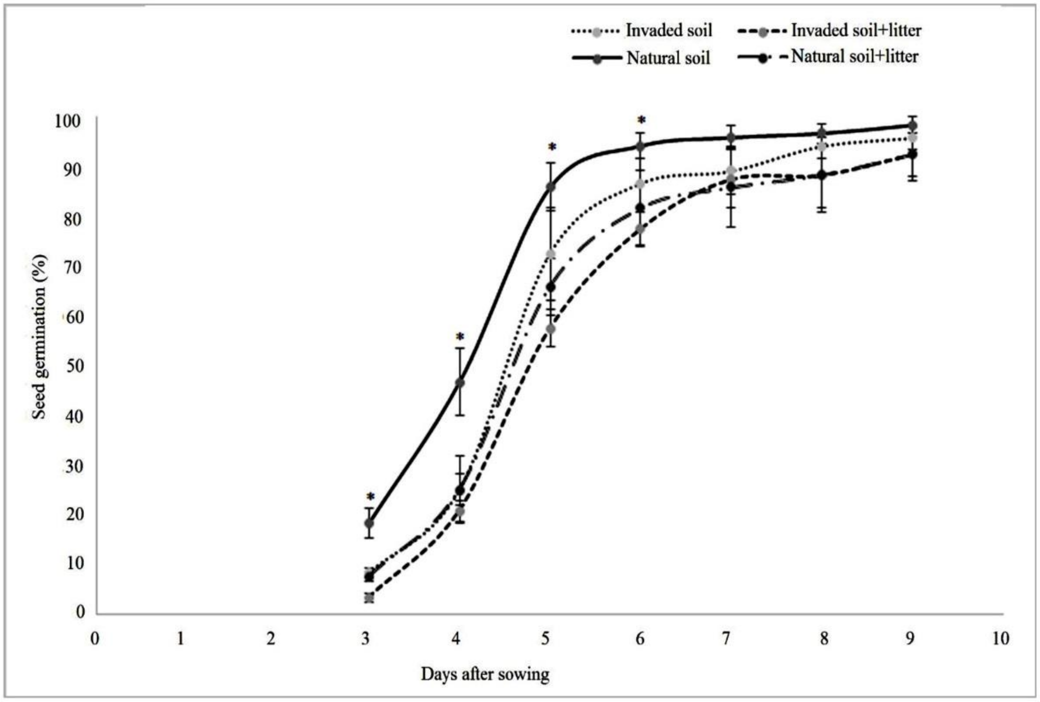


**Fig.** 1 *S. wallichii* seed germination in pots containing natural or *A. adenophora* invaded soil in the presence and absence of *A. adenophora* leaf litter. Data are means ± SE. * above the error bars indicate significant differences (*p≤0.05*) by two-way ANOVA analysis.

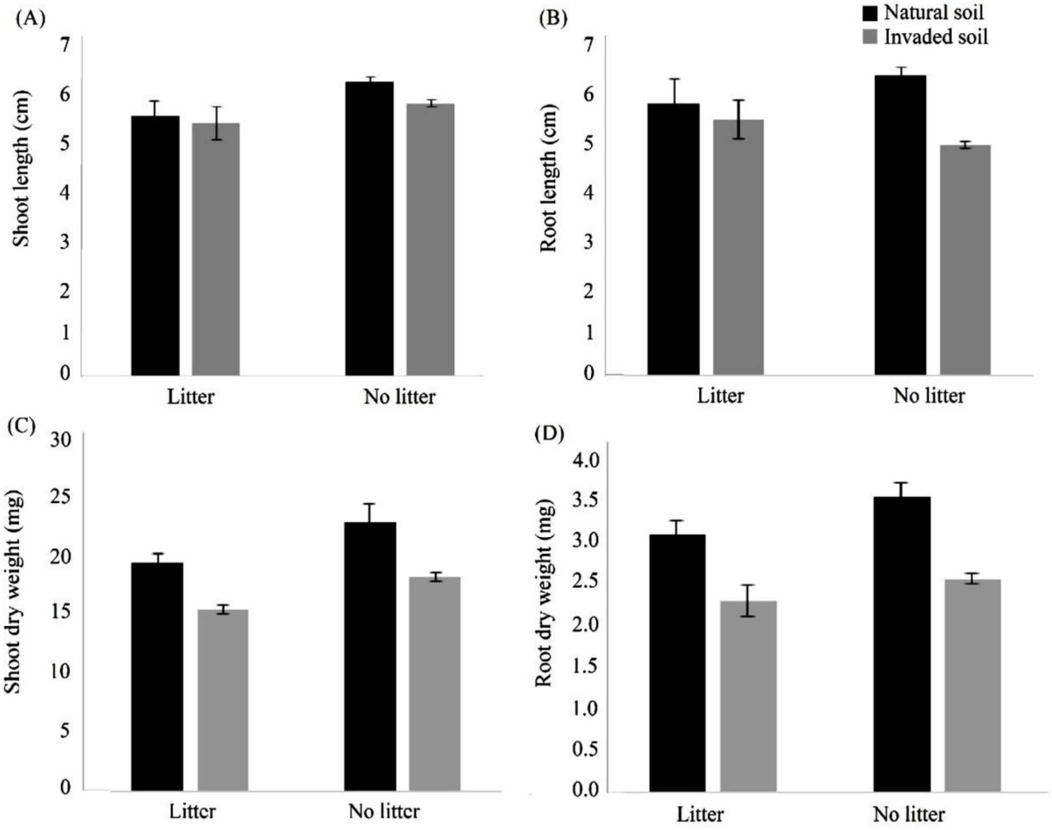


**Fig. 2** *S. wallichii* seedling growth in pots containing natural or *A. adenophora* invaded soil in the presence or absence of *A. adenophora* leaf litter: (A) Shoot length (B) Root length (C) Shoot dry weight and (D) Root dry weight. Data are means ± SE.

**Table 1** Two-way ANOVA results of the effect of soil, litter VOCs and their interaction on *S. wallichii* seed germination percentage (Detailed ANOVA statistics is given in Appendix I)

| **Factor** | **Days after sowing** | | | | | |
|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 | 8 |
| | ***p values*** | | | | | |
| **Soil** | <0.001*** | 0.028* | 0.107 | 0.263 | 0.665 | 0.813 |
| **Litter** | <0.001*** | 0.028* | 0.016* | 0.050* | 0.321 | 0.194 |
| **Soil × litter** | 0.254 | 0.115 | 0.695 | 0.743 | 0.474 | 0.813 |

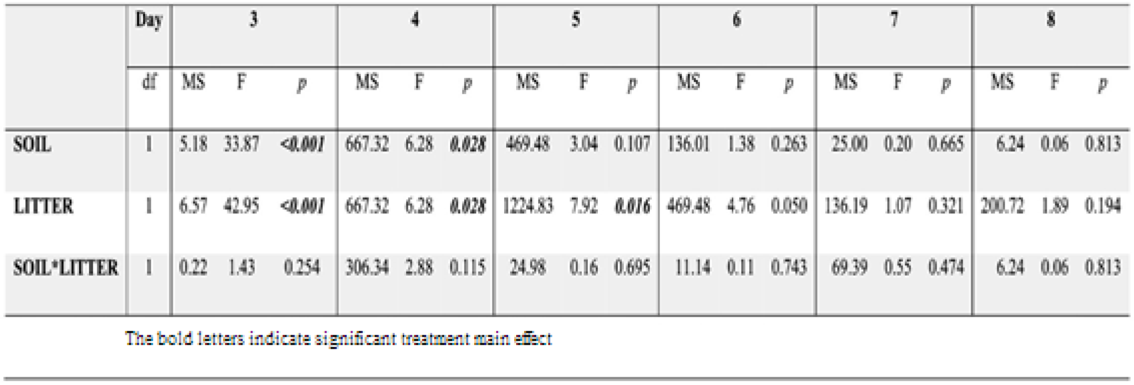
*, *** indicate significant differences at $p \leq 0.05$ and $0.001$, respectively.

**Table 2** Two-way ANOVA results of the effect of soil, litter and their interaction on *S. wallichii* shoot/root length and dry weight (Detailed ANOVA statistics is given in Appendix II)

| **Factor** | ***p values*** | | | |
|---|---|---|---|---|
| | **Shoot length** | **Root length** | **Shoot dry weight** | **Root dry weight** |
| **Soil** | 0.250 | 0.022* | < 0.001*** | < 0.001*** |
| **Litter** | 0.039* | 0.940 | 0.004** | 0.035* |
| **Soil × litter** | 0.540 | 0.130 | 0.650 | 0.560 |

*, **, *** indicate significant differences at $p \leq 0.05$, $0.01$ and $0.001$, respectively.

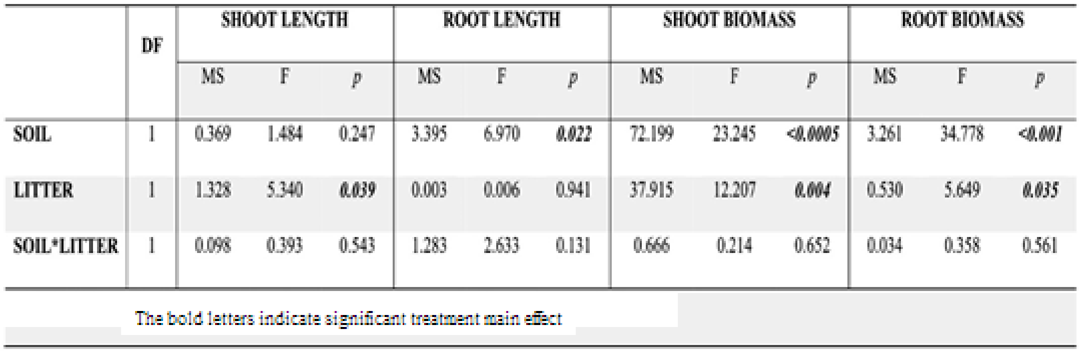
**SUPPLEMENTARY TABLE 1:** Two way ANOVA, Effect of soil, litter and soil*litter on *S. wallichii* seed germination (%)



| | Day | 3 | | | 4 | | | 5 | | | 6 | | | 7 | | | 8 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | MS | F | *p* | MS | F | *p* | MS | F | *p* | MS | F | *p* | MS | F | *p* | MS | F | *p* |
| **SOIL** | 1 | 5.18 | 33.87 | ***<0.001*** | 667.32 | 6.28 | ***0.028*** | 469.48 | 3.04 | 0.107 | 136.01 | 1.38 | 0.263 | 25.00 | 0.20 | 0.665 | 6.24 | 0.06 | 0.813 |
| **LITTER** | 1 | 6.57 | 42.95 | ***<0.001*** | 667.32 | 6.28 | ***0.028*** | 1224.83 | 7.92 | ***0.016*** | 469.48 | 4.76 | 0.050 | 136.19 | 1.07 | 0.321 | 200.72 | 1.89 | 0.194 |
| **SOIL*LITTER** | 1 | 0.22 | 1.43 | 0.254 | 306.34 | 2.88 | 0.115 | 24.98 | 0.16 | 0.695 | 11.14 | 0.11 | 0.743 | 69.39 | 0.55 | 0.474 | 6.24 | 0.06 | 0.813 |

The bold letters indicate significant treatment main effect

**SUPPLEMENTARY TABLE 2:** Two way ANOVA, Effect of soil, litter and soil*litter on *S. wallichii* shoot root length and biomass



| | DF | SHOOT LENGTH | | | ROOT LENGTH | | | SHOOT BIOMASS | | | ROOT BIOMASS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | F | *p* | MS | F | *p* | MS | F | *p* | MS | F | *p* |
| **SOIL** | 1 | 0.369 | 1.484 | 0.247 | 3.395 | 6.970 | ***0.022*** | 72.199 | 23.245 | ***<0.0005*** | 3.261 | 34.778 | ***<0.001*** |
| **LITTER** | 1 | 1.328 | 5.340 | ***0.039*** | 0.003 | 0.006 | 0.941 | 37.915 | 12.207 | ***0.004*** | 0.530 | 5.649 | ***0.035*** |
| **SOIL*LITTER** | 1 | 0.098 | 0.393 | 0.543 | 1.283 | 2.633 | 0.131 | 0.666 | 0.214 | 0.652 | 0.034 | 0.358 | 0.561 |

The bold letters indicate significant treatment main effect

# Paper V

**Thapa, L.B**., Kaewchumnong, K., Sinkkonen, A. and Sridith, K. “Soaked in rainwater” effect of *Ageratina adenophora* on seedling growth and development of native tree species in Nepal (Un-submitted Manuscript, 2016)

# “Soaked in rainwater” effect of *Ageratina adenophora* on seedling growth and development of native tree species in Nepal

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## Abstract

*Ageratina adenophora* is considered one of the most problematic invasive species spreading worldwide replacing native vegetation. There is hardly any information on its effect on native, dominant tree species. A more severe problem is that previous tests have typically used crushed or grinded leaves that may contain compounds that do not exist on soil surface. To avoid these drawbacks, we used entire leaves and two native trees (*Schima wallichii* and *Alnus nepalensis*) as target species. Our pot experiment was designed to simulate soaking of *A. adenophora* leaves in rainwater as it happens in nature at times of native seed germination in Nepal. Seedling growth (length and dry weight) of the target species was inhibited by *A. adenophora* fresh leaves and leaf extract. Inhibition was shown after seedling exposure and the effect remained permanent. In conclusion, *A. adenophora* leaves can suppress the growth of native tree species. This may create problems in the establishment and survival of native seedlings in invaded areas.

**Key words**: *Ageratina adenophora*, native species, invasion, leaf extract, seedling growth

## Introduction

Above ground parts of plants may release organic and inorganic metabolites in liquid form in rain, mist, dew and soil percolates (Tukey 1970; Molina et al. 1991). The leachate can be toxic to other plants, and they may alter soil microbial process (Molina et al. 1991; Reigosa et al. 1999; Bais et al. 2006). A donor plant that releases chemicals can produce positive or negative effects on other associated members. They may be connected to nutrient availability or toxin effect against other plant species. Both altered nutrient availability and toxin effects of invasive species on native species have been considered as important invasion mechanisms worldwide (Callaway and Ridenour 2004; Callaway et al. 2008; Ni et al. 2012; Del Fabbro et al. 2014).

*Ageratina adenophora* (Spreng.) R.M. King and H. Rob. (Syn. *Eupatorium adenophorum*), one of the worst invasive species, has created severe problems in native ecosystems (Wang and Wang 2006; Dogra et al. 2010). This species has invaded and created severe impacts on forest ecosystem in subtropical ranges of Nepal (Tiwari et al. 2005; Thapa et al. 2016). Earlier studies have indicated allelopathic inhibition of *A. adenophora* on crop plants and weeds (Ming-Zhi et al. 2011; Wan et al. 2011; Zhang et al. 2012; Kundu 2013; Bardwaj et al. 2014), native plant seedlings might also have been affected along the invaded patches but literature regarding the impacts of *A. adenophora* on native tree seedlings are scarce. Several putative allelochemicals expected to have negative impacts on native species are isolated and identified from *A. adenophora* (Yang et al. 2011; Zhang et al. 2012; Zhang et al. 2013). However, the chemicals have normally been identified from plant tissues or extracts made from crushed plant parts, which makes it difficult to link the observed toxin effect to an ecologically significant phenomenon in nature. Therefore, a study that investigates the inhibitory effect of extracts made from entire, non-crushed leaves is crucial to mimic the situation happened in nature.

*Schima wallichii* (DC.) Korth. and *Alnus nepalensis* D.Don. are two native trees distributed along the tropical to subtropical vegetation in central Nepal (Dobremez and Shakya 1975; Ohsawa et al. 1986). *A. nepalensis* is a deciduous tree growing commonly along streams, degraded or landslide areas at altitudinal range from 500 to 3000 m above sea level (Orwa et al. 2009). Seeds of *A. nepalensis* are small and winged produced in large number in female cone which are dispersed by wind. *S. wallichii* is another important evergreen tree growing in a wide range of climates, habitats and soil types from 900 to 2000 m above sea level (Orwa et al. 2009). Its seeds are winged, light and are dispersed by wind when capsules rupture. In case of both species, sexual reproduction is frequent and germination often starts so that the seeds soak in small puddles. The plant communities dominated by these natives are severely invaded by *A. adenophora* in lower montane vegetation of central Nepal (Tiwari et al. 2005; Thapa et al. 2016). *A. adenophora* invasion in *Schima-Alnus* plant communities has raised questions about its effect on native species in these plant communities.

The objective of the current study is to fill the gap between field observations and previous laboratory tests where leaves were crushed or grinded before making an extract for germination and seedling growth experiments. As *A. adenophora* fresh leaves have a potential to produce toxic substances, we used extracts made of entire, non-crushed fresh leaves of *A. adenophora*. As *S. wallichii* and *A. nepalensis* are dominant native trees in central Nepal, we selected them as target species to measure the effect of *A. adenophora*. We hypothesized that our "soaked in rainwater" extract inhibits early seedling growth of these two selected native tree species. For ecological relevancy, our tests included a treatment where non-soaked leaves of *A. adenophora* were used on soil surface, and an *A. adenophora* root extract treatment. We also hypothesized that also these treatments inhibit early growth of the selected target species.

## Methods

### *Sample collection*

Natural soil, seeds of native species, leaves and roots of *A. adenophora* were collected from *Alnus-Schima* forest at Champadevi hill forest, Kathmandu, Central Nepal (27˚42′ N and 85˚19′ E). Seeds were collected in March/April, 2014 and the soil was collected in June 2014. The collected seeds were air dried and stored at 5˚C in plastic bags until use. Leaves and roots of *A. adenophora* were freshly collected before preparation of extract each time.

*Seedling and extract preparation*

Seeds of both native species were soaked in water and allowed to germinate on moist double layered filter paper in a tray. The tray was covered with a lid and left at room temperature (26±2˚C). After germination, 30 mm long seedlings of *S. wallichii* and 10 mm long seedlings of *A. nepalensis* were transferred to experimental pots containing natural soil collected as a composite sample from *Schima-Alnus* forest.

Extracts were prepared in a way that resembles soaking in rainwater in the field during the seedling growing season of the native plant species. Freshly 10 grams each of collected leaves and roots of *A. adenophora* were soaked in 100 ml rain water at room temperature for 24 h without crushing or drying. Plant parts were removed and the liquids were used immediately. For the fresh leaf treatment, leaves were not soaked but collected just before use.

*Effect of leaf/root extract and fresh leaves on native seedling growth*

Pot experiment was conducted in a greenhouse of Central Department of Botany, Tribhuvan University, Kirtipur, Nepal from July to September - 2014. Seedlings of native species were transferred to polythene pots (8 cm diameter and 10 cm height) with holes at bottom containing 450 g of natural soil. Three seedlings were grown in each pot and 4 treatments were made: (i) Normal water (control) (ii) Leaf extract (iii) Root extract and (iv) Fresh leaves.

Pots for the fresh leaf treatment contained 1 cm thick layer of fresh leaves on the surface which was changed every second day. Twenty ml of normal rain water was poured to the control pot and fresh leaf pots. Equal volume (20 ml) of fresh leaf or root extract was added in the pots. Temperature in the greenhouse varied between from 21-38˚C. Normal day light duration was approximately 12 h and moisture content in the greenhouse varied between 52- 85%. The duration of the experiment was 48 days for *S. wallichi* and 84 for *A. nepalensis*. Shoot length was measured weekly and final measurement was taken at the end of experiment. Root and shoot length was measured separately on the final day. Root and shoot were dried separately at 70˚C for 24 h in hot air oven and dry weight was measured.

## Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's significant difference (Tukey's HSD) tests. All statistical analyses were conducted using software SPSS (version 20). Statistical significance was determined at $p \leq 0.05$.

## Results

### *Shoot and root length*

Shoots of *S. wallichii* were shorter in treatment with *A. adenophora* leaf extract but roots were shorter in both leaf extract and fresh leaves treatments (Fig. 1). In *A. nepalensis,* both leaf extract and fresh leaves of *A. adenophora* inhibited shoot length while there was no effect on root. Root extract did not show inhibition to shoot and root length of both native species.

### *Shoot and root dry weight*

*A. adenophora* leaf extract and fresh leaves inhibited shoot dry weight in both native species while the root extract did not affect shoot dry weight (Fig. 2a). In *S. wallichii* roots were lighter by leaf extract, root extract and fresh leaves of *A. adenophora*. In *A. nepalensis* root extract of *A. adenophora* did not inhibit root dry weight while the weight was reduced by both leaf extract and fresh leaves (Fig 2b).

### *Weekly growth pattern*

The inhibitory effect of *A. adenophora* leaf extract on shoot length of *S. wallichii* was pronounced from the third week of seedling transfer and the effect was visible until the end of the experiment. Other treatments did not show any inhibition to *S. wallichii* shoot length throughout the development except in the third week there was inhibition of root extract (Fig. 3a). Similarly, the shoot elongation pattern in *A. nepalensis* showed inhibition by fresh leaves and leaf extract from the fourth week of seedling transfer. Inhibition was more pronounced at later weeks (Fig. 3b). The root extract did not show any effect on shoot elongation. There was continuous shoot elongation in the control treatment for both target species.

## Discussion

Our results show that fresh leaves and leaf extract of *A. adenophora* are inhibit seedling growth of both test species more than root extracts (Fig. 1, 2 and 3). The inhibitory effect on seedling growth of both native species after the third week of seedling transfer demonstrates that the seedlings are affected soon after the exposure starts. As natural soil was used in the experiment, the results also indicate that the natural soil gets contaminated by chemicals in the extracts of *A. adenophora*, or that the chemicals change soil processes and properties, such as microbial activity and nutrient availability. Weekly growth pattern (Fig. 3) indicates that the inhibition remains permanent during the whole seedling development. Noticeably, the pattern of inhibition is different in roots and shoot growth depending on native species and extract origin (Fig. 1 and 2). Comparatively, *A. nepalensis* roots showed resistance to the effect of fresh leaves and leaf extract of *A. adenophora* in terms of their length while their dry weight was reduced compared with other treatments (Fig. 1b and Fig 2b). Except root dry weight, *S. wallichii* shoot length, shoot dry weight and root length were not affected by root extract. *A. adenophora* plant parts and can be severely affected by *A. adenophora* leachate in the field.

Normally plant parts which are selected for extract preparation are crushed freshly or dried plant materials are grinded to make powder from which water extracts are prepared to conduct extract experiments. The extract is also filtered and filtrate is applied to test species in several laboratory experiments. In our study, we avoided unnecessary and non-natural release of chemicals from crushed tissues. We just soaked fresh leaves or roots of *A. adenophora* to imitate natural process as the rainwater soaks the plant parts in rainy days in nature. This design can be recommended in later studies. Our "soaked in rainwater" technique should be tested for possible harmful allelochemicals rather than using crushed parts or powder forms.

Using this "soaked in rainwater" technique, we allowed *S. wallichii* and *A. nepalensis* seedlings to grow in natural soil collected from a *Schima-Alnus* forest. As hypothesized, heavier plants were found with longer roots and shoots in control pots. As weaker plants having reduced growth of root and shoot and root were found in *A. adenophora* leaf extract and fresh leaves, our results demonstrate altered quality of natural soil in the presence of liquid from soaked *A. adenophora* material. The result supports the hypothesis that natural soil contamination may occur through leaching of chemicals from *A. adenophora* leaves with rain water. It further suggests that the growing seedlings of native trees absorb harmful chemicals leached from *A. adenophora* through contaminated soil directly or the chemicals may alter soil nutrients or soil microbial communities that could play an important role to bring changes in seedling growth (Mangla and Callaway 2008; Richardson and Simpson 2011; Xu et al. 2012; van der Putten et al. 2013).

Leaf extract of *A. adenophora* has earlier been found toxic to certain crop plants, e.g. barley, maize, rice (Ming-Zhi et al. 2011; Wan et al. 2011) and some weeds such as *Amaranthus retroflexus* (Ma et al. 2012). Various allelochemicals have been isolated from *A. adenophora* aerial parts such as quinic acid derivatives, 5-*O-trans-o*-coumaroylquinic acid methyl ester together with chlorogenic acid methyl ester, macranthoin F and macranthoin G (Zhang et al. 2013); α-phellandrene, camphene, ρ-cymene, 2-carene, α-pinene, limonene, and (z)-3-hexen-1-ol (Zhang et al. 2012) and monoterpenes such as ($1\alpha$,$6\alpha$,$7\alpha$)-8-hydroxy-2-carene-10-oic acid and ($1\alpha$,$6\alpha$)-10-hydroxy-3-carene-2-one (Xu et al. 2014).

In the field, *A. adenophora* is found evergreen throughout the year. Phenological coincidence in the field is that the mature seeds of both *A. nepalensis* and *S. wallichii* start germination with the initiation of pre-monsoon and at the same time *A. adenophora* growth is found luxuriant with new leaves. It can be expected that when native seeds produce seedlings in *A. adenophora* invaded sites they should be affected by water leachates from *A. adenophora*. This mechanism of *A. adenophora* may suppress the growth of native seedlings and the benefit is *A. adenophora* invade *Alnus-Schima* natural forests successfully. Both the selected native species grow on landslide area with varying climatic and soil conditions (Sharma et al. 1998; Orwa et al. 2009). The habitats of these two native species are similar to the areas where *A. adenophora* prefers to grow. This similarity of habitat between native and invasive species indicates that native species can suffer from *A. adenophora* impacts.

Furthermore, *A. nepalensis* hosts nitrogen fixing bacteria (*Frankia*) that have an ability to develop endosymbiotic relationship with this host plant forming root nodules (Chaia et al. 2010; Santi et al. 2013). *Frankia* infects host roots through root hairs and in response of its penetration pre-nodules are formed by mitotic cell division in cortical zone near infected root hairs during the process of nodulation (Santi et al. 2013). Beside inhibition of *A. nepalensis* growth by *A. adenophora*, the fresh leaves of *A. adenophora* might also alter either *Frankia* population in the soil or nodule producing mechanisms. It is reported that *A. adenophora* reduces population of certain rhizospheric microflora (Dutta and Deb 1986). The current findings would encourage the study of *Frankia* population in *A. adenophora* invaded and non-invaded sites as well as the interaction of *A. adenophora* allelochemicals with nodule physiology of *A. nepalensis*.

In conclusion, natural soil contaminated by *A. adenophora* leaves has an inhibitory effect on the normal growth of seedlings of native trees (*S. wallichii* and *A. nepalensis*). As both native trees and invasive *A. adenophora* prefer similar habitats, the growth and even survival of the native tree seedlings may be problematic. Further investigation on identifying allelochemicals from non-crushed extracts of *A. adenophora* is recommended. Studies about impacts of such allelochemicals on native seedling growth, soil microbial activities and *A. nepalensis* root nodule physiology can help in understanding further details of *A. adenophora* impacts on native flora.

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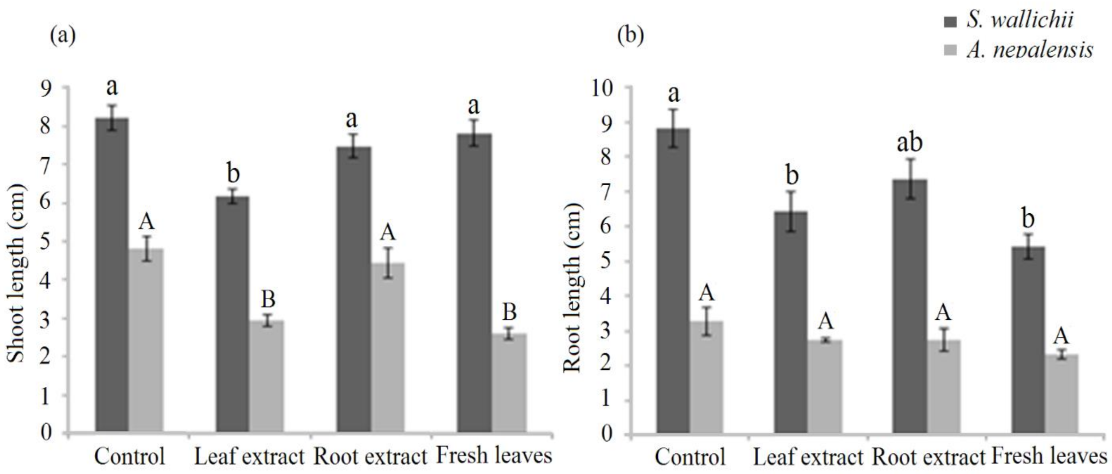

Fig. 1 Effect of *A. adenophora* on shoot (a) and root (b) length of *S. wallichii* and *A. nepalensis*. Error bars represent standard errors of means. Letters above the error bars indicate significant differences.

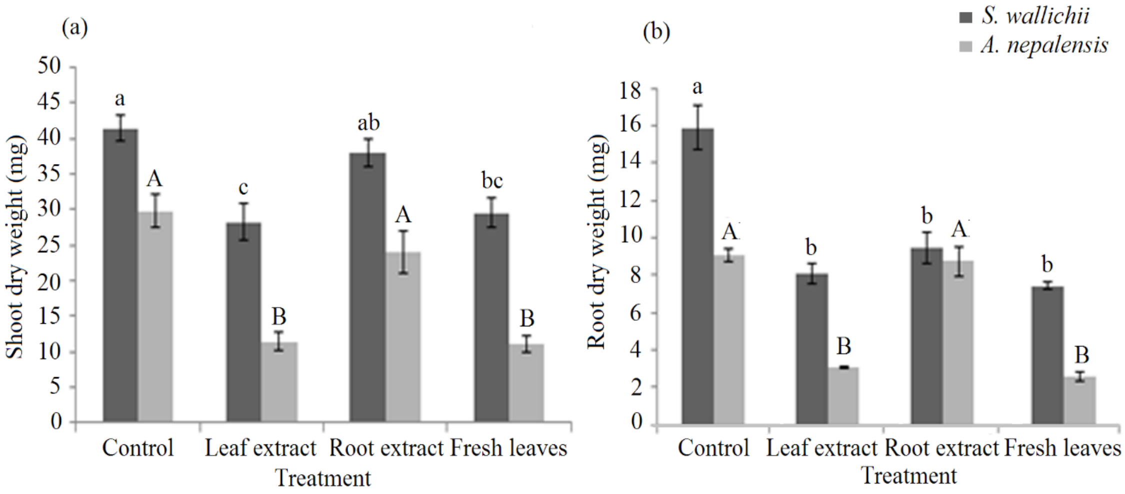

Fig. 2 Effect of *A. adenophora* on shoot (a) and root (b) dry weight of *S. wallichii* and *A. nepalensis*. Error bars represent standard errors of means. Letters above the error bars indicate significant differences.

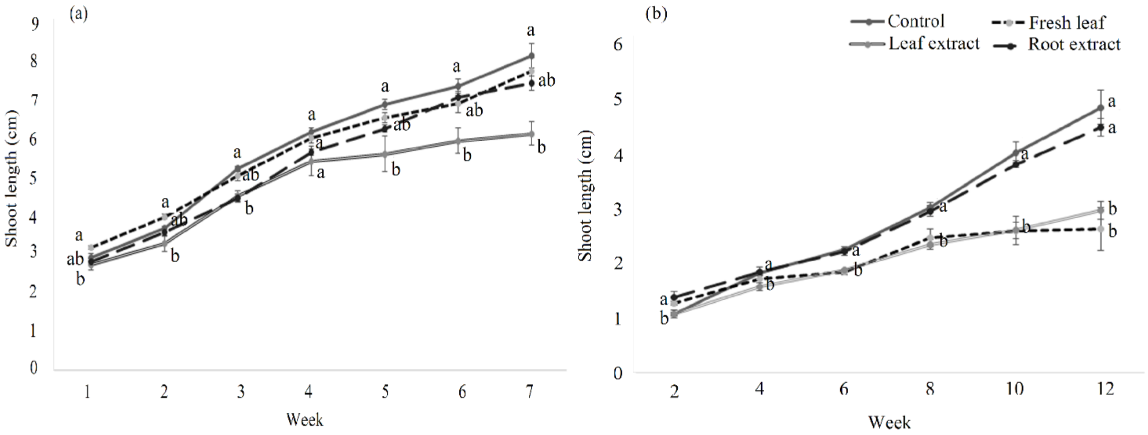

**Fig. 3** Weekly growth pattern of shoot length of *S. wallichii* (a) and *A. nepalensis* (b). Letters above the error bars indicate significant differences.

**Supplementary table 1**

One-way ANOVA test statistics: effects of *A. adenophora* treatments on *S. wallichii* and *A. nepalensis* seedling growth.

| Species | df | Shoot length | | | Shoot dry weight | | | Root length | | | Root dry weight | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | *p*-value | MS | *F* | *p*-value | MS | *F* | *p*-value | MS | *F* | *p*-value |
| ***S. wallichii*** | 3 | 3.85 | 9.06 | 0.001*** | 203.83 | 9.18 | 0.001*** | 10.58 | 7.98 | 0.002** | 75.50 | 24.97 | 0.000*** |
| ***A. nepalensis*** | 3 | 5.90 | 15.78 | 0.000*** | 432.31 | 19.41 | 0.000*** | 0.74 | 2.11 | 0.139 | 62.14 | 61.55 | 0.000*** |

*, **, *** indicate significant treatment main effects at p≤0.05, p≤0.01 and p≤0.001, respectively

**Supplementary table 2**

One-way ANOVA test statistics: effects of *A. adenophora* treatments on *S. wallichii* and *A. nepalensis* weekly growth of shoot length

| Week | df | ***S. wallichii*** | | | Week | df | ***A. nepalensis*** | | |
|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | *p*-value | | | MS | *F* | *p*-value |
| **1** | 3 | 0.194 | 5.36 | 0.010* | **2** | 3 | 0.107 | 4.383 | 0.020* |
| **2** | | 0.615 | 6.08 | 0.006* | **4** | | 0.076 | 3.749 | 0.033* |
| **3** | | 0.489 | 3.27 | 0.049* | **6** | | 0.244 | 19.159 | 0.000*** |
| **4** | | 0.734 | 3.14 | 0.050* | **8** | | 0.571 | 9.185 | 0.001** |
| **5** | | 1.350 | 4.15 | 0.024* | **10** | | 2.898 | 18.598 | 0.000*** |
| **6** | | 1.889 | 7.22 | 0.003** | **12** | | 5.909 | 15.818 | 0.000*** |

*, **, *** indicate significant treatment main effects at p≤0.05, p≤0.01 and p≤0.001, respectively

# VITAE

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2. **Thapa, L. B.,** Kaewchumnong, K., Sinkkonen, A. and Sridith, K. 2016. Plant Communities and *Ageratina adenophora* Invasion in Lower Montane Vegetation, Central Nepal. International Journal of Ecology & Development 31 (2): 35-49

3. **Thapa, L. B.**, Thapa, H. and Gharti Magar, B. 2015. Perception, trends and impacts of climate change in Kailali district, Far West Nepal. International Journal of Environment 4 (4): 62-76.

4. **Thapa, L. B.,** Dhakal, T. M. and Chaudhary, R. N. 2014. Wild edible plants used by endangered and indigenous Raji tribe in western Nepal. International Journal of Applied Science and Biotechnology 2 (3): 243-252. doi: : 10.3126/ijasbt.v2i3.10969.

5. **Thapa, L. B.**, Dhakal, T. M., Chaudhary, R. N. and Thapa, H. 2013. Medicinal plants used by Raji ethnic tribe of Nepal in treatment of gastrointestinal disorders; Our Nature 11 (2): 177-186. doi: 10.3126/on.v11i2.9645.

6. **Thapa, L. B.** 2012. Indigenous knowledge on common medicinal plants among Raji community of Surkhet district, mid-western Nepal. Nepalese Journal of Biosciences 2: 88-92. doi: 10.3126/njbs.v2i0.7494.

7. **Thapa, L.B.** and Prasad B.N. (2001). Effect of GA3 and ABT – 6 on seed germination and seedling growth in *Nardostachys grandiflora* DC and its ethnomedicinal uses. Ecoprint 8: 99-101.