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EFFECT OF INVASIVE *Ageratina adenophora* ON SPECIES RICHNESS AND COMPOSITION OF SAPROTROPHIC AND PATHOGENIC SOIL FUNGI

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ABSTRACT

Belowground modification of soil microbial community by invasive plants is well evident. Similar instances of *Ageratina adenophora* invasion have been reported. This study was aimed to determine the effect of *A. adenophora* invasion on species richness, species or community composition and occurrence frequency of soil fungi. These parameters were analyzed using culture method on invaded and uninvaded soils. Species richness of soil fungi was lower in the *A. adenophora* invaded soil compared to the uninvaded soil. The occurrence frequency of particular fungi was different for those two soil conditions. *A. adenophora* also altered soil fungi species composition in the invaded soil by replacing saprophytic fungi and accumulating pathogenic fungi. Thus, *A. adenophora* is associated to lower species richness of saprophytic soil fungi and high occurrence frequency of pathogenic soil fungi. This study concluded that the invasive *A. adenophora* modifies belowground soil fungi communities as one of the mechanisms involved in the successful invasion of *A. adenophora*.

Keywords: Belowground modification, soil fungi, species composition, species richness

INTRODUCTION

Uncoupling of ecosystem dynamics and consequent threat to biodiversity loss are attributed to invasion of Alien Plants (IAPs) (Webster *et al.* 2006; Boy & Witt 2013). The Invasive Alien Species have adopted multiple strategies to proliferate (Holzmueller & Jose 2009) in an introduced range and able to compete with native plants for resources and space (Mack & D' Antonio 2003). Various experimental evidences showed that modification in soil biota is one of strategies involved in the invasion of IAPs (Xiao *et al.* 2014a).

The IAPs release myriads of chemicals from their above and belowground parts into soils by leaching process and exudation (Dayakar *et al.* 2009). These chemicals are capable of altering soil biophysical properties and ultimately, plant diversity (Garbeva 2008; Doornbos 2012; Thapa *et al.* 2016a). In response to this, aboveground biota sharing the same niche with the IAPs invariably regulates the changes (Darrah 1993) by applying a feedback system (Van der Putten *et al.* 1993).

Soil microbial community has either negative or positive feedback to the plants (Bonanomi *et al.* 2005). For example, negative feedback may involve pathogenic effect, production of bioactive compounds and production of allelochemicals, while positive feedback involves root fungus mutualism and secretion of plant growth promoting substances (Bias *et al.* 2006). On the other hand, regulation in nutrient cycle is a result from plant-soil feedback system (Johnson *et al.* 1997). Such a plant-soil feedback system determines diversity and relative abundance of the above and belowground organisms (Van der Putten *et al.* 1993).

Plant soil microbe studies in IAPs shows that they can modify soil microbial communities (Kourtev *et al.* 2002; Van der Putten *et al.* 2007) for successful invasion (Reinhart & Callaway 2004; Li *et al.* 2006; Boudiaf *et al.* 2013). Negative feedback mechanisms due to IAPs may result to the decrease in competitive abilities of native species (Ehrenfeld *et al.* 2005). The negative feedbacks are also recognized in biomass and growth of native

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plants (Stinson *et al.* 2006), which might be host-specific, leading to a decline of native plants population (Didham *et al.* 2007).

Ageratina adenophora (Spreng.) R. M. King and H. Robinson, an invasive alien plant of Asteraceae family, is a perennial, erect or decumbent subshrub (Tiwari *et al.* 2005), natively found in Mexico and Costa Rica and spread worldwide (Niu *et al.* 2007; Xue *et al.* 2010; Inderjit *et al.* 2011). This species contains an array of bioactive constituents such as terpenoids, flavonoids, phenyl propanoids and their derivatives (Kundu *et al.* 2013).

Several mechanisms have been suggested as the reasons of successful invasion of IAPs including *A. adenophora* in their novel range. Most studies on IAPs focused on aboveground vegetation changes (Levine *et al.* 2003). A wide range of belowground biotic interactions can play important role in determining plant interactions and ecosystem function (Callaway *et al.* 2001). Therefore, it is important to understand the interactions between IAPs and soil biotic community and their interactions (Wolfe & Klironomos 2005). Moreover, an integrated understanding of how aboveground and belowground biota interact with IAPs is necessary to manage and restore alien invaded native communities (Wolfe & Klironomos 2005).

There are some studies on modifications in the belowground biotic community associated with *A. adenophora* invasion, for example Mangla and Callaway (2008) and Niu *et al.* (2007). In Nepal, severe invasion of *A. adenophora* occurred from tropical to subtropical regions affecting native *Schima-Alnus* and other types of vegetation (Tiwari *et al.* 2005; Thapa *et al.* 2015; 2016b; 2017). Hence, this study hypothesized that *A. adenophora* is responsible for changing species richness, species or community composition and occurrence frequency of the native soil fungi as invasion mechanisms of *A. adenophora* in Nepalese forests.

MATERIALS AND METHODS

The experiment was designed for comparing species richness of soil fungi, species composition and occurrence frequency between *A. adenophora* invaded and uninvaded soil. The invaded soil consisted of invaded non-rhizosphere soil and rhizosphere-contained invaded soil. The experiments were conducted at the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal in March and August 2014.

Sampling Site and Method of Soil Sampling

Soil samples were collected from Champadevi Community Forest located at southwest of Kathmandu valley, Nepal. Altitude of the location varied from 1,400 – 2,300 m asl with annual mean temperature of 18 °C and annual precipitation of 1,343 mm. *Schima wallichii*, *Alnus nepalensis*, *Myrsine capitelata*, *Maesa chisia*, *Castanopsis indica* and *Quercus* sp. were common native trees in the forest. The forest was invaded by *A. adenophora* for 40 - 50 years and the invasion has become a serious challenge for the native diversity of the area due to its severe colonization (Thapa *et al.* 2016b).

Uninvaded soil was collected from the forest site located at 27°42′06″ N and 85°19′14″ E with altitude of 1,600 m. Rhizosphere-contained invaded soil was collected by uprooting *A. adenophora*. A total of 30 plants were uprooted from three sampling sites of the invaded patches nearby the uninvaded site, 10 plants from each sampling site (distance between each sampling site was at least 50 m). The soil around root surfaces was collected in sterile plastic bags and a composite sample was prepared. Invaded non-rhizosphere and uninvaded soil samples were collected from 10 random points at the invaded and uninvaded patches of the forest. Soil samples were collected from soil depth of 10 cm below soil surface. Composite sample of each invaded non-rhizosphere and uninvaded soil was prepared. Freshly collected soils were sieved separately through sterile mesh (2 mm size), and stored in a refrigerator at 4 °C for 5 days, until use.

Fungi Culture, Isolation and Identification

Serial dilution method (Benson 2002) followed by pour plate technique were adopted for culturing and isolating fungi from all types of composite soil samples. Dilutions of 10^{-3} and 10^{-5} were used for plating (Aneja 2003). Czapek Dox Agar [with 30 mg/L (Amoxicillin)] and Potato Dextrose Agar were used for culture, isolation and pure culture. The culture plates were incubated at 25±5 °C (Gallenkamp Economy incubator size 1) for 15 days. There were 90 replication plates for each soil sample, where the presence or absence (1/0) of particular fungi was recorded in each replication plates. Fungi grown in the plates were observed at day 3, 7 and 15 and identified based on standard literature (Barnett & Hunter 1960; Gilman 1975; Watanabe 2010). A data matrix of species was prepared

Statistical Analysis

Species richness of soil fungi in different soil samples was compared using One-way Analysis of Variance (ANOVA). Frequency rank curve was used to compare the occurrence frequency of fungi found in different soil samples. Species composition of soil fungi was analyzed using Non-metric Multidimensional (NMDS) technique. The analyses were carried out using R software (version 2.15.3) (R Core Team 2015). The acceptable significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Species Richness

A total of 34 soil fungi species were found in soil samples (Table 1). Twenty-nine four species were reported from division Ascomycota followed by three species from class Zygomycota, one species from Basidiomycota and one Actinomycetes.

Species richness of soil fungi in the uninvaded soil was greater (28 species) than that in the invaded soil ($p < 0.05$). Twenty soil fungi species were recorded in the rhizosphere-contained invaded soil. Twenty two soil fungi species were recorded in the invaded non-rhizosphere soil (Table 1). These results indicated that *A. adenophora* reduced species richness of soil fungi. These findings also clarified the interactions between soil fungi and invasion of *A. adenophora*, which was still contradictory (Mangla & Callaway 2008).

Czapek Dox Agar medium was used to culture and isolate soil fungi targeting saprophytic or pathogenic soil fungi, as this media was proven to be appropriate for culturing and isolating common saprophytic and pathogenic fungi (Abildgren *et al.* 1987). This study proved that soil fungi enumeration favored Czapek Dox Agar medium.

Other studies showed that *A. adenophora* increased the abundance of mycorrhizal soil fungi which suggested that mycorrhizal

Table 1 Total numbers of soil fungi species found in different soil type

Soil type	Total species
Uninvaded soil	28[a]
A. adenophora invaded non-rhizosphere soil	22[b]
A. adenophora invaded rhizosphere soil	20[b]

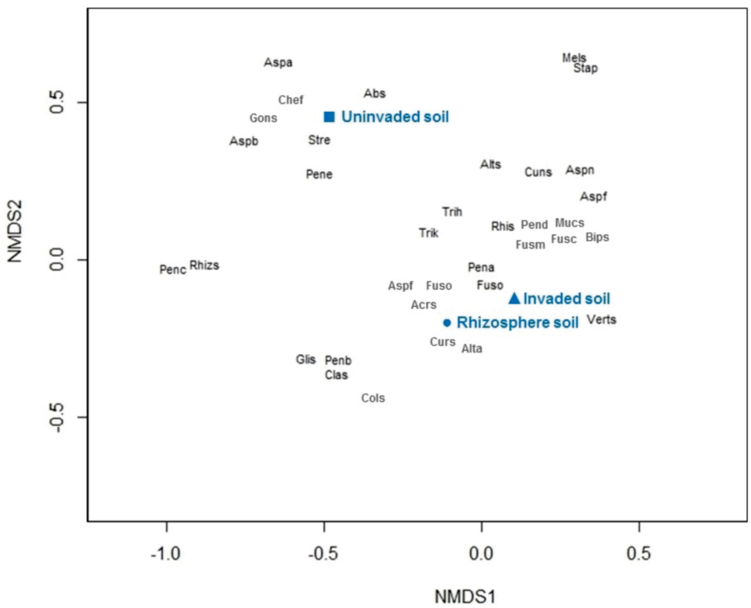


Figure 1 Species composition of soil fungi in the invaded soil, rhizosphere-contained invaded soil and uninvaded soil based on NMDS analysis (Final stress = 0.0002163944, K = 2, distance measure = "Jaccard" distance, trymax = 1,000)

Table 2 Soil fungi species and respective species codes depicted on NMDS space in Figure 1

SN	Fungi name	Abbreviation
1	*Absidia* sp.	Abs
2	*Acremonium* sp.	Acrs
3	*Alternaria alternata*	Alta
4	*Alternaria* sp.	Alts
5	*Aspergillus a*	Aspa
6	*Aspergillus b*	Aspb
7	*Aspergillus fumigates*	Aspf
8	*Aspergillus niger*	Aspn
9	*Aspergillus fumigates*	Aspf
10	*Bipolaris* sp.	Bips
11	*Curvularia* sp.	Curs
12	*Cladosporium* sp.	Clas
13	*Cunninghamella* sp.	Cuns
14	*Chaetomium funicola*	Chaf
15	*Colletotrichum* sp.	Cols
16	*Fusarium oxysporum*	Fuso
17	*Fusarium moniliforme*	Fusm
18	*Fusarium ciliatum*	Fusc
19	*Gliocladium* sp.	Glis
20	*Gonytrichum* sp.	Gons
21	*Melanospora* sp.	Mels
22	*Mucor* sp.	Mucs
23	*Penicillum a*	Pena
24	*Penicillium b*	Penb
25	*Penicillium c*	Penc
26	*Penicillium d*	Pend
27	*Penicillium e*	Pene
28	*Rhizophus* sp.	Rhis
29	*Rhizoctonia* sp.	Rhizs
30	*Staphylotrichum* sp.	Stap
31	*Streptomyces* sp.	Stre
32	*Trichoderma harzianum*	Trih
33	*Trichoderma koningii*	Trik
34	*Verticillium* sp.	Verts

Note: a, b, c, d and e are arbitrarily given specific names for unidentified fungi (consis ting more than one species) to species level

colonization induced positive feedback to enhance the invasiveness of *A. adenophora* (Niu *et al.* 2007; Yu *et al.* 2011; and Xiao *et al.* 2014b). Comparing the results of those studies with the results of present study, it is suggested that *A. adenophora* can replace certain groups of soil fungi commonly found in the uninvaded soil.

Analysis of soil fungi species composition (Fig. 1; Table 2) and frequency ranking curve (Fig. 3) showed the response of soil fungi species toward rhizosphere-contained soil, invaded soil and uninvaded soil.

Species Composition and Occurrence Frequency

NMDS analysis showed that species composition of soil fungi varied in accordance with soil types. Species compositions recorded in the invaded non-rhizosphere soil and the rhizosphere-contained invaded soil were different from that in the uninvaded soil. Soil fungi species such as *Curvularia* sp., *Alternaria alternata, Colletotrichum* sp., *Acremonium* sp., *Aspergillus fumigates, Verticillium* sp. and *Fusarium moniliforme* occurred more frequently in the rhizosphere-contained invaded and the invaded non-rhizosphere soils. Other soil fungi species such as *Absidia* sp. and *Chaetomium funicola* were exclusively occurred in the uninvaded soil (Fig. 1 & 2).

Species belonging to genera *Alternaria, Colletotrichum, Fusarium, Verticillium, Acremonium* and *Curvularia* were contributors in species composition of soil fungi in the invaded soil, whereas the species belonging to genera *Aspergillus, Penicillium, Absidia, Chaetomium* were major contributors in forming species composition of soil fungi in the uninvaded soil (Fig. 1 & 3). These results supported hypothesis of soil biota alteration as proposed by various researchers (Wolfe & Klironomos 2005; Si *et al.* 2013).

Goodness of fit for environmental factors (represented by soil types) in Figure 1 was significant in ordination space ($R^2 = 0.29, p < 0.01$). Permutational ANOVA (PERMANOVA) showed that species composition of soil fungi significantly varied in accordance with soil types ($p < 0.05$).

NMDS analysis showed that species composition of soil fungi was changed by the invasion of *A. adenophora* (Fig. 1). Alteration of species composition could be caused by differences in species richness in the invaded and uninvaded soils. Species composition of soil fungi in the rhizosphere-contained invaded soil and invaded non-rhizosphere soil was not different (Fig. 1). Species richness of soil fungi in those two soil types was similar (Table 1).

Frequency rank analysis showed that *A. alternata, Penicillium* sp.*, Curvularia* and *Fusarium oxysporum* were the frequently occurred soil fungi in the rhizosphere-contained invaded soil (Fig. 3). Similarly, *F. oxysporum* frequently occurred in the invaded non-rhizosphere soil followed by *Curvualria* sp., *Penicillium* sp. and *A. alternata* (Fig. 3). *Rhizophus* sp. and *Cunninghamella* sp. were the least frequent in rhizosphere-contained soil. *Rhizoctonia* sp., *Rhizophus* sp., *Verticillium* sp. were the least occurred soil fungi species in the invaded non-rhizosphere soil.

Trichoderma harzianum was the most frequently found soil fungi in the uninvaded soil followed by *Penicillium* sp. and *T. knoningii* (Fig. 3). The least

Figure 2 Fungal plate of *Chaetomium funicola*

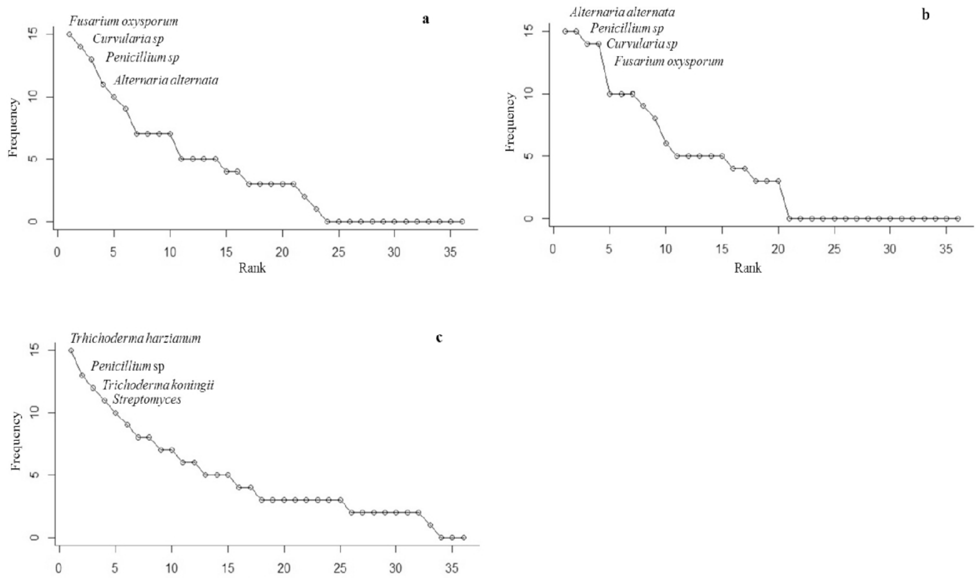
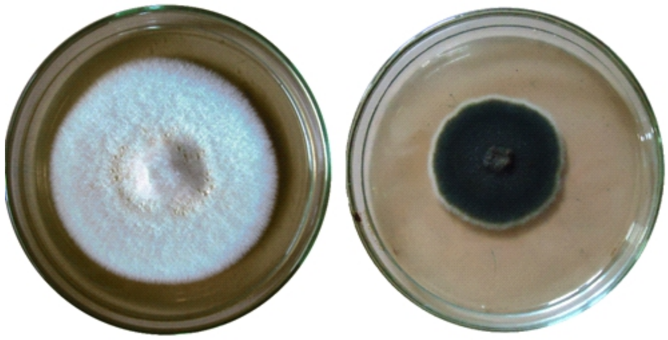


Figure 3 Frequency rank curve of (a) rhizosphere-contained invaded soil; (b) invaded non-rhizosphere soil; and (c) uninvaded soil



(a) (b)

Figure 4 Fungal plates of *Fusarium* (a) and *Alternaria* (b)

frequently found soil fungi species in the uninvaded soil were *Curvularia* sp., *Alternaria* sp., *Staphylotrichum* sp. and *Aspergillus* sp.

Occurrence frequency in this study showed a similar tendency of accumulation of pathogenic soil fungi in the invaded soil. NMDS and frequency analysis showed that *Fusarium, Colletotrichum* and *Alternaria* species were very frequently found in the invaded soil (Fig. 1, 3 & 4) and these genera are common pathogens (Chalermpongse 1987). Previous studies in warm tropical humid monsoonal climate of India also reported that *A. adenophora* and *Chromolaena odorata* accumulated pathogenic fungi (Mangla & Callaway 2008; Mei *et al.* 2014).

On the other hand, soil fungi belonging to genera *Penicillium, Aspergillus* and *Chaetomium* were common decomposers (Fu-qiang *et al.* 2004) found in the uninvaded soil (Fig. 1 & 3). This indicated that saprophytic soil fungi in the invaded soil might be reduced by *A. adenophora* where pathogenic soil fungi increased. The reduction of saprophytic soil fungi might be important mechanism behind the invasion and affliction of native species by *A. adenophora*.

Plant species could determine rhizosphere microbes (both bacteria and fungi) via root exudates, phytoanticipins, phytoalexins or allelochemicals secreted by the plants (Bever *et al.* 2010). Presence of allelochemicals in root exudates or leachates from aerial parts such as leaf and litter of *A. adenophora* (Wan *et al.* 2011; Zhang *et al.* 2013) could be responsible for the decrease of species richness, the alteration of soil fungi species composition and the alteration of occurrence frequency of saprophytic fungi.

Inderjit *et al.* (2011) found that *A. adenophora* is responsible for higher mortality of native species in China and India. Similarly, Thapa *et al.* (2017) reported that *A. adenophora* reduced the growth and development of native seedlings Nepal. This study suggested that changes in species richness and species composition of soil fungi might also be responsible in seedling mortality, growth and development of aboveground native plant.

In the field, *A. adenophora* is found in thick stands in the invaded areas of Nepal. *A. adenophora* deposits litter in the soil and leaches substances from aerial parts, including green leaves, during rainy days (Thapa *et al.* 2017). Litter accumulation, decomposition in soil and leached substances from aerial parts may have antifungal properties against certain fungi (Broeckling *et al.* 2007). Various allelochemicals in aerial parts of *A. adenophora* such as phenolics, sesquiterpens (Zheng *et al.* 2012) might affect soil microflora or alter soil quality (Katherine *et al.* 2006). Additionally, *A. adenophora* might alter the cycle of soil nutrient which could have affected the abundance or distribution of particular soil fungi species or community in the soil.

Plant-soil feedback mechanism is important to explain vegetation dynamics and ecosystem function including plant invasiveness. Positive feedback is evident through colonization of mycorrhizal fungi by *A. adenophora* (Niu *et al.* 2007; Yu *et al.* 2011; Xiao *et al.* 2014b). There might be negative feedback to the native species through accumulation of pathogenic soil fungi and decrease of saprophytic soil fungi in the invaded soil by *A. adenophora.*

CONCLUSIONS

A. adenophora invasion led to the decrease of soil fungi species richness and altered soil fungi species composition. The invasion facilitated the occurrence of saprophytic fungi as well as accumulated several pathogenic soil fungi. The accumulation of pathogenic fungi could have detrimental effect on the growth and development of native species of higher plant. Changes in soil fungi species and communities could be one of the mechanisms involved in the successful invasion of *A. adenophora*.

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