**Austroeupatorium inulifolium Invasion Alters Soil Microbial Populations to Facilitate its Own Growth**

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**Abstract:** It is a known fact that invasive plants perform better in their introduced range than in the native range. The invasion-driven modifications of the soil microbial community and allelopathic compounds are considered as factors contributing to their success over co-occurring natives. Here we present evidence to show that Austroeupatorium inulifolium invasion has altered the soil microbial population to facilitate its own growth. A pot experiment showed higher growth increments in Austroeupatorium when grown in soil with a history of Austroeupatorium invasion compared to that with no history of invasion. The native, Psiadia ceylanica showed no parallel growth increments when grown under similar conditions. In support, the enzyme activities (catalase and dehydrogenase) and soil microbial biomass carbon also recorded higher values in invaded soils compared to un-invaded soils. Further, Austroeupatorium showed higher dependence on arbuscular mycorrhizal fungi (AMF) than that of the native, Psiadia. The results suggest that Austroeupatorium invasion has enhanced soil microbial populations and functions in order to take the advantage over other natives.

**Keywords:** soil microbes, invasion, Sri Lanka, Austroeupatorium inulifolium, enzyme activities

**INTRODUCTION**

Invasive plants are mostly non-native species with an ability to spread successfully outside their native range (Richardson et al., 2000). Invasions are known to incur negative impacts not only on native plants and animals, but on ecosystem functions as well. Plant and soil communities are closely linked, though underlying mechanisms are still poorly understood (Piper et al., 2015). More studies are focused towards evaluating the above-ground impacts of invasions compared to below-ground aspects. However, few studies revealed that invasive plants can alter the soil microbial population to receive positive feedback to facilitate their invasive nature, thus inhibiting the native flora (Kourtev et al., 2002; Reinhart et al., 2003; Coykendall and Houseman, 2014). Invasive plants also possess allelochemicals to suppress the growth of co-existing native flora (Uddin et al., 2014). These allelochemicals may also have a role in increasing the population and growth of microbes.

Interactions between plants and soil microbes are considered as critical determinants of community composition and ecosystem functions of both above- and below-ground (Broz et al., 2007). Studies have shown that invasions can modify the structure and function of soil microorganisms (Lorenzo et al., 2010; Castro-Diez et al., 2009; Dassonville et al., 2011), thereby imposing positive (nutrient cycling, nitrogen fixation etc.) and/or negative (pathogens, parasites etc.) feedbacks to influence the growth and survival of invasive and non-invasive plants (Zhang et al., 2010; Van Der Heijden et al., 2008). Studies also revealed that changes in soil microbial communities (in terms of composition and abundance) including arbuscular mycorrhizal fungi (AMF) driven by invasive plants may influence invasive and native plants inversely due to their host specificity (Bever, 2002; Kliromonos, 2003; Reinhart et al., 2003). Some invasive species are more responsive towards AMF compared to co-occurring natives, while some are less responsive. However, both situations could generate positive feedbacks enhancing the invasive nature of the plant (Bever, 2003; Vogelsang and Bever, 2009). A study, conducted in highly degraded man-made grasslands in the central highlands of Sri Lanka, reported that Austroeupatorium inulifolium increased the AMF populations significantly (Madawala, 2014). Kourtev et al. (2002) showed that exotic invasive
species can cause profound impacts on the structural and functional aspects of microbial communities in soil. In contrast, others observed that soil microbial populations are rather resistant to any environmental changes including invasions (Carey et al., 2015).

For the past 7-8 years, many land-use types in the Knuckles Conservation Area (KCA), including highly degraded, man-made grasslands, have been invaded by an exotic shrub, Austroeupatorium inulifolium. Studies suggest that Austroeupatorium invasion can alter the structure and the diversity of these grassland communities, soil edaphic properties and other ecosystem functions including, litter loading and decomposition rates (Piyasinghe et al., 2014). The present study investigated whether the A. inulifolium invasion has altered the soil microbial populations in order to facilitate its growth while at the same time making the environment inhospitable for co-occurring native, Psiadia ceylanica.

**METHODOLOGY**

A pot experiment was conducted using two shrub species belonging to the family Asteraceae, Austroeupatorium inulifolium (an exotic invasive with a creamy-white inflorescence) and Psiadia ceylanica (a native, non-invasive) to evaluate any differences in growth when raise them in soils with and without a history of Austroeupatorium invasion. Psiadia ceylanica, bearing a yellow-coloured inflorescence, found frequently along the boundaries of grasslands invaded by Austroeupatorium (Figure 1). The pot experiment was conducted under glasshouse conditions at the Department of Botany, University of Peradeniya, Sri Lanka. The soil was collected from Austroeupatorium-invaded (with a history of Austroeupatorium for the last 7-8 years) and un-invaded grasslands at KCA. Soil samples (to a depth of 20 cm) were collected from 3 representative locations each of invaded and un-invaded sites and were composited. Potting mixtures were prepared by combining grassland (invaded and un-invaded) soils with river sand separately in 1:2 ratio. One portion of each potting mixture (invaded/un-invaded) was sterilized by autoclaving at 120°C for 30 minutes. There were 4 treatments used in the experiment viz., invaded/un-sterilized (In-U), invaded/sterilized (In-S), un-invaded/un-sterilized (Un-U) and un-invaded/sterilized (Un-S). The seedlings (raised from seeds) of Austroeupatorium and Psiadia were transplanted in plastic pots filled with differently-treated soils (as mentioned above), with 6 replicates for each treatment. The pots were arranged in completely randomized design in the glasshouse and their positions were changed weekly to avoid any position effect. Plants were watered as required. No fertilizers were added at any time. Shoot heights were recorded weekly. After 14 weeks, plants were destructively harvested and dry weights of root and shoot were recorded.

![Figure 1: The inflorescences of Austroeupatorium inulifolium (Left) and Psiadia ceylanica (Right).](image)

Photo Credit: Inoka Piyasinghe.
Root colonization of AMF

At the final harvest, fine root samples were taken from each individual to quantify AMF colonization. Root samples were washed with water to remove soil particles and other debris, and preserved in 50% ethanol in tightly sealed plastic vials until further processing. Root samples were cleared (in 10% KOH) and stained with Chlorazol black E (CBE) (Brundett et al., 1996) before observing them under a binocular microscope. Mycorrhizal colonization was estimated by counting the number of interceptions crossing AMF structures (vesicles, arbuscules etc.) as mentioned in McGonigle et al. (1990).

Soil microbial biomass carbon and enzyme activities

Three well represented soil samples collected from each invaded and un-invaded sites were analyzed for soil microbial biomass carbon (MBC) by chloroform-fumigation extraction method (Vance et al., 1987). Soil samples were also analyzed for catalase and polyphenolic oxidase using titration methods (according to methods given in Jin et al., 2009 and Wang et al., 2013, respectively), and the dehydrogenase activity using a spectrophotometric method (Tabatabai, 1994). All chemical analyses were carried out in the laboratories at the National Institute of Fundamental Studies, Kandy.

Data analysis

Plant height and dry total biomass were used in calculating the height increments (%) and relative growth rates (RGR; g per day), respectively. Prior to the use of parametric analysis, growth parameters were transformed to meet the assumptions of ANOVA. Two-way ANOVA was carried out to evaluate the significant differences between treatments (In-S, In-U, Un-S and Un-U) and species (Austroeupatorium and Psiadia). Mean separation was done using Tukey’s HSD ad-hoc test. Edaphic parameters (enzyme activities and soil microbial biomass carbon) were analyzed using one-way ANOVA. All statistical analyses were carried out with Minitab 17.0 software package.

RESULTS

Growth Increments

During the experimental period, Austroeupatorium grew faster in invaded/unsterilized soil (In-U) compared to other treatments (In-S, Un-S and Un-U). Plants grown in In-U started to boost their growth increments only after 8 weeks of growth (Figure 2). In contrast, the native Psiadia showed no differences in their growth increments between treatments throughout the experimental period. Furthermore, Austroeupatorium demonstrated higher growth increments than that of the native, Psiadia throughout the test period (Figure 2).

![Figure 2: Cumulative increase in height (as a percentage) of Austroeupatorium and Psiadia during the experimental period grown under differently-treated soils; invaded/sterilized (In-S), invaded/un-sterilized (In-U), un-invaded/sterilized (Un-S) and un-invaded/un-sterilized (Un-U). Error bar represents the standard error of the mean (SEM) values.](image-url)
In spite of their height increment differences during the experimental period, the relative growth rates (calculated using total dry biomass data after 14 weeks of growth) showed no significant differences between treatments as well as between species (Figure 3).

**Dependence on AMF**

Higher percentage root colonization of AMF was recorded in *Austroeupatorium* plants when grown in both sterilized and un-sterilized invaded soils (29 and 34% colonization in In-S and In-U, respectively) than that in un-invaded soils (3 and 9% in Un-S and Un-U soils, respectively). In contrast, *Psiadia* showed rather low AMF colonization in both invaded and un-invaded (In and Un) soils (≈ 1 - 9%) indicating its low response towards AMF (Figure 4). However, plants grown in sterilized soils too recorded AMF colonization in roots, perhaps due to infected seedlings at the time of transplanting.

**Enzyme activities**

Higher catalase and dehydrogenase activities were recorded in invaded soils compared to that of un-invaded soils (Figure 5). However, the polyphenolic oxidase activity was not significantly different between the two soil types (ANOVA; F=0.14; p=0.727; df=2). Microbial biomass carbon too showed significantly higher values in invaded soils than that in un-invaded soils (Figure 5).

![Figure 3](image1.png)

**Figure 3:** Relative growth rate (g per day) of *Austroeupatorium* and *Psiadia* grown under differently-treated soils; invaded/sterilized (In-S), invaded/un-sterilized (In-U), un-invaded/sterilized (Un-S) and un-invaded/un-sterilized (Un-U). Error bar represents the standard error of the mean (SEM) values (ANOVA; F=0.001, p=0.998, df=5).

![Figure 4](image2.png)

**Figure 4:** Percentage root colonization of arbuscular mycorrhizal fungi (AMF) in *Austroeupatorium* and *Psiadia* grown in differently-treated invaded and un-invaded soils; In-S, In-U, Un-S and Un-U (ANOVA; F=18.66; p<0.001; df=5). Vertical bars represent standard error of mean (SEM) values.
**DISCUSSION**

*Austroeupatorium* plants grew better when subjected to soils with a history of *Austroeupatorium* invasion indicating its facilitative effect compared to other treatments. Phenotypic plasticity in terms of height is considered as one of the vital traits shown by invasive species to compete for light, space and other resources especially in early stages of their life cycle (Balandier et al., 2007; Robakowski and Bielinis, 2011). Greater height increments under invaded/unsterilized soils suggest that *Austroeupatorium* alters its soil micro-biota to earn the advantage for its own invasive behaviour. The co-occurring native, *Psiadia* showed no added advantage when grown in differently-treated soils. Previous studies too have demonstrated similar effects with other invasive species to suggest that they alter the soil microbial community to generate a positive feedback to enhance its competitiveness at the expense of co-occurring native species (Zhang et al., 2010; Coykendall and Houseman, 2014). However, studies suggest that these soil chemical and microbiological changes following invasions may not necessarily remain constant or else accumulate over time (Strayer, 2012; Dostal et al., 2013), while stressing that ecological and adaptation processes may increase or weaken these impacts of invaders in their introduced
range with time (Souza-Alonso et al., 2015). However, present results suggest that even after 7-8 years of invasion, modifications to the soil microbial community still exist.

Austroeupatorium seems to be more responsive towards AMF compared to the native Psidia, indicating its higher dependence on soil microbes. Root–AMF association is a chemically modulated process (Harrison, 2005), and therefore it seems that sterilization may not destroy such chemical signals enabling Austroeupatorium roots to colonize even under sterilized soils. A previous study conducted in man-made grasslands at KCA has demonstrated that the AMF abundance enhanced following Austroeupatorium invasion (Madawala, 2014). In agreement, the present study also confirmed that the Austroeupatorium invasion has increased the AMF colonization potential in Austroeupatorium. In addition to higher AMF abundance, present results also demonstrated higher soil microbial populations and activities following invasion. Previous studies confirmed that microbial communities may change under different plant species (Priha et al., 1999; Grayston et al., 2001). These variations may have resulted due to the differences in root inputs (rhizosphere exudates and root turnover), and the quality and quantity of aboveground litter inputs (Coleman et al., 2000). Studies also revealed that invasion by mycotrophic plants could enhance AMF abundance and richness when they replace poor hosts in the existing native flora (Lekberg et al., 2013; Madawala, 2014).

Microbial biomass carbon is a measure of the carbon content within the living component of the soil organic matter (i.e. mainly fungi) and therefore high percentage of microbial biomass carbon indicates presence of an increased microbial abundance. Apart from few limitations, soil microbial biomass carbon is considered as a useful indicator in quantifying the soil microbial populations (Schloter et al., 2003). According to results of the present study, invaded soils showed a higher microbial biomass carbon, further confirming increased soil microbial populations following the Austroeupatorium invasion.

Soil enzyme activity has been used as an effective indicator of functional capability of the soil microbiota (Kourtev et al., 2002; Waldrop et al., 2000; Zhang et al., 2013). Therefore, any changes to the microbial composition could reflect in the composition and activity of soil enzymes. The present analysis demonstrated higher catalase and dehydrogenase activities in invaded soils compared to un-invaded soils. Catalase decomposes peroxide which is formed during the respiration and oxidation of organic matter, and therefore considered as an important indicator of soil fertility and metabolic activity of aerobic organisms (Burns, 1982; Kızılkaya and Hepşen, 2007). Being an intracellular enzyme, dehydrogenase can be considered as a good indicator of overall soil microbial activity, and showed positive correlation with soil microbial biomass carbon (Yuan and Yue, 2012; Salazar et al., 2011). Significantly higher catalase and dehydrogenase activities in Austroeupatorium invaded soil compared to un-invaded soil further suggested a higher abundance and activity of microbes once the landscape is invaded by Austroeupatorium.

CONCLUSION

Austroeupatorium invasion seems to increase the soil microbial population and their activities. Austroeupatorium seems to take the advantage of this altered condition to perform better than that of co-occurring natives. In contrast, Psidia ceylanica did not acquire any gain out of this situation suggesting that Austroeupatorium spread had altered soil microbial communities to boost its invasive nature in the introduced range.

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