

SHORT COMMUNICATION

***Austro eupatorium inulifolium* invasion increases arbuscular mycorrhizal abundance in *Cymbopogon*-dominated grasslands in Knuckles Conservation Area**

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Revised: 13 March 2014; Accepted: 21 March 2014

Abstract: Plant invasions can interfere with above- and below-ground dynamics of ecosystems, which eventually alter the ecosystem functions. However, relatively less attention has been drawn to assess the impacts of invasion on the soil microbial community. *Austro eupatorium inulifolium* has been spreading into different land use types in the Knuckles Conservation Area (KCA) for the last few years, including *Cymbopogon*-dominated grasslands. Therefore, the study investigated whether this invasion has altered the arbuscular mycorrhizal fungal (AMF) abundance and the diversity in these grasslands through isolation and identification of AMF spores in three different habitats, namely less- and highly-invaded grasslands (LIG and HIG, respectively) and nearby lower montane forest patch (LMF), which is free of *Austro eupatorium* invasion. The results revealed that the invasion has significantly increased the AMF spore abundance. However, the LMF reported the highest AMF diversity compared to invaded grasslands. AMF richness showed no difference between the habitats. The results highlighted the importance of investigating the changes caused to the soil microbial community due to invasions and their implications for site restoration.

Keywords: Arbuscular mycorrhizae, diversity, exotic plants, invasion, root colonization.

INTRODUCTION

Exotic plant invasions are often associated with marked shifts in the vegetation structure and composition in their introduced range (Levine *et al.*, 2003). However, only a few attempts have been made to study the potential alterations of soil microbial communities that may be associated with such vegetation changes. Exotic plant invasions could alter the composition and functions of the soil microbial community due to changes in plant inputs

to the soil (van der Putten *et al.*, 2007). It is well known that belowground biotic interactions play a major role in plant distribution and ecosystem functions (Bever *et al.*, 1997; Stinson *et al.*, 2006). Therefore, it is of critical importance to understand the impacts of invasions on the soil microbial communities.

Among soil microorganisms, arbuscular mycorrhizal fungi (AMF) play a vital role in sustaining the plant growth in an ecosystem. Sanon *et al.* (2012) observed a clear reduction of the AMF density in soil invaded by the exotic plant *Amaranthus viridis* when compared with an un-invaded soil nearby. On the contrary, Lekberg *et al.* (2013) observed an increase in the AMF abundance and diversity in grass-dominated communities invaded by the exotics, *Centaurea stoebe* and *Euphorbia esula*. For the past few years, many land-use types in the KCA including man-made grasslands have been invaded by an exotic shrub, *Austro eupatorium inulifolium*. Studies showed that *Austro eupatorium* invasion has not only altered the structure and diversity of these grassland communities but also the soil fertility levels, litter loading and decomposition rates as well (Piyasinghe *et al.*, 2013). The present study investigated whether the *A. inulifolium* invasion has altered the AMF abundance and diversity in *Cymbopogon*-dominated grasslands in the Knuckles Conservation Area (KCA) in Sri Lanka.

METHODS AND MATERIALS

The study took place in highly degraded, man-made grasslands at Riverston in the KCA, Sri Lanka. For the present study, 3 plant communities were selected:

two grassland communities with different levels of *Austroeuropatorium* invasion (less- and highly-invaded grasslands, hereafter referred to as LIG and HIG, respectively) and a lower-montane forest (LMF) patch bordering these grasslands. From each of the plant community (LIG, HIG and LMF) dominated by *Cymbopogon nardus*, *Austroeuropatorium* and native plant species, respectively 10 composite soil samples were taken within 500 m of each other. Based on the visual observations, LIG and HIG had an invasive coverage of < 30 and > 80 %, respectively. The forest-grassland edge had an *Austroeuropatorium* coverage of < 5 %, which decreased rapidly towards the forest interior (Haluwana & Madawala, 2013). Some basic soil chemical properties of the 3 vegetation types are given in the Table 1. Root samples of *Austroeuropatorium* and *Ageratina riparia* (another exotic invasive plant dominating the ground layer of LMF) were extracted and preserved in 70 % ethyl alcohol until processed.

Table 1: Basic soil chemical parameters at the study sites

Soil parameters	LIG *	HIG *	LMF #
Soil pH	5.48 ± 0.11 ^b	5.91 ± 0.27 ^a	5.5 ± 0.04
Total C (%)	4.20 ± 0.22 ^a	4.22 ± 0.29 ^a	4.8 ± 0.01
Total N (%)	0.37 ± 0.09 ^b	0.44 ± 0.10 ^a	0.085 ± 0.001
Total P (%)	0.099 ± 0.01 ^a	0.098 ± 0.02 ^a	0.065 ± 0.001

Significant differences were indicated using different letters following one-way ANOVA. LMF not included in the analysis. Average values (n = 18)* are indicated with standard errors of mean.

* Piyasinghe *et al.* (2013)

Gunaratne *et al.* (2010)

The soil samples were analyzed to determine the total number of AMF spores in 100 g of dry soil using wet sieving and decanting, followed by sucrose centrifugation. Spores (categorized into 45, 63 and 125 µm diameter classes) were counted under a compound microscope and different morphotypes were identified to the genus level with the aid of the descriptions available at <http://invam.caf.Wvu.edu/info/Taxonomy/species.htm>. The roots of *Austroeuropatorium* and *A. riparia* were cleared in 10 % KOH, stained with 0.05 % w/v trypan blue (Kormanik & McGraw, 1982) and the percentage colonization of AMF (McGonigle *et al.*, 1990) was determined.

Using the spore counts and different spore morphotypes, the species density (number of AMF spores present in 100 g of dry soil), species richness (number of AMF morphotypes present in 100 g of dry soil) and diversity indices such as Simpson's dominance

index (Is) and Shannon's diversity index (H) were calculated. Evenness values [evenness = H/Ln (number of morphotypes)] were also calculated. Percentage root colonization was estimated by the number of cells infected over the total number of cells counted. AMF spore data were statistically analyzed using ANOVA in Minitab 16.0. The means were separated using Tukey's test. Root colonization percentages were compared using a t-test with arcsine transformed data.

RESULTS

A total of 31 AMF morphotypes were detected in all three study sites. Out of this, 24 morphotypes were identified to their generic levels, while 7 remained as un-identified. A total of 30, 29 and 28 morphotypes (belonging to three different size classes) were isolated from LIG, HIG and LMF, respectively. Out of the 30 morphotypes at LIG, 7 *Glomus* (74.0 % of the total isolates of LIG), 12 *Scutellospora* (17.6 %) and 4 *Gigaspora* (7.1 % of immature spores) species were recorded. There were seven un-identified morphotypes (1.3 %). In HIG, 7 *Glomus* (65.7 %) 12 *Scutellospora* (28.9 %), 4 *Gigaspora* (3.7 %) species and 6 unidentified morphotypes (1.8 %) were recorded. In LMF, 7 *Glomus* (51.1 %), 13 *Scutellospora* (24.3 %), 4 *Gigaspora* (23.0 %) species and 4 unidentified morphotypes (1.67 %) were detected.

The highest number of spores (15,439) was recovered from HIG, followed by LIG and LMF (11,480 and 2,808, respectively). The average spore counts (abundance) were significantly higher in both invasive grasslands (LIG and HIG) compared to that of the LMF (one-way ANOVA; df = 9, F = 6.36, p = 0.003). The abundance of AMF spores in forest soils was markedly low. Spore size distribution also varied among the three vegetation types. The total spore count was significantly dominated by medium-sized spores (63 µm) in both LIG and HIG (≈ 79 and 64 %, respectively) but not in LMF (45 %). In all three vegetation types, the lowest contribution to the total spore count was from the large-sized spores (125 µm) (≈ 4, 3 and 12 % in LIG, HIG and LMF, respectively). Small- and large-sized spore counts (45 and 125 µm) showed no significant differences among the vegetation types (Figure 1).

The average number of spore morphotypes (richness) demonstrated no significant difference between the vegetation types (Figure 2). However, large-sized spore morphotypes were significantly less in all vegetation types studied compared to that of small- and medium-sized spores.

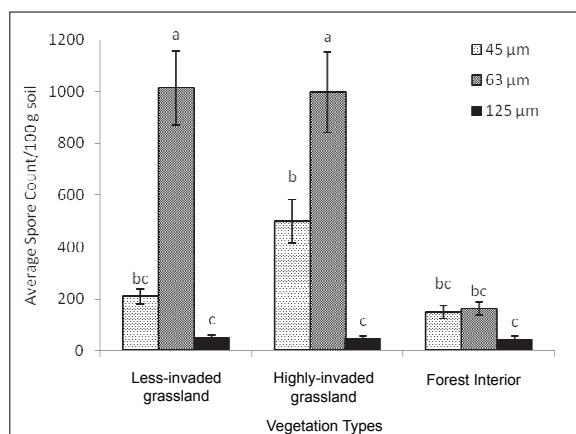


Figure 1: Average arbuscular mycorrhizal fungal spore counts in less-invaded and highly-invaded grasslands, and lower montane forest soils (LIG, HIG and LMF, respectively) at the Knuckles Conservation Area, Sri Lanka. Bars with different letters indicate significant differences at $p \leq 0.001$ (two-way ANOVA; $df = 9$, $F = 18.8$ and 51.6 for vegetation type and spore size, respectively).

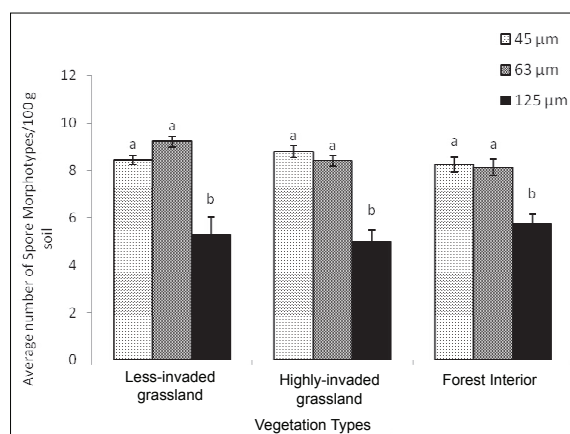


Figure 2: Average number of arbuscular mycorrhizal spore morphotypes under each spore size category (45, 63 and 125 µm) in less-invaded, highly-invaded and lower montane forest soils at the Knuckles Conservation Area, Sri Lanka. Bars with different letters indicate significant differences at $p \leq 0.001$ (two-way ANOVA; $df = 9$, $F = 69.4$ spore size).

Table 2: Total number of arbuscular mycorrhizal spores, spore morphotypes, diversity indices (Shannon's and Simpson's diversity indices) and evenness values in the three vegetation types studied (less-invaded grassland, highly-invaded grassland and lower-montane forest) at the Knuckles Conservation Area, Sri Lanka.

	Less-invaded grassland (LIG)	Highly-invaded grassland (HIG)	Lower-montane forest (LMF)
Total number of spores	11,480	15,439	2,808
Number of spore morphotypes	30	29	28
Simpson's diversity index	0.787	0.849	0.906
Shannon's diversity index	2.059	2.271	2.682
Evenness	0.605	0.674	0.805

In spite of the low spore abundance, LMF showed a higher AMF diversity compared to invaded grasslands, HIG and LIG (Table 2). *Austro eupatorium* roots showed a poor AMF colonization (9 %) compared to that of *A. riparia* (28 %).

DISCUSSION

The present study investigated the alterations of AMF abundance, richness and diversity due to the *Austro eupatorium inulifolium* invasion. The results showed that AMF spore abundance was significantly

higher in invaded grasslands (both LIG and HIG) indicating the ability of the invaded grassland community to support a high AMF population compared to that of the native, mixed plant community present in the forest nearby. The root colonization data revealed that *Austro eupatorium* is not a good host for AMF. Previous studies have also shown that most invaders are either non-mycorrhizal or have a low inherent dependency on mycorrhizae (van der Putten *et al.*, 2007; Seifert *et al.*, 2009). The high abundance and richness of forbs observed in the *Austro eupatorium*-invaded grasslands (Haluwana & Madawala, 2013) may have contributed to

the high AMF abundance in these grasslands. The ability of forbs to harbour AMF has been reported by Wilson and Harnett (1998). The present results also support the fact that AMF abundance would differ among different plant communities possibly due to the variations in host quality.

Contrary to the thinking that AMF richness may decrease due to the dominance of a single plant species and the reduction of AMF niches, all three vegetation types in this study showed a similar AMF spore richness. However, in all three vegetation types, large-sized spores contributed less to the AMF richness compared to small- and medium-sized spores. Lekberg *et al.* (2013) observed an increase in both AMF abundance and richness followed by an invasion in rangelands in the USA. However, the present results demonstrated that plant diversity is not always the determining factor for AMF richness as suggested by previous studies (Landis *et al.*, 2004; Lekberg *et al.*, 2013). The changes in supply of resources might be the main driving force behind soil microbial diversity (Waldrop *et al.*, 2006). In spite of the low AMF spore abundance, LMF had the highest AMF diversity compared to invaded grasslands. The present results support the positive relationship between above- and below-ground diversity.

In summary, the results showed that invaded grasslands have the ability to harbour higher AMF populations than multi-species forests, possibly due to the higher abundance of mycorrhizal forbs. However, plant diversity may be a strong predictor of AMF diversity. This study confirms that *Austroepatorium inulifolium* invasion has altered the soil microbial community, which could further enhance invasion and ensuing impacts on the ecosystem.

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