Original article

Changes in arbuscular mycorrhizal fungal communities during invasion by an exotic invasive plant

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A B S T R A C T

Exotic invasive plants can show strong plant–soil feedback responses, but little is known about time scales for significant changes in soil microbial communities to occur after invasion. Previous work has suggested that plant invasions can modify arbuscular mycorrhizal (AM) fungal community structure. However, there is a lack of understanding about how long it takes for these changes to develop. To test this we investigated temporal changes in AM fungal communities colonising the invasive plant Vincetoxicum rossicum (Apocynaceae). We hypothesised that AM fungal community structure would change in a particular direction during the invasion process. We collected soil from two sites with a long history of invasion by this plant, with each site having paired invaded and uninvaded plots. Soil from these plots was used in a glasshouse experiment to characterise AM fungal community structure in the roots of V. rossicum at different times throughout a simulated growing season. AM fungal community structure differed between invaded and uninvaded plots. However, contrasting with our hypothesis, AM fungal communities colonising V. rossicum growing in soil from uninvaded plots did not change towards those in plants growing in previously invaded soil. Our data suggest that changes to AM fungal communities in the presence of V. rossicum require longer than the first growing season after establishment to develop.

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1. Introduction

Studies on plant–soil feedback, where the soil microbial community changes in response to a particular plant species and subsequently affect plant growth, show that exotic invasive plants often benefit from positive feedback (van der Putten et al., 2007a; Pendergast et al., 2013; Bardgett and van der Putten, 2014). It has been hypothesised that soil mutualisms drive this positive feedback (Klironomos, 2002; Zhang et al., 2010; Bever et al., 2012). However, little is known about the time scale required for significant changes to occur in mutualistic soil microbial communities after establishment of an exotic plant invader. Hawkes et al. (2013) recently showed that the direction of plant–soil feedbacks can change over relatively short periods of time. This indicates that investigating the temporal dynamics of key soil microbial communities is important to understand invasiveness and environmental impacts of exotic plant invaders.

Arbuscular mycorrhizal (AM) fungi (Phylum Glomeromycota) establish obligate mutualisms with most land plants, where plants provide the fungal partners with carbon in exchange for benefits, including nutrient uptake and pathogen protection (Smith and Read, 2008). The symbiosis falls along a continuum from mutualism to parasitism (Johnson et al., 1997; Klironomos, 2003; Kiers et al., 2011); however, a meta-analysis indicated that AM fungal associations are generally beneficial in terms of increasing plant biomass (Hoeksema et al., 2010). AM fungal community structure is determined by a range of biotic and abiotic factors, including plant host (Eom et al., 2000; Lekberg et al., 2007; Jansa et al., 2008; Hausmann and Hawkes, 2010), its neighbours (Hausmann and Hawkes, 2009; Lekberg et al., 2012, 2013), land use, and soil type and pH (Lekberg et al., 2007; Schreiner and Mihara, 2009; Oehl et al., 2010; Bunn et al., 2014). Large inter- and intra-annual variability in AM fungal communities has also been observed in field studies (Husband et al., 2002; Liu et al., 2009; Dumbrell et al., 2011; Sánchez-Castro et al., 2012; Helgason et al., 2014). AM plants are colonised by multiple fungal species and species isolates, some

more beneficial than others (van der Heijden et al., 1998; Eom et al., 2000; Lewandowski et al., 2013). In addition, selective recruitment between particular plant species and AM fungal isolates has been demonstrated (Bever et al., 2009; Kiers et al., 2011; Fellbaum et al., 2014), which may contribute to positive feedback (Bever et al., 2012).

Exotic invasive plants (hereafter invasive plants) can depend on mutualisms in their native range, but this may not hinder invasion success in a novel range (Richardson et al., 2000; Moora et al., 2011; Wandrag et al., 2013; Nuñez and Dickie, 2014). One theory is that highly mycorrhizal and successful invasive plants may be less selective and associate with a wide range of AM fungi across locations (van der Putten et al., 2007b; Pringle et al., 2009; Nuñez and Dickie, 2014). However, AM fungal communities can change in the presence of invasive plants (Zhang et al., 2010; Pendergast et al., 2013). For example, *Centaurea maculosa* imposed significant changes to AM fungal communities in Montana grasslands (Mummey and Rillig, 2006). Little is known about how quickly AM fungal communities change in response to invasive plants. This knowledge may contribute to better understand feedbacks, invasiveness, and impacts of plant invasions (Levine et al., 2006; Kardol et al., 2013).

Vincetoxicum rossicum (Kleopow) Barbar. (Apocynaceae) (syn. *Cynanchum rossicum* (Kleopow) Borhidi; dog-strangling vine) is a highly invasive perennial plant in parts of North America, including southern Ontario in Canada (Sheeley and Raynal, 1996; Cappuccino et al., 2002). It becomes dominant and can outcompete surrounding vegetation (Cappuccino, 2004; Douglass et al., 2009; Anderson, 2012). Originally from the Ukraine and southwest Russia (Pobedimova 1952 in DiTommaso et al., 2005), in North America *V. rossicum* can establish under a range of light, moisture, and climate conditions, as well as in many different soil and vegetation types (DiTommaso et al., 2005; Averill et al., 2011; Sanderson and Antunes, 2013; Sanderson et al., 2015). *V. rossicum* is highly mycorrhizal dependent and readily associates with many AM fungal species in its invaded range (Smith et al., 2008; Bongard et al., 2013).

The main objective of this study was to determine whether AM fungal community structure changes in response to *V. rossicum*. We collected soil from two field sites with paired plots that had either no record of invasion or decades of invasion by this species in Canada. This soil was used in a glasshouse experiment to monitor changes in AM fungal communities colonising *V. rossicum* roots over the course of 29 weeks (i.e., equivalent to one growing season). We hypothesised that at the onset of the experiment AM fungal community structure colonising plants growing in soils from invaded and uninvaded field plots would be different. However, if AM fungal communities change in response to *V. rossicum*, then we expected that the AM fungal community structure in plants growing in the uninvaded soil would become more similar to those in the invaded soil over time. In addition, we measured plant growth responses throughout the experiment. We expected that general soil biotic effects of *V. rossicum* invasion would have growth effects on this plant species in soils with different invasion histories.

2. Materials and methods

2.1. Soil and seed collection

Soil was collected from Toronto Zoo, ON, Canada (N 43°49′7″, W −79°11′8″) at each of two sites approximately 1 km apart (hereafter referred to as site 1 and 2). Given that *V. rossicum* invasion in this area was homogeneous, two sites were considered to be sufficient to assess patterns in AM fungal communities. Within sites, soil was collected from two paired plots: one with no record

of *V. rossicum* invasion ('uninvaded') and the other with a dense population of *V. rossicum* ('invaded'). *V. rossicum* had been present for at least 20 years in the invaded plots, which were last mown or managed in the early 1990s (J. Bell, Toronto Zoo Manager, personal communication). Therefore, these plots are considered to have been trained by *V. rossicum* for multiple decades. The two sites were chosen within a small geographic area to minimise environmental and soil differences. Since the sites had similar management histories, it is an assumed that plant communities in the uninvaded plots are representative of those present prior to invasion by *V. rossicum* and that all locations were at the same successional stage (see Table S1). Approximately 60 L of soil was collected from each plot from the top 20–30 cm, covering an area of approximately 3 m². Soil was homogenised by sieving (4 mm) and stored in air tight, opaque containers for transport back to the laboratory where they were kept at 4 °C until the start of the experiment four days later. On site, all containers, spades and soil sieves were scrubbed and soaked in diluted bleach for at least 20 min to prevent cross-contamination between plots. A subsample of soil from each site indicated that both sites had the same soil type: a Till Plain slightly alkaline (mean pH 7.93 ± 0.05) fine sandy loam. Soil fertility was similar among all four plots (Table S2).

Seeds of *V. rossicum* were collected five weeks prior to soil collection from opened seedpods within the invaded plots. Pappi were removed and seeds were stored in paper envelopes at 4 °C for approximately two weeks. Seeds were stratified between sheets of moist filter paper in the dark at 4 °C for 18 days (Smith et al., 2008). Prior to planting, seeds were surface disinfected in 10% bleach for three minutes and rinsed in sterile water.

2.2. Experimental design

The experiment was conducted in a glasshouse and each site was considered separately to assess reproducibility of patterns across sites. We used a completely randomised factorial design with two crossed factors: 'invasion' (soil with two categories: invaded and uninvaded) and 'time' (with 5 categories: harvests 1, 2, 3, 4, and 5, corresponding to 9, 13, 19, 24, and 29 weeks after planting) with four replicates per invasion–time combination. Four sterile control pots were also prepared for each plot, consisting of autoclaved soil (90 min at 121 °C and 18 psi), making a total of 8 controls and 48 experimental units per site. All potting equipment was disinfected by soaking in diluted bleach for at least 20 min. For each site, soil was sieved and thoroughly mixed with sterile sand (non-calcareous "B" sand, Hutcheson Sand and Mixes, Huntsville, ON, Canada) and turface (calcined, non-swelling illite and silica clay, Turface Athletics MVP, Profile Products LLC, Buffalo Grove, IL, USA) in a 1:1:1 ratio. This substrate was divided equally into 2.8 L pots (Nursery products Inc., C300 pots 18 cm tall, 16 cm diameter). Control pots contained sterile soil, sand, and turface in a 1:1:1 ratio and were used to assess potential cross contamination or glasshouse effects. Pots were lined with a 2 mm mesh to prevent substrate loss and placed on saucers.

Four *V. rossicum* seeds were placed into each pot approximately 5 mm below the soil surface using sterile tweezers. Where multiple seeds germinated in a pot, one seedling was randomly selected to grow and the other seedlings were repeatedly cut at soil level using sterile scissors. Plants other than *V. rossicum* were pulled out immediately after germinating. Plants were watered with reverse osmosis water between two and seven days each week over the course of the experiment and received a 14/10 day/night photoperiod with temperature ranging between 20 and 24 °C. Pots were randomised monthly. At week 11 (between harvests 1 and 2) 200 mg of 20-2-20 (Plant Products, ON, Canada) fertiliser was added to each pot (Plant Products, Brampton, ON, Canada).