Fungal Ecology 26 (2017) 114–124

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Characterisation of above-ground endophytic and soil fungal communities associated with dieback-affected and healthy plants in five exotic invasive species

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ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form
15 December 2016
Accepted 10 January 2017
Available online 9 February 2017

Corresponding Editor: Barbara J. Schulz

Keywords:
Fungal community structure
Latent pathogens
Plant population decline
Plant health
ITS1 region
454 sequencing
Weeds

ABSTRACT

In Australia, several well-established invasive plant species have experienced unexplained dieback. To investigate this issue, we used internal transcribed spacer (ITS) amplicon pyrosequencing to characterise fungal communities within stems (endophytes) and soils associated with dieback-affected and healthy plants from populations of five exotic invasive species (*Jatropha gossypiifolia*, *Mimosa pigra*, *Parkinsonia aculeata*, *Tamarix aphylla* and *Vachellia nilotica*) across northern Australia. *M. pigra* and *P. aculeata* were sampled from multiple geographic regions. A total of 353 and 4926 fungal operational taxonomic units (OTUs) were identified in stem and soil samples, respectively. Members of Ascomycota were common, representing 75% of stem and 49% of soil OTUs. Four common endophytes, including *Cladosporium perangustum*, were at least 50% more prevalent in healthy than dieback-affected samples for the five invasive species combined. Fungal community structure within stem and soil samples varied among invasive species. For the two species sampled across multiple regions, *M. pigra* had similar fungal communities within stems among regions, but a significant difference in associated soil fungi, suggesting that host plant rather than environment determined endophytic communities in this species. Irrespective of the invasive species and sample type (stem *vs.* soil), no significant differences were observed in fungal richness, diversity or community structure between dieback-affected and healthy plants, either locally or regionally. Our work failed to identify fungi that were either unique or relatively more abundant in dieback than healthy plants in these invasive species. Future investigations of biotic factors other than fungi, such as bacteria, archaea and oomycetes, may provide more insights into the mechanism of the dieback phenomenon affecting these species.

1. Introduction

There is an increasing number of cases globally where the plant health of well-established exotic invasive plant species has declined, subsequently resulting in at least local population reductions (Li et al., 2009; Boyce et al., 2014; Meyer et al., 2014). In Australia, this includes several of the most serious invaders, and has been observed across the geographic range of some of these (van Klinken and Heard, 2012; Aghighi et al., 2014). Despite this, we lack a clear understanding of the mechanisms that cause such decline, although it is speculated that biotic factors, such as fungi, oomycetes or bacteria, may play a critical role. It is becoming clear from a few recent studies that pathogens (particularly soil-borne) may be limiting recruitment in invasive plant populations (Nijjer et al., 2007; Diez et al., 2010; Dostál et al., 2013). However, we have limited knowledge of above- and/or below-ground microbial communities associated with deteriorating health of established invasive plants (Flory and Clay, 2013). Even for those studies where

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http://dx.doi.org/10.1016/j.funeco.2017.01.003
1754-5048/

pathogens are linked to invasive plant population decline (Diez et al., 2010; Dostál et al., 2013), interpretations have been solely based on correlations, rather than the identity of pathogens. Characterising microbial communities, isolating and identifying putative pathogens and endophytes, and conducting manipulative experiments are necessary to determine whether biotic factors are involved. Considering that fungi are an important group of plant pathogens and represent the most frequently isolated endophytes, it is logical as a first step to investigate fungal communities that may contribute to such plant decline.

In recent years, fungal endophytes have received increased attention from researchers not only because of their ubiquity and diversity among plant species (Schulz and Boyle, 2005; Kohout et al., 2013; Siddique and Unterseher, 2016), but also because of the potential for them to have both positive and negative effects on host plants. For example, some endophytic species defend plants against biotic and abiotic stress (Rodriguez et al., 2009; Raghavendra and Newcombe, 2013; Cosme et al., 2016), while others are reported as latent pathogens (Slippers and Wingfield, 2007; Junker et al., 2012). Most studies investigating the diversity and functional role of fungal endophytes have focused on economically important crops or indigenous plant species (reviewed in Porras-Alfaro and Bayman, 2011). In contrast, fewer attempts have been made to investigate fungal endophytes in invasive plant species (White and Backhouse, 2007; Shipunov et al., 2008; Mei et al., 2014). Nonetheless, these studies suggest that invasive plants do harbour taxonomically diverse fungal endophytes (White and Backhouse, 2007; Shipunov et al., 2008), some of which may positively or negatively affect plant growth and development (Raghavendra et al., 2013), and even contribute to the success of plant invasions (Aschehoug et al., 2014).

Changes in the fungal endophytic community, particularly in favour of those with pathogenic capabilities, may negatively affect plant health. For example, several important pathogenic species in the Botryosphaeriaceae can colonise their host as non-pathogenic endophytes (Slippers and Wingfield, 2007; Sakalidis et al., 2011). However, under favourable conditions such as drought and insect damage, these fungi can emerge from latency, proliferate, colonise vascular tissues and cause considerable damage to plants (Slippers and Wingfield, 2007). In a recent study, Linaldeddu et al. (2011) demonstrated that experimentally reducing soil moisture levels under natural conditions for over one year not only reduced overall fungal endophytic diversity, but also increased abundance of a potentially pathogenic fungus *Biscogniauxia mediterranea* (causing charcoal rot) in woody tissues of declining *Quercus suber* (cork oak) trees, compared to asymptomatic controls.

Changes in soil fungal communities associated with invasive plant species may also have important consequences for plant health. The role of soil fungi in the initial stages of plant invasions has been extensively studied (Mangla and Callaway, 2008; Lekberg et al., 2013), but relatively little is known about their role in established plant populations. Soil harbours a diverse community of fungi, including plant pathogenic and beneficial species, which collectively influence plant health and development (Raaijmakers et al., 2009). *Fusarium* spp. and *Verticillium* spp., which are widely distributed in soil and known to cause root rot diseases in agroecosystems, may either live as resting spores or as saprotrophs in soil (Klosterman et al., 2009; Burgess and Bryden, 2012). However, water stress and high soil temperatures can alter host plant susceptibility, and offer favourable conditions for proliferation and infection by these fungi (Ghaemi et al., 2011). In turn, these fungi can out-compete other antagonistic and beneficial fungi (e.g., soil mutualists) for resources (Kosiak et al., 2004), causing changes in soil fungal community. Several studies have correlated soil fungal community structure with the health of crop species (e.g. Li et al., 2014; Penton et al., 2014). However, we are unaware of any study documenting similarities or differences in soil fungal community structure in relation to plant health in invasive species.

In Australia, populations of several highly invasive and widespread exotic species, with the potential for causing significant adverse economic or ecological impacts, have experienced reductions in abundance in the past decade due to naturally occurring dieback, sometimes also referred as decline (defined here as a phenomenon that deteriorates plant health, often resulting in death of plant parts or whole plants, and not easily explained by any specific biotic or abiotic stress; Steinrucken et al., 2016). The dieback phenomenon is widely observed across northern Australia in the invasive plant, *Parkinsonia aculeata* (van Klinken and Heard, 2012). Similar observations of dieback have also been reported for the invasive species *Rubus anglocandicans* (European Blackberry) from populations in Western Australia (Aghighi et al., 2014), *Mimosa pigra* in the Northern Territory (Sacdalan, 2015) and *Vachellia nilotica* subsp. *indica* in central Queensland (Haque, 2015). Previous culture-based studies, particularly in *P. aculeata*, *M. pigra* and *V. nilotica*, indicated that dieback-affected plants harbour taxonomically diverse communities of fungal endophytes, including several potential pathogens. These include members of the Botryosphaeriaceae, which are capable of damaging plants, particularly when under stress (Diplock, 2015; Haque, 2015; Sacdalan, 2015). Further, Haque (2015) reported that endophytic isolates identified as *Cophinforma* spp. (Botryosphaeriaceae) were not only more abundant in dieback-affected than in healthy plants of *V. nilotica*, but were pathogenic on 2-week old seedlings, suggesting that these fungi could be potentially associated with the dieback.

In this study, we focused on populations of five major invasive species in arid (*Tamarix aphylla*), semi-arid (*Jatropha gossypiifolia, P. aculeata, V. nilotica*), and wet-dry tropical (*M. pigra*) Australia. We sampled living stems and soil from around the base of plants in paired dieback-affected and healthy sites within populations of these plant species (only *P. aculeata* and *M. pigra* were sampled from multiple geographic regions) and used internal transcribed spacer (ITS) amplicon pyrosequencing to characterise fungal communities. We addressed the following questions: (1) does the stem and soil fungal community diversity and structure differ among the five invasive plant species and among geographic regions for the two invasive species, *M. pigra* and *P. aculeata*?; and (2) does fungal richness, diversity and community structure vary between dieback-affected and healthy plants for these five invasive plant species, both locally and regionally?

2. Materials and methods

2.1. Focal invasive species and study sites

All five invasive species are small to medium-sized, woody plants. *V. nilotica* subsp. *indica*, *M. pigra* and *P. aculeata* (all Fabaceae) are native to tropical, semi-arid America. In Australia, *P. aculeata* is widely distributed across northern Australia (van Klinken and Heard, 2012), *M. pigra* is largely restricted to wetlands in the wet-dry tropics (Lonsdale, 1992) and *V. nilotica* is largely restricted to semi-arid Queensland (March, 2000). *T. aphylla* (Tamaricaceae) is native to North Africa and Asia. In Australia, the largest infestation is on the Finke River, in the arid interior (Brown and Grace, 2006). *J. gossypiifolia* (Euphorbiaceae) is native to tropical America and is widely distributed in the Northern Territory and northern Queensland (Bebawi et al., 2007).

Study sites were located where each species forms dense infestations and where dieback has been observed (Fig. 1, Table S1). Together these sites spanned diverse climate regions and a large