South African Journal of Botany 116 (2018) 103–109

Contents lists available at ScienceDirect

South African Journal of Botany

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

Re-establishment of *Protea repens* after clearing invasive *Acacia saligna*: Consequences of soil legacy effects and a native nitrophilic weedy species

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ARTICLE INFO

Article history:
Received 19 July 2017
Received in revised form 22 December 2017
Accepted 13 February 2018
Available online xxxx

Edited by T Kraaij

Keywords:
Biological invasion
Invasive acacias
Legacy effects
Nitrophilic weedy species

ABSTRACT

Invasive Australian acacias can alter soil chemistry and microbial communities in areas they invade. After clearing invasive acacias, these changes can persist, and previously invaded areas can become dominated by nitrophilic weedy species. Restoration of viable native plant communities in cleared sites often fails due to a lack of native species re-establishment. Therefore, to improve restoration outcomes, it is important to understand the effects of soil chemical and biotic legacies, and of nitrophilic weedy species, on native species re-establishment. To investigate the effect of soil chemical legacies, we germinated and grew *Protea repens* seedlings (a native proteoid shrub) as an indicator species in soil taken from areas cleared of *Acacia saligna* in lowland fynbos, as well as from non-invaded areas under controlled conditions. To investigate the effect of soil biotic legacies, we sterilized half the soil from each cleared or non-invaded area. We grew *Ehrharta calycina* (a native nitrophilic weedy grass species) in half of each treatment and measured the effect of treatments on *P. repens* germination and growth. Germination percentage, root and shoot dry mass of *P. repens* did not significantly differ between altered and native soil chemistry. The germination percentage of *P. repens* was significantly greater (93%) in the presence of soil microbial communities than in their absence. The presence of *E. calycina* significantly increased (29%) the root-to-shoot ratio of *P. repens* than their absence. Since the legacy of altered soil chemistry did not have a direct negative effect on *P. repens* germination and growth; we conclude that restoration efforts do not always have to manage altered soil chemistry after clearing invasive *A. saligna*.

1. Introduction

Australian acacias have been introduced to many parts of the world for various purposes (Richardson et al., 2011). A majority of these introductions have resulted in naturalization and ultimately widespread invasion (Richardson and Rejmánek, 2011). Most of the areas invaded by Australian acacias (e.g. South African lowland fynbos and Portuguese dune systems) have nutrient-poor soils characterized by low levels of nitrogen (Musil, 1993; Marchante et al., 2009). Invasive Australian acacias are "ecosystem transformers", as they can alter ecosystem processes and functions (sensu Richardson et al., 2000; Ehrenfeld, 2003). Invasion by acacias can alter the soil chemistry through mechanisms such as nitrogen fixation and litter production (Witkowski, 1991; Musil, 1993; Yelenik et al., 2004; Marchante et al., 2008); change the soil microbial community composition and function using mechanisms such as introduction of novel microbes and deposition of allelochemicals (Inderjit and Van Der Putten, 2010; Crisóstomo et al., 2013; Lorenzo et al., 2013; Rodríguez-Echeverría et al., 2013), exclude native species through competition (González-Muñoz et al., 2012; Gaertner et al., 2012a), and create feedback loops that favor their dominance (Gaertner et al., 2014).

It is often assumed that the negative impacts of invasive species will diminish after clearing the invasive species (Wittenberg and Cock, 2005). However, the negative impacts of invasive acacias can persist for long periods despite clearing the invasive species – i.e. they become legacy effects (Marchante et al., 2009; Rodríguez-Echeverría et al., 2013; Lazzaro et al., 2014; Souza-Alonso et al., 2014; Nsikani et al., 2017). Legacy effects include measurable changes in biological, soil chemical or physical conditions that persist after clearing the invasive species (sensu Corbin and D'Antonio, 2004).

Efforts to restore functional native plant communities are underway after clearing invasive acacias (Marchante et al., 2009; Le Maitre et al., 2011; Gaertner et al., 2012a). Restoration measures often include removing the nitrogen-rich invader litter left over after clearing (Marchante et al., 2009; Le Maitre et al., 2011), removing *Acacia* seedlings that germinate following clearing of adult plants (Marchante et al., 2010; Krupek et al., 2016) and re-introducing native species (Marchante et al., 2004; Gaertner et al., 2012a, 2012b). However, it has proved difficult

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https://doi.org/10.1016/j.sajb.2018.02.396
0254-6299/

to successfully restore functional native plant communities in cleared areas due to a lack of native species re-establishment (Galatowitsch and Richardson, 2005; Marchante et al., 2011). The lack of native species re-establishment is often associated with depleted native soil seed banks (Holmes and Cowling, 1997; Le Maitre et al., 2011; González-Muñoz et al., 2012).

The legacy of altered soil chemistry after clearing invasive acacias such as elevated nitrogen availability has been shown to persist for up to ten years (Marchante et al., 2009; Nsikani et al., 2017). Several studies have suggested that this legacy effect might directly have negative consequences for native species re-establishment by affecting their germination, growth, and/or indirectly by giving a competitive advantage to nitrophilic weedy species (Marchante et al., 2008, 2009; Le Maitre et al., 2011). Several vegetation surveys have reported that alien and/or native nitrophilic weedy species often dominate areas cleared of invasive acacias (Galatowitsch and Richardson, 2005; Blanchard and Holmes, 2008; Gaertner et al., 2012b; Fill et al., 2017). Soil microbial communities can influence the germination and growth of species during restoration (Balshor et al., 2017). Several studies have suggested that the legacy of altered soil microbial communities can have a negative influence on native species re-establishment through different mechanisms such as accumulation of pathogenic microbes and/or disruption of beneficial mutualisms (Bever et al., 1997; Eppinga et al., 2006; Callaway et al., 2008).

Currently there is limited knowledge on the effect of soil chemical and biotic legacies and nitrophilic weedy species after clearing invasive acacias on the re-establishment of proteoid shrubs. Furthermore, after clearing invasive acacias, previous studies have often used field observations instead of controlled experiments to reach conclusions about the effect of soil legacies and nitrophilic weedy species on the re-establishment of proteoid shrubs (e.g. Gaertner et al., 2012b; Fill et al., 2017). However, native species re-establishment under field conditions is affected by multiple interacting factors such as water availability and light. Using *Acacia saligna* (Labill.) H. L Wendl. (Fabaceae) invasions in the South African lowland fynbos as case study, we conducted a greenhouse experiment to investigate how germination and growth of a native proteoid shrub is affected by the invasive species' (i) soil chemical legacy, (ii) soil biotic legacy, and (iii) a native nitrophilic weedy species.

2. Materials and methods

2.1. Study sites

The study was conducted using soils collected from the Cape Flats Sand Fynbos (CFSF) of the Western Cape Province in South Africa, where invasion by *A. saligna* is common. The climate in the CFSF is Mediterranean with cool wet winters and hot dry summers and the soils are broadly classified as well-drained eolian acidic sands (Rebelo et al., 2006). Native CFSF vegetation consists of evergreen shrublands dominated by a mixture of ericoid and proteoid shrubs, and restioid (aphyllous graminoid) growth forms (Rebelo et al., 2006). Cape Flats Sand Fynbos is a critically endangered vegetation type with approximately 2% of the total historical area statutorily conserved, and it is the most transformed of the sand fynbos types occurring in the Greater Cape Floristic Region (Rebelo et al., 2006). We selected three study sites in the CFSF, namely, Blaauwberg Nature Reserve (33°4605.16″S; 18°27010.08″E), Youngsfield (34°0030.30″S; 18°29016.20″E) and Penhill (33°5900.39″S; 18°43037.74″E). The study sites varied in size from 1.5 to 9.5 km^2. Youngsfield and Penhill had similar soil types, whereas Blaauwberg Nature Reserve differed slightly (Table 1). Mean annual precipitation was between 361 and 1018 mm at the three study sites (Table 1).

In each study site, we selected non-invaded areas and previously invaded areas that had been cleared of invasive *A. saligna*. Each cleared area had experienced at least one fire in the last ten years (Table 1). The time between clearing and soil collection was between two and ten years, and the cleared areas received between one and seven follow-up treatments, in which re-emerging *A. saligna* seedlings were removed through weeding or herbicides (Table 1). Only one cleared area (Penhill) was immediately burnt after clearing. Cleared areas were dominated by nitrophilic weedy species such as *Briza maxima* L. and *Ehrharta calycina* Sm. (M. M. Nsikani, unpublished data). Non-invaded areas were characterized by mature native fynbos plant communities and were free from *A. saligna* invasion. We believe that the non-invaded areas closely represent the environmental characteristics of cleared areas prior to invasion and clearing. Non-invaded areas were kept free from *A. saligna* invasion through management and not as a result of different environmental characteristics. Each cleared area was characterized by different soil chemistry from its reference non-invaded area (Nsikani et al., 2017).

2.2. Study species

The native shrubs of the Proteaceae family are among the species that are most affected by *A. saligna* invasion in the CFSF (Holmes and Cowling, 1997). *Protea repens* was historically widespread in the CFSF, but its range has been severely reduced by invasive acacias (Witkowski, 1991). Therefore, we chose *P. repens* as a representative of proteoid shrubs for purposes of this study. *Ehrharta calycina* (Sm.)(Poaceae) is a native re-sprouting perennial grass that often dominates sites cleared of invasive acacias and disturbed environments (Yelenik et al., 2004; Fill et al., 2017). Therefore, we chose it as a representative of nitrophilic weedy species for purposes of this study. Seeds of *P. repens* and *E. calycina* harvested from populations of wild plants were obtained from Vula Environmental Services.

2.3. Study design

In each cleared or non-invaded area, we chose five random soil collection points ($n = 30$). In each soil collection point, organic-horizon material was removed and 44 l of soil excavated using a shovel to a depth of 10 cm (modified from Lehnhoff and Menalled, 2013). The soil from each cleared or non-invaded area was thoroughly mixed to obtain a representative soil sample. Half the soil from each cleared or

Table 1
History of study sites including the years after initial clearing; years since fire; whether the cleared site was burnt after initial *A. saligna* clearing; number of follow-up *A. saligna* clearing treatments; mean annual precipitation; and soil type.

Study site	Years after initial clearing	Years since fire	After clearing burn?	Number of follow-up treatments	Mean annual precipitation (mm)	Soil type (http://bgisviewer.sanbi.org)
Blaauwberg Nature Reserve	2	4	No	1	361	Grayish sandy soil
Youngsfield	6	6	No	4	1018	Soils with a sandy texture, leached and with sub-surface accumulation of organic matter and aluminum with/without oxides, either deep or on hard or weathering rock
Penhill	10	7	Yes	7	556	Soils with a sandy texture, leached and with sub-surface accumulation of organic matter and aluminum with/without oxides, either deep or on hard or weathering rock