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

M.M. Nsikani [a,*], B.W. van Wilgen [a], S. Bacher [a,b], M. Gaertner [a,c,d]

[a] *Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa*
[b] *Department of Biology, Ecology & Evolution Unit, University of Fribourg, Ch. du Musée 10, CH-1700 Fribourg, Switzerland*
[c] *Invasive Species Unit, Environmental Resource Management Department, City of Cape Town, Westlake Conservation Office, Ou Kaapse Weg, Tokai 7966, South Africa*
[d] *Nürtingen-Geislingen University of Applied Sciences (HFWU), Schelmenwasen 4-8, 72622 Nürtingen, Germany*

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

* Corresponding author.
 E-mail address: mllue06@gmail.com (M.M. Nsikani).

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

non-invaded area was sterilized to remove soil microbial communities using an autoclave machine at 80 °C in three 60-minute autoclaving periods with 24 h in-between each period (modified from Meiman et al., 2006), whilst the other half was not (soil microbial communities remained). We chose to use a whole soil treatment rather than inoculating sterile soil with live field soil because we were interested in measuring the total soil-mediated effects of *A. saligna* invasion including both biotic and abiotic components. We acknowledge that the use of soil sterilization through autoclaving and other available methods often presents a challenge because the process is known to alter soil characteristics under some circumstances and this can confound the effect of sterilizing (Troelstra et al., 2001; Perkins et al., 2013). To determine the effect of autoclaving on soil characteristics that could influence the germination and growth of *P. repens*, five soil samples were taken in the autoclaved and non-autoclaved soil from each cleared or non-invaded area, and analyzed for % carbon, % nitrogen, nitrate, ammonium and available phosphorus (Bemlab Pty Ltd., Somerset West).

The soil was taken to a 40- m² greenhouse tunnel with open ends to allow for ventilation, searched for *P. repens* and *E. calycina* seeds, and those that were found were removed. Seedling trays (40 × 27.5 × 11.5 cm) were filled with the soil. Fifty seeds of *P. repens* were planted in each seedling tray. This number was chosen to ensure sufficient germination numbers because germination numbers of *P. repens* are often low (Brown, 1993). The seeds of *P. repens* were pre-treated by soaking them in a smoke water solution for 18 h before planting to improve germination (Brown, 1993). To investigate the competitive or facilitative responses on germination and biomass of *P. repens* when grown together with nitrophilic weedy species, twenty *E. calycina* seeds per tray were sown into half the number of trays with sterilized or non-sterilized soil from each cleared or non-invaded area. Overall, a fully factorial and completely randomized experimental design was set up in the greenhouse with six replicates per treatment (three study sites × two invasion statuses × two soil microbial community conditions × two nitrophilic weedy species conditions × six replicates = 144 seedling trays).

Each tray was initially watered to field capacity and subsequently given 600 ml of water every two days. The location of trays was rotated in the greenhouse every two weeks. The number of germinating *P. repens* seedlings was recorded every seven days. Ten *E. calycina* seedlings were left in each tray after germination, and the rest were weeded out together with non-target species that germinated. *Protea repens* seedlings were harvested five months after planting. The soil from the roots was removed by wet sieving, and the roots separated from the shoots. All plant material was oven dried at 60 °C for 48 h and weighed.

In this study, we did not determine how the soil chemistry differed between each cleared area and its reference non-invaded area because results of those differences have been established in a previous study (Nsikani et al., 2017). Therefore, we based the legacy of altered soil chemistry in this study on those results.

2.4. Statistical analyses

The effects of soil chemical and biotic legacies, and *E. calycina* on *P. repens* root-to-shoot ratio, root and shoot dry mass were analyzed as explanatory variables using linear mixed-effects ANOVA models with Satterthwaite's approximation for denominator degrees of freedom using the *lmerTest* package (Kuznetsova et al., 2017) in R version 3.4.3 (R Development Core Team, 2017). Study site was included as a random effect to account for variability between study sites. Residual and Q-Q plots were applied to examine data normality. We first fitted different models with two and three-way interactions of the explanatory variables and included study site as random effects. To determine the explanatory variables that best explain variation in *P. repens* root-to-shoot ratio, root and shoot dry mass, we compared the different models using the anova( ) function. We only selected a model if its AICc value was less than the AICc value of all the other models even if

it did not include all the explanatory variables. If an additional parameter provides little or no increase in model fit, then the more complex model fits the data equally well or worse as the simpler model. The more complex model with the additional parameter should not be considered for ecological inference, since nothing is explained by the additional complexity (Burnham and Anderson, 2002; Richards, 2008). For each explanatory variable, only one model remained after model selection, and we report the results of these best-fitting models. Significant mean differences were separated with Tukey's HSD test using the *lsmeans* package (Lenth, 2016). The effect of soil sterilization on % carbon, % nitrogen, nitrate, ammonium and available phosphorus was also analyzed as an explanatory variable using linear mixed-effects ANOVA models with Satterthwaite's approximation for denominator degrees of freedom using the *lmerTest* package (Kuznetsova et al., 2017). Study site nested with invasion status was included as a random effect to account for variability between the cleared-non-invaded pairs in each study site.

3. Results

Germination percentage, root and shoot dry mass of *P. repens* did not significantly differ between altered, and native soil chemistry as "invasion status" was not selected in any best-fitting model for any response variable. Germination percentage of *P. repens* was significantly higher (93%; $p < 0.001$) in the presence of soil microbial communities than in their absence (Fig. 1). There was a significant interaction between the effects of soil microbial communities and *E. calycina* on root ($p = 0.018$; Fig. 2) and shoot ($p < 0.001$; Fig. 3) dry mass of *P. repens*. Root-to-shoot ratio of *P. repens* was significantly higher (29%; $p < 0.001$; Fig. 4) in the presence of *E. calycina* than in their absence. Soil sterilization led to a significant increase in % nitrogen (38%; $p = 0.017$; Fig. 5), ammonium (83%; $p < 0.001$; Fig. 5) and available phosphorus (61%; $p = 0.041$; Fig. 5).

4. Discussion

Our results suggest that the legacy of altered soil chemistry does not have direct negative consequences on the re-establishment of native proteoid shrubs because the germination and growth of *P. repens* was
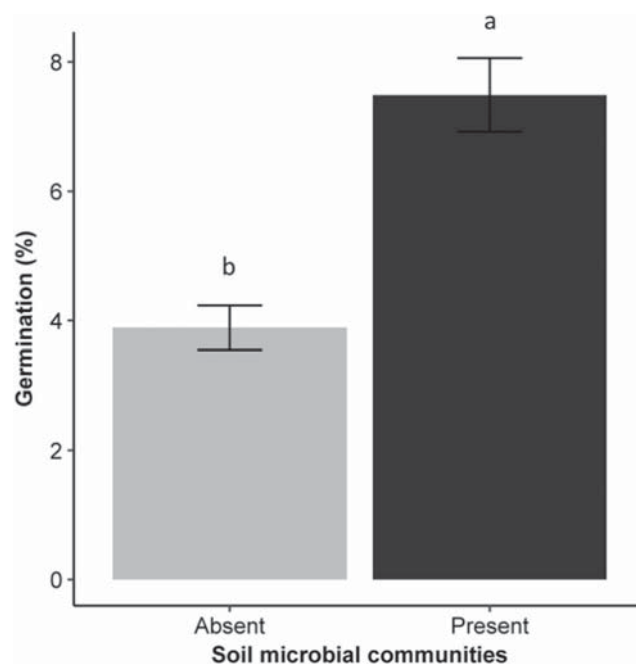

Fig. 1. The effect of soil microbial communities on the germination percentage of *P. repens*. Mean values with the same letter are not significantly different. Error bars represent ± SE.
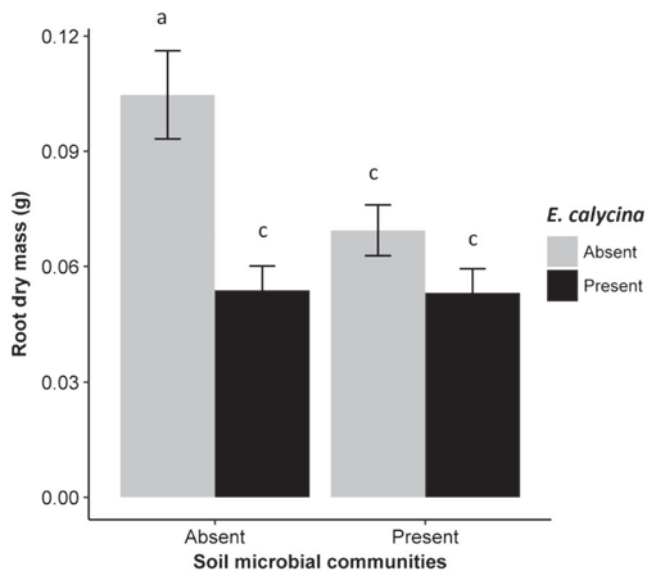
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Fig. 2. The effects of soil microbial communities and *E. calycina* on the root dry mass of *P. repens*. Mean values with the same letter are not significantly different. Error bars represent ± SE.
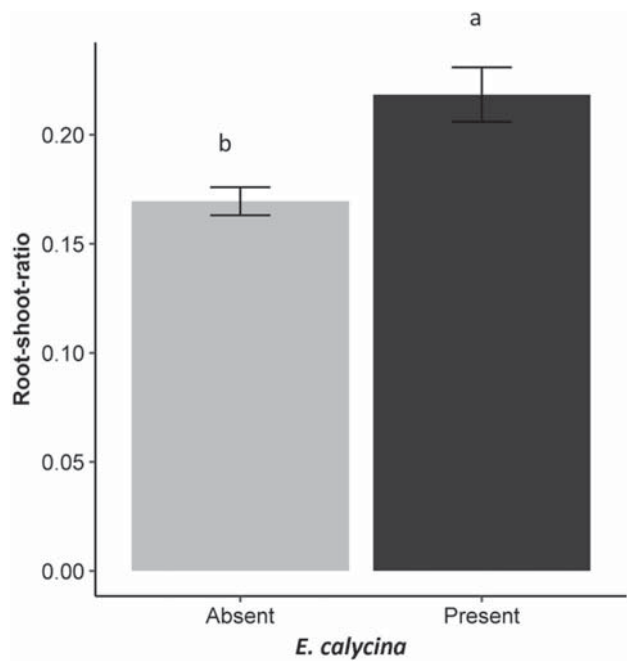

Fig. 4. The effect of *E. calycina* on the root-to-shoot ratio of *P. repens*. Mean values with the same letter are not significantly different. Error bars represent ± SE.

the same despite differences in soil chemistry between previously invaded and non-invaded areas. Our results support the findings of Musil (1993) and Holmes (2008) which suggested that the growth of *Protea* seedlings is often unaffected by changes in soil chemistry. Furthermore, Holmes (2008) suggested that if total soil available nitrogen is less than double that in native areas, it is unlikely to hinder the establishment of fynbos seedlings (Holmes, 2008). Our findings, put together with those of these other authors, follow the general principle that plants from infertile areas such as South African lowland fynbos, that have relatively low growth and nutrient absorption rates, are often unresponsive to changes in soil characteristics (Grime and Hunt, 1975; Chapin, 1980). The re-establishment of other typical native fynbos species besides proteoid shrubs, irrespective of whether their seeds were sown or not, was also not negatively affected by the legacy

of altered soil chemistry after clearing invasive acacias (Gaertner et al., 2012b; Mostert et al., 2016). The general unresponsiveness of native seedlings to altered soil chemistry has also been observed with other invasive species (e.g. *Psidium cattleianum*, *Macaranga mappa*, *Falcataria moluccana Melastoma septemnervium* and *Clidemia hirta*; Ostertag et al., 2009; and *Acacia dealbata*; Lorenzo et al., 2017). However, our results conflict with the findings of Witkowski (1991) which suggested that *P. repens* seedlings displayed an increase in growth in response to increased nutrient availability. We believe that this is a result of differences in the levels of soil nutrients, particularly nitrogen, explored by the two studies. Witkowski (1991) looked at the effect of growing *P. repens* in soils with nitrogen levels between 50 and 200 g/m^2, which were much higher than those in this study (Nsikani et al., 2017) and in Musil (1993).

Our results suggest that the presence of soil microbial communities can improve *P. repens* germination. Our findings are in line with the results of a study by Lozano et al. (2017) that suggested that soil microbial communities improved the germination of several annual plants in a Mediterranean ecosystem, when compared to soils in which microbial communities were absent. However, our results are contrary to those from several studies where the presence of soil microbial communities either had a negative (Crist and Friese, 1993; O'Hanlen-Manners and Kotanen, 2004) or no effect on native species germination (Rudgers and Orr, 2009). Prior to soil contact, microbial communities can be found on internal seed tissues (Gallery et al., 2007) and the surface (Kremer, 1986). Microbial communities on the seed surface can play a role in preventing pathogen infection when the seeds are in the soil (Kremer, 1987). However, seeds may also become infected by microbes such as ascomycetous fungi after burial in the soil, and these may prevent pathogenic attacks on seed contents and/or slow decay of seed enclosing structures (Kluger et al., 2008; Dalling et al., 2011). It is crucial for beneficial microbes to establish first so as to limit subsequent pathogenic infection (Dalling et al., 2011). We suspect that in the presence of soil microbial communities in our study, *P. repens* seeds were infected by beneficial microbes after burial in the soil. These microbes could have played a role in maintaining seed viability, thereby improving germination. However, this requires more testing and direct evidence. The effect of soil microbial communities on germination
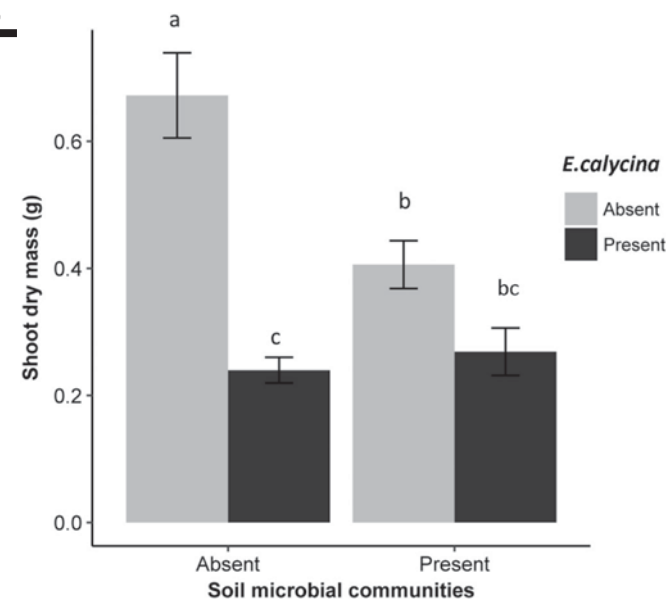

Fig. 3. The effects of soil microbial communities and *E. calycina* on the shoot dry mass of *P. repens*. Mean values with the same letter are not significantly different. Error bars represent ± SE.
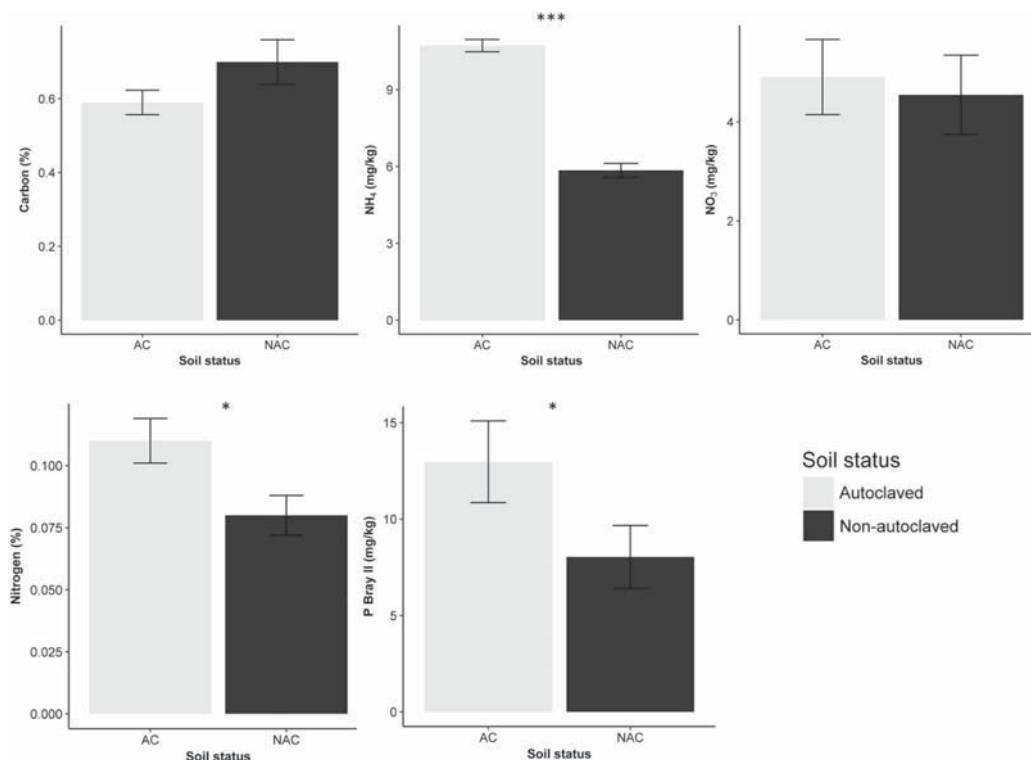
Fig. 5. Results of linear mixed-effects ANOVA models of the different soil nutrients before and after soil sterilization. Mean values with the same letter are not significantly different. Error bars represent ± SE. Significance indicated in bold as: *p < 0.05; ***p < 0.001.

is largely unexplored and the identity, and function of most seed-associated microbial communities is currently unknown (Lopez-Velasco et al., 2013). More research is required to elucidate the effects of microbial communities on germination rates during restoration projects.

The significant interaction between the effects of soil microbial communities and *E. calycina* on *P. repens* root and shoot dry mass suggests that, in the absence of *E. calycina*, the absence of soil microbial communities leads to significantly higher root and shoot growth than their presence. However, the presence of *E. calycina* overwhelms this beneficial effect and significantly reduces *P. repens* root and shoot growth. We suspect that growth reduction in the presence of soil microbial communities might have been caused by pathogenic microbes that accumulated during invasion and/or disruption of native mutualistic associations, although further research would be required to be able to explain this (Bever et al., 1997; Eppinga et al., 2006; Inderjit and Van Der Putten, 2010). A study conducted by Mangla et al. (2008) suggested that invasion by *Chromolaena odorata* led to the accumulation of the soil pathogen *Fusarium spp.*, which reduced the seedling growth of a native species. However, mycorrhizas are absent from the *Protea* family (Allsopp and Stock, 1993), and proteoid shrubs are considered much less dependent on soil microbial symbionts than most South African fynbos taxa owing to their specialized roots that are efficient in nutrient uptake (Lamont, 1986, Stock et al., 1990). It is also possible that in the absence of soil microbial communities, *P. repens* could have responded to soil nutrient changes imposed by soil sterilization (Fig. 5; Perkins et al., 2013). Future studies could use other techniques such as soil inoculation instead of soil sterilization to further elucidate the role of soil microbial communities on *P. repens* germination and growth.

The reduction in *P. repens* root and shoot growth, when *E. calycina* was present, but soil microbial communities were absent, was most likely a consequence of competition for soil moisture and nutrients (D'Antonio and Vitousek, 1992). Grasses are good competitors against woody species' seedlings because of their rapid uptake of water and nutrients (D'Antonio and Vitousek, 1992) as a result of their dense

and shallow root systems (Phillips, 1963). In *Banksia* woodlands in south-west Australia, *E. calycina* invasion is one of the major causes of poor native species establishment because *E. calycina* suppresses native seedling growth by out-competing them for soil moisture (Fisher et al., 2009). Our results suggest that in the presence of soil microbial communities, the presence or absence of *E. calycina* does not make a difference to *P. repens* root and shoot growth. We suspect that pathogenic microbes that were most likely accumulated during invasion had a significant negative effect on *P. repens* root and shoot growth that was equal to the effect of *E. calycina* presence (Reinhart and Callaway, 2006).

The increase in root-to-shoot ratio (when grown with *E. calycina*) could be a response by *P. repens* seedlings to avoid competition from *E. calycina* for moisture and nutrients, by investing more into root growth, thereby accessing water and nutrients lower in the soil profile (Reynolds and D'Antonio, 1996; Casper and Jackson, 1997). The increase in root-to-shoot ratio is most likely a result of diverting photosynthates into the root system to support lateral root growth towards soil pockets that have more water and nutrients (Drew and Saker, 1975). However, this is not always the case because a shortage of certain minerals such as potassium, magnesium and manganese can lead to reduction in root growth (Ericsson et al., 1996). In a nursery experiment conducted in Denmark, *Quercus robur* seedlings were grown together with grasses, and this led to an increase in their root-to-shoot ratio (Andersen et al., 2000). However, Newton and Cole (1991) suggested that competition was not solely responsible for the alteration of biomass allocation because allocation of photosynthates to roots and shoots can be constant over a wide range of competition intensities.

We acknowledge that growing *P. repens* alone and with *E. calycina* is not an adequate test for competition because an increase in the density of *P. repens* could have given the same outcome (Li and Wilson, 1998). We suggest that future studies should manipulate the densities of both *P. repens* and *E. calycina*. Furthermore, future studies should use a variety of native species (representing the major fynbos guilds) and nitrophilic weedy species (alien and native).

5. Conclusions and implications for restoration

Overall, our results suggest that the legacy of altered soil chemistry after clearing invasive *A. saligna* does not necessarily have direct negative consequences on the re-establishment of native proteoid shrubs. While the soil microbial communities after clearing invasive *A. saligna* may have a positive effect on the germination of native proteoid shrubs, the legacy of altered soil microbial communities and presence of nitrophilic weedy species could have negative impacts on their growth. Our results provide some insight on the effect of soil legacies of invasive acacias, and nitrophilic weedy species on native species re-establishment, but given that the results of studies from elsewhere are often inconclusive or contradictory, it is clear that further research is needed.

Since the legacy of altered soil characteristics does not always have a direct negative effect on the re-establishment of native proteoid shrubs, we do not see a need for management interventions to always attempt to restore native soil chemistry. Furthermore, to our knowledge there is no restoration strategy that can be successfully used to restore overall native soil chemistry, although methods to immobilize elevated nitrogen are available. If elevated nitrogen availability, legacy of altered soil microbial communities and nitrophilic weedy species prove to be barriers to native species re-establishment during restoration; we urge practicing restoration ecologists to incorporate management actions such as soil carbon addition, soil microbial treatments and prescribed burning into their restoration programs (reviewed in Nsikani et al., 2018).

Conflicts of interest

None.

Appendix A

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sajb.2018.02.396.

References

Allsopp, N., Stock, W.D., 1993. Mycorrhizal status of plants growing in the Cape Floristic Region, South Africa. Bothalia 23, 91–104.

Andersen, L., Rasmussen, H.N., Brander, P.E., 2000. Regrowth and dry matter allocation in *Quercus robur* (L.) seedlings root pruned prior to transplanting. New Forests 19, 205–214.

Balshor, B.J., Garrambone, M.S., Austin, P., Balazs, K.R., Weihe, C., Martiny, J.B., Huxman, T.E., McCollum, J.R., Kimball, S., 2017. The effect of soil inoculants on seed germination of native and invasive species. Botany 95, 469–480.

Bever, J.D., Westover, K.M., Antonovics, J., 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology 85, 561–573.

Blanchard, R., Holmes, P.M., 2008. Riparian vegetation recovery after invasive alien tree clearance in the Fynbos Biome. South African Journal of Botany 74, 421–431.

Brown, N.A.C., 1993. Promotion of germination of fynbos seeds by plant-derived smoke. New Phytologist 123, 575–583.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. second edition. Springer-Verlag, New York.

Callaway, R.M., Cipollini, D., Barto, K., Thelen, G.C., Hallett, S.G., Prati, D., Stinson, K., Klironomos, J., 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89, 1043–1055.

Casper, B.B., Jackson, R.B., 1997. Plant competition underground. Annual Review of Ecology and Systematics 28, 545–570.

Chapin, F.S., 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11, 233–260.

Corbin, J.D., D'Antonio, C.M., 2004. Effects of exotic species on soil nitrogen cycling: implications for restoration. Weed Technology 18, 1464–1467.

Crist, T.O., Friese, C.F., 1993. The impact of fungi on soil seeds: implications for plants and granivores in semiarid shrub-steppe. Ecology 74, 2231–2239.

Crisóstomo, J.A., Rodríguez-Echeverría, S., Freitas, H., 2013. Co-introduction of exotic rhizobia to the rhizosphere of the invasive legume *Acacia saligna*, an intercontinental study. Applied Soil Ecology 64, 118–126.

Dalling, J.W., Davis, A.S., Schutte, B.J., Elizabeth Arnold, A., 2011. Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. Journal of Ecology 99, 89–95.

D'Antonio, C.M., Vitousek, P.M., 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23, 63–87.

Drew, M.C., Saker, L.R., 1975. Nutrient supply and the growth of the seminal root system in barley: II. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. Journal of Experimental Botany 26, 79–90.

Ehrenfeld, J.G., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6, 503–523.

Eppinga, M.B., Rietkerk, M., Dekker, S.C., De Ruiter, P.C., Van der Putten, W.H., 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. Oikos 114, 168–176.

Ericsson, T., Rytter, L, Vapaavuori, E., 1996. Physiology of carbon allocation in trees. Biomass and Bioenergy 11, 115–127.

Fill, J.M., Kritzinger-Klopper, S., van Wilgen, B.W., 2017. Short-term vegetation recovery after alien plant clearing along the Rondegat River, South Africa. Restoration Ecology (in press).

Fisher, J.L., Loneragan, W.A., Dixon, K., Delaney, J., Veneklaas, E.J., 2009. Altered vegetation structure and composition linked to fire frequency and plant invasion in a biodiverse woodland. Biological Conservation 142, 2270–2281.

Gaertner, M., Holmes, P.M., Richardson, D.M., 2012a. Biological invasions, resilience and restoration. In: van Andel, J., Aronson, J. (Eds.), Restoration Ecology: The New Frontier, 2nd edition Blackwell Publishing Ltd., Oxford, pp. 265–280.

Gaertner, M., Nottebrock, H., Fourie, H., Privett, S.D.J., Richardson, D.M., 2012b. Plant invasions, restoration, and economics: perspectives from South African fynbos. Perspectives in Plant Ecology, Evolution and Systematics 14, 341–353.

Gaertner, M., Biggs, R., Te Beest, M., Hui, C., Molofsky, J., Richardson, D.M., 2014. Invasive plants as drivers of regime shifts: identifying high-priority invaders that alter feedback relationships. Diversity and Distributions 20, 733–744.

Galatowitsch, S., Richardson, D.M., 2005. Riparian scrub recovery after clearing of invasive alien trees in headwater streams of the Western Cape, South Africa. Biological Conservation 122, 509–521.

Gallery, R.E., Dalling, J.W., Arnold, A.E., 2007. Diversity, host affinity and distribution of seed-infecting fungi: a case-study with neotropical *Cecropia*. Ecology 83, 582–588.

González-Muñoz, N., Costa-Tenorio, M., Espigares, T., 2012. Invasion of alien *Acacia dealbata* on Spanish *Quercus robur* forests: impact on soils and vegetation. Forest Ecology and Management 269, 214–221.

Grime, J.P., Hunt, R., 1975. Relative growth-rate: its range and adaptive significance in a local flora. Journal of Ecology 63, 393–422.

Holmes, P.M., Cowling, R.M., 1997. Diversity, composition and guild structure relationships between soil stored seed banks and mature vegetation in alien plant invaded South African fynbos shrubland. Plant Ecology 133, 107–122.

Holmes, P.M., 2008. Optimal ground preparation treatments for restoring lowland Sand Fynbos vegetation on old fields. South African Journal of Botany 74, 33–40.

Inderjit, van der Putten, W.H., 2010. Impacts of soil microbial communities on exotic plant invasions. Trends in Ecology & Evolution 25, 512–519.

Kluger, C.G., Dalling, J.W., Gallery, R.E., Sanchez, E., Weeks-Galindo, C., Arnold, A.E., 2008. Prevalent host-generalism among fungi associated with the seeds of four neotropical pioneer species. Journal of Tropical Ecology 24, 332–351.

Kremer, R.J., 1986. Microorganisms associated with velvetleaf (*Abutilon theophrasti*) seeds on the soil surface. Weed Science 34, 233–236.

Kremer, R.J., 1987. Identity and properties of bacteria inhabiting seeds of broadleaf weed species. Microbial Ecology 14, 29–37.

Krupek, A., Gaertner, M., Holmes, P.M., Esler, K.J., 2016. Assessment of post-burn removal methods for *Acacia saligna* in Cape Flats Sand Fynbos, with consideration of indigenous plant recovery. South African Journal of Botany 105, 211–217.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. LmerTest package: tests in linear mixed effects models. Journal of Statistical Software 82, 1–26.

Lamont, B., 1986. The significance of proteoid roots in proteas. Acta Horticulturae 185, 163–170.

Lazzaro, L., Giuliani, C., Fabiani, A., Agnelli, A.E., Pastorelli, R., Lagomarsino, A., Benesperi, R., Calamassi, R., Foggi, B., 2014. Soil and plant changing after invasion: the case of *Acacia dealbata* in a Mediterranean ecosystem. Science of the Total Environment 497, 491–498.

Le Maitre, D.C., Gaertner, M., Marchante, E., Ens, E.J., Holmes, P.M., Pauchard, A., O'Farrell, P.J., Rogers, A.M., Blanchard, R., Blignaut, J., Richardson, D.M., 2011. Impacts of invasive Australian acacias: implications for management and restoration. Diversity and Distributions 17, 1015–1029.

Lehnhoff, E.A., Menalled, F.D., 2013. Impacts of Tamarix-mediated soil changes on restoration plant growth. Applied Vegetation Science 16, 438–447.

Lenth, R.V., 2016. Least-squares means: the R package lsmeans. Journal of Statistical Software 69, 1–33.

Li, X., Wilson, S.D., 1998. Facilitation among woody plants establishing in an old field. Ecology 79, 2694–2705.

Lopez-Velasco, G., Carder, P.A., Welbaum, G.E., Ponder, M.A., 2013. Diversity of the spinach (*Spinacia oleracea*) spermosphere and phyllosphere bacterial communities. FEMS Microbiology Letters 346, 146–154.

Lorenzo, P., Pereira, C.S., Rodríguez-Echeverría, S., 2013. Differential impact on soil microbes of allelopathic compounds released by the invasive *Acacia dealbata* Link. Soil Biology and Biochemistry 57, 156–163.

Lorenzo, P., Rodríguez, J., González, L., Rodríguez-Echeverría, S., 2017. Changes in micro-habitat, but not allelopathy, affect plant establishment after *Acacia dealbata* invasion. Journal of Plant Ecology 10, 610–617.

Lozano, Y.M., Armas, C., Hortal, S., Casanoves, F., Pugnaire, F.I., 2017. Disentangling above- and below-ground facilitation drivers in arid environments: the role of soil microorganisms, soil properties and microhabitat. New Phytologist 216, 1236–1246.

Mangla, S., Inderjit, Callaway, R.M., 2008. Exotic invasive plant accumulates native soil pathogens which inhibit native plants. Journal of Ecology 96, 58–67.

Marchante, H.S., Marchante, E.M., Buscardo, E., Maia, J., Freitas, H., 2004. Recovery potential of dune ecosystems invaded by an exotic *Acacia* species (*Acacia longifolia*). Weed Technology 18, 1427–1433.

Marchante, E., Kjøller, A., Struwe, S., Freitas, H., 2008. Short- and long-term impacts of *Acacia longifolia* invasion on the belowground processes of a Mediterranean coastal dune ecosystem. Applied Soil Ecology 40, 210–217.

Marchante, E., Kjøller, A., Struwe, S., Freitas, H., 2009. Soil recovery after removal of the N$_2$-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration. Biological Invasions 11, 813–823.

Marchante, H., Freitas, H., Hoffmann, J.H., 2010. Seed ecology of an invasive alien species, *Acacia longifolia* (Fabaceae), in Portuguese dune ecosystems. American Journal of Botany 97, 1780–1790.

Marchante, H., Freitas, H., Hoffmann, J.H., 2011. Post-clearing recovery of coastal dunes invaded by *Acacia longifolia*: is duration of invasion relevant for management success? Journal of Applied Ecology 48, 1295–1304.

Meiman, P.J., Redente, E.F., Paschke, M.W., 2006. The role of the native soil community in the invasion ecology of spotted (*Centaurea maculosa* auct. non Lam.) and diffuse (*Centaurea diffusa* Lam.) knapweed. Applied Soil Ecology 32, 77–88.

Mostert, E., Gaertner, M., Holmes, P.M., Rebelo, A.G., Richardson, D.M., 2016. Impacts of invasive alien trees on threatened lowland vegetation types in the cape floristic region, South Africa. South African Journal of Botany 108, 209–222.

Musil, C.F., 1993. Effect of invasive Australian acacias on the regeneration, growth and nutrient chemistry of South African lowland fynbos. Journal of Applied Ecology 30, 361–372.

Newton, M., Cole, E.C., 1991. Root development in planted Douglas-fir under varying competitive stress. Canadian Journal of Forest Research 21, 25–31.

Nsikani, M.M., Novoa, A., van Wilgen, B.W., Keet, J.H., Gaertner, M., 2017. *Acacia saligna*'s soil legacy effects persist up to 10 years after clearing: implications for ecological restoration. Austral Ecology 42, 880–889.

Nsikani, M.M., van Wilgen, B.W., Gaertner, M., 2018. Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N$_2$-fixing woody species: implications for ecological restoration. Restoration Ecology 26, 235–244.

O'Hanlen-Manners, D.L., Kotanen, P.M., 2004. Evidence that fungal pathogens inhibit recruitment of a shade-intolerant tree, white birch (*Betula papyrifera*) in understory habitats. Oecologia 140, 650–653.

Ostertag, R., Cordell, S., Michaud, J., Cole, T.C., Schulten, J.R., Publico, K.M., Enoka, J.H., 2009. Ecosystem and restoration consequences of invasive woody species removal in Hawaiian Lowland Wet Forest. Ecosystems 12, 503–515.

Perkins, L.B., Blank, R.R., Ferguson, S.D., Johnson, D.W., Lindemann, W.C., Rau, B.M., 2013. Quick start guide to soil methods for ecologists. Perspectives in Plant Ecology, Evolution and Systematics 15, 237–244.

Phillips, W.S., 1963. Depth of roots in soil. Ecology 44, 424–429.

R Development Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna (Available from URL: http://www.R-Project.org).

Rebelo, A.G., Boucher, C., Helme, N.A., Mucina, L., Rutherford, M.C., 2006. Fynbos biome. In: Mucina, L., Rutherford, M.C. (Eds.), The Vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria, pp. 53–219.

Reinhart, K.O., Callaway, R.M., 2006. Soil biota and invasive plants. New Phytologist 170, 445–457.

Reynolds, H.L., D'Antonio, C., 1996. The ecological significance of plasticity in root weight ratio in response to nitrogen: opinion. Plant and Soil 185, 75–97.

Richards, S.A., 2008. Dealing with over dispersed count data in applied ecology. Journal of Applied Ecology 45, 218–227.

Richardson, D.M., Rejmánek, M., 2011. Trees and shrubs as invasive alien species – a global review. Diversity and Distributions 17, 788–809.

Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D., West, C.J., 2000. Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6, 93–107.

Richardson, D.M., Carruthers, J., Hui, C., Impson, F.A.C., Miller, J.T., Robertson, M.P., Rouget, M., Le Roux, J.J., Wilson, J.R.U., 2011. Human-mediated introductions of Australian acacias – a global experiment in biogeography. Diversity and Distributions 17, 771–787.

Rodríguez-Echeverría, S., Afonso, C., Correia, M., Lorenzo, P., Roiloa, S.R., 2013. The effect of soil legacy on competition and invasion by *Acacia dealbata* link. Plant Ecology 214, 1139–1146.

Rudgers, J.A., Orr, S., 2009. Non-native grass alters growth of native tree species via leaf and soil microbes. Journal of Ecology 97, 247–255.

Souza-Alonso, P., Gonzalez, L., Cavaleiro, C., 2014. Ambient has become strained. Identification of *Acacia dealbata* Link volatiles interfering with germination and early growth of native species. Journal of Chemical Ecology 40, 1051–1061.

Stock, W., Pate, J., Delfs, J., 1990. Influence of seed size and quality on seedling development under low nutrient conditions in five Australian and South African members of the Proteaceae. Journal of Ecology 78, 1005–1020.

Troelstra, S.R., Wagenaar, R., Smant, W., Peters, B.A.M., 2001. Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. New Phytologist 150, 697–706.

Witkowski, E.T.F., 1991. Growth and competition between seedlings of *Protea repens* (L.) L. and the alien invasive, *Acacia saligna* (Labill) Wendl. in relation to nutrient availability. Functional Ecology 5, 101–110.

Wittenberg, R., Cock, M.J.W., 2005. Best practices for the prevention and management of invasive alien species. In: Mooney, H.A., Mack, R.N., McNeely, J.A., Neville, L.E., Schei, P.J., Waage, J.K. (Eds.), Invasive Alien Species. A New Synthesis. Island Press, Washington, p. 368.

Yelenik, S.G., Stock, W.D., Richardson, D.M., 2004. Ecosystem level impacts of invasive *Acacia saligna* in the south African fynbos. Restoration Ecology 12, 44–51.

http://bgisviewer.sanbi.org [Cited 02 January 2018].