Interactions between native and exotic plants in the context of grassland restoration and the importance of below-ground processes

Monique Ellie Smith

PhD at School of Biological Sciences, University of Adelaide, SA

Supervisors: José M. Facelli, Timothy Cavagnaro and Leanne Rosser

Summary

Australia's lowland temperate grasslands and open grassy woodlands are one of our rarest ecosystems. This has spurred an interest in restoring these systems; however, this can be very challenging with many barriers preventing the establishment of resilient native plant communities. My PhD project aimed to identify and overcome some of these challenges that limit restoration efforts and to develop a deeper understanding of the processes hindering native grass establishment and to improve the effectiveness of techniques used in the restoration of native grasslands. Funding from the Australian Flora Foundation went towards 4WD car hire, to access my field site, and soil chemical analysis, carried out by an external company. These

two components covered several aspects of my thesis including two field trials, aimed at reducing invasive plant competition, which are currently in review at international journals. Therefore, I will be focussing on the glasshouse study which has been published in *Plant & Soil* (Smith et al., 2018).

In this paper, we used soil from a degraded old-field and a remnant grassland to explore home-field advantages in plant-soil feedbacks and plant responses to the abiotic (e.g. nutrients) and biotic (e.g. bacteria and fungi) soil conditions. We also recorded the responses of the soil microbial community to changes in soil conditions and plant species. Plants (both native and invasive species) grew larger when microbes were added (as a whole soil inoculant) to its sterilized home soil. However, this relationship is complex, with microbial communities changing in response to the plant species and soil type. The apparent home-field advantage of the soil microbes shown in this study may restrict the utility of inoculants as a management tool. In the very least, these findings demonstrate the importance of soil amendments, both abiotic and biotic, in ecological restoration and greater consideration of these should lead to more successful and sustainable restoration outcomes in grassland habitats. This was a very detailed experiment so here I will only present relevant results but see the published version for more information.

Introduction

The importance of native ecosystems is being ever more realised as human-induced environmental change leads to ecosystem degradation. For example, the majority of Australia's lowland temperate grasslands and open grassy woodlands have been destroyed, mainly due to their suitability for agriculture. This type of anthropogenic change has spurred increased efforts to restore ecosystems. However, the sites used for ecological restoration are often degraded and success can be limited. In previously cultivated landscapes (old-fields), for example, the legacy of farming practices can persist for decades. This can include increased nutrient availability, soil compaction, changes to soil microbial communities, destruction of native seed bank and invasion of exotic species (Facelli & Pickett, 1990; Cramer et al., 2008). Such legacies often challenge restoration efforts thus highlighting the need for restoration techniques based on solid (i.e. mechanistic) ecological knowledge and which enhance native flora establishment.

Soil microbes, such as bacteria and fungi, can affect plants either positively or negatively through pathogenic effects, aeration of soils and controlling nutrient cycles (Wolfe & Klironomos, 2005; Ferrazzano & Williamson, 2013). Symbiotic relationships are also very important: around 80% of vascular plants rely on soil microbes to aid nutrient uptake (Ferrazzano and Williamson 2013). Therefore, it is understandable why soil inoculations, i.e. introducing mutualistic soil microbes, has received so much attention in ecological restoration (Neuenkamp et al., 2018). However, it is important to understand how the function diversity of inoculated microbial communities may change depending on what type of soil they are introduced to. This is particularly important in severely degraded sites such as old-fields where the soil properties can be very different to native areas.

Here I present results of a study in which we attempted to separate the abiotic and biotic components of both remnant and old-field soil of South Australian grasslands and measure their impact on plant performance of two grass species. We chose a native perennial species *Rytidosperma auriculatum* (J. M. Black; Lobed Wallaby Grass) and an invasive annual *Avena barbata* (Pott ex Link; bearded oat). This was carried out in a glasshouse experiment where soil treatments were made up of whole-soil inoculant and from an old-field and a remnant grassland transplanted into home and away (sterilized) soil. We also had sterilized controls to separate soil abiotic factors from biotic factors. By growing wallaby grass and bearded oat separately in each of eight soil treatments we sought to answer the following questions

1) do the plant-soil interactions show a home-field advantage when inoculants and plants are added to their home soil?

Further, by using DNA sequencing techniques to characterize the bacterial and fungal communities we were able to ask the following questions:

2) do microbial communities differ between the inoculant sources (old-field and remnant)? and
3) do the communities change depending on which bulk soil they are added to or which plant species they are exposed to?

Overall, this study aims to have applied outcomes by building on our understanding of mechanisms involved in invasive species dominance and the requirements of native species for restoration projects.

Methods

Soil and seed collection

Soil and seed collection was carried out at Para Woodlands Reserve, South Australia. The reserve lies in a region with a Mediterranean-type climate with mean annual (winter dominated) rainfall of 450 mm, and a mean annual air temperature of 23.6 °C. The reserve is an active restoration site with some areas degraded by previous farming practices, mainly cereal cropping, livestock grazing and regular fertilizer application until farming ceased in 2004. Areas of remnant vegetation in comparatively good condition are present nearby and have lower soil nutrients than the neighbouring old-field areas (Rosser, 2013), though grazing likely occurred in these areas prior to 2004. These remnant areas are classified as open grassy woodlands dominated by grasses, such as, *Rytidosperma* species Steud., *Austrostipa* species S.W.L. Jacobs & J. Everett or *Themeda triandra* (R.Br.) Stapf with an over-story of *Eucalyptus camaldulensis* (Dehnh.) and *E. leucoxylon* F. Muell.

Soil was collected during June 2016 from two locations within Para Woodlands: an old-field and a remnant grassland. Soil collection occurred within three 20 x 20 m plots at each site, taking soil from the base of either invasive (old-field) or native (remnant) grasses. All soil was collected to a depth of 10 cm, sieved (<3 mm) and stored at 4 °C until further processing.

Rytidosperma auriculatum was chosen for this experiment because it is an Australian native, winter-growing grass, common in the region and is widely used for restoration at Para Woodlands and surrounding areas. Its performance was compared to the annual, winter-growing, *A. barbata*, which is originally from central Asia and the Mediterranean and now invasive worldwide. *Avena barbata* has been shown to be a strong competitor in this region, reducing species richness and the occurrence of Wallaby grass species (Lenz et al., 2003) and it was the dominant species in the old-field site as indicated by 100 % cover in a pilot study (data not shown). Seed collection occurred in Spring 2015 from Para Woodland's seed orchard (*R. auriculatum*) or from the old-field site (*A. barbata*).

Glasshouse-based microcosm experiment

Each soil treatment was made up of 85 % sterilised bulk soil, from the old-field and remnant areas (Figure 1). It was important to use sterilised soil to separate the chemical from the biological properties in the soil. The bulk soil was then mixed with two inoculant types,

either unsterilized (referred to as 'live' hereafter) or sterilized (referred to as 'mock' hereafter; used as a control) inoculum. This inoculum, which made up the remaining 15 % of soil treatments, was collected from both the old-field and remnant sites (referred to as 'inoculant sources' hereafter; Figure 1). This gave a total of eight inoculation treatments (2 bulk soils x 2 inoculant types x 2 inoculant sources). Soils were sterilised by twice-autoclaving for one hour at 121 °C.

Seeds of the two test species were germinated in the dark on trays of vermiculate and paper towel in a germination cabinet at 12 °C, with regular watering over a period of two weeks. All pots were planted with one seedling of equal size and any seedling that died in the first two weeks was replaced. The pots were then arranged in a randomised block design in the greenhouse, with one replicate from each treatment combination per block. Pots were watered to 75 % field capacity thrice weekly.
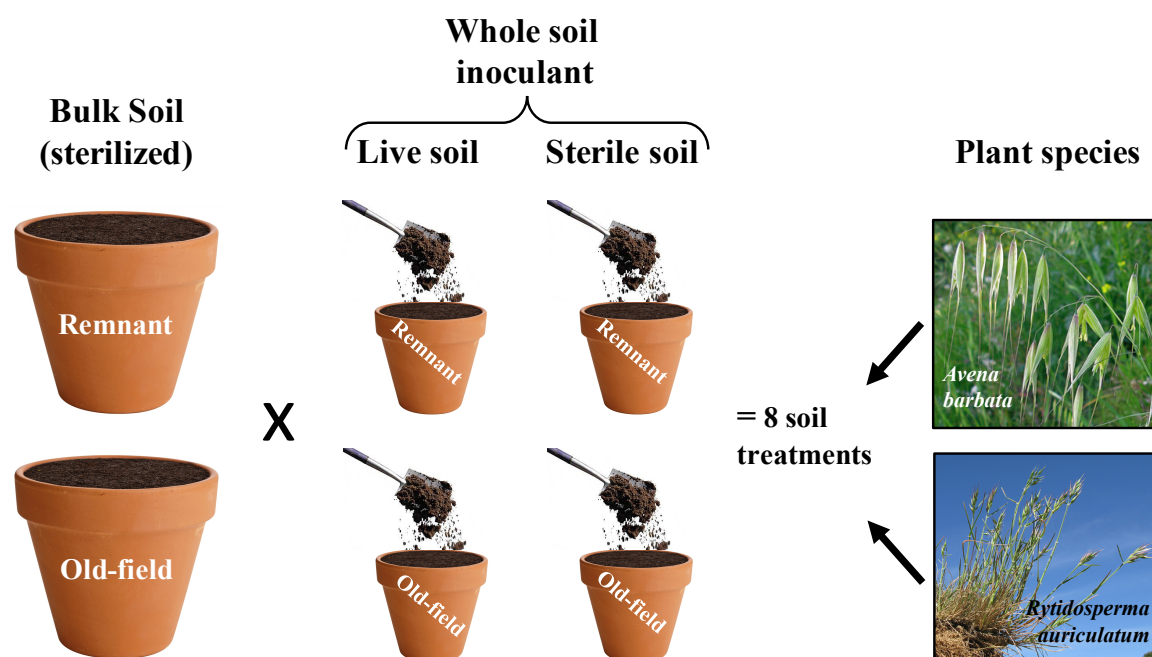

Figure 1. Experimental design including two bulk soil types (old-field and remnant), two inoculant types (live and sterile) and two inoculant sources (old-field and remnant) to make eight soil treatments. Ten replicate pots were then planted with seedlings from either *Rytidosperma auriculatum* (native) or *Avena barbata* (invasive).

To account for the differential responses to AM (Bethlenfalvay et al. 1982; Miller et al. 2014; Ronsheim 2012; Smith et al. 2018), harvest was timed to phenology of species, using first flowering as the trigger. This differed between the annual *A. barbata* (57 days) and the

perennial *R. auriculatum* (81 days). The shoots were cut at the base and soil cores (10 mm diameter by 70 mm deep) were collected (sterilizing equipment between each sample collection) and frozen for genetic analysis. Roots were then rinsed separately to remove adhering soil. All other plant material was oven dried at 70 °C for at least 48 hours before being weighed.

Statistical analysis – soil and plant material

To determine how soil properties at the beginning of the experiment were explained by main effects, i.e. the different bulk soils, inoculant sources and inoculant types, principal component analysis (PCA) was performed. PCA plots provide a visual representation of the similarity of groups and help to identify properties (and the correlation of properties) that separate groups from each other (Bruckner & Heethoff, 2017). In addition, linear models were also carried out on each soil property using the 'lm' function in the base R and the main effects, bulk soil, inoculant source and inoculant type, included.

To get compare how each plant species responded to the different soil microbial communities, we used a measure called microbial growth response (MGR). This was calculated using the individual total dry biomass of the live-inoculated plants and the mean total dry biomass of the corresponding mock-inoculated plants (equation 1). Values above zero indicate higher growth when plants are grown with the live-inoculant and values below zero indicate less growth with the live-inoculant, zero indicates no difference (Watts-Williams & Cavagnaro, 2012). The effects of plant species, bulk soil type and inoculant source on the MGR were then tested for using a linear model. Where significant differences were detected with analysis of variance (ANOVA) tests, we made planned pairwise comparisons (i.e. carried out a few targeted comparisons of interest between levels of the factors; henceforth planned comparisons) in the interests of testing our research questions, rather than every possible combination.

$$\%MGR = \frac{dry\ weight\ (\text{live}) - mean\ dry\ weight\ (\text{mock})}{mean\ dry\ weight\ (\text{mock})} \times 100$$

(1)

Microbial community

Our interest was on determining any differences in community composition between the soil inoculation sources and whether these communities changed after exposure to the different grass species or bulk soils, therefore, only samples from the live-inoculum treatments were used. This included three subsamples of the pre-experimental soil mixtures and five randomly selected samples from the experimental pots at the time of harvest from both plant species (N = 52). The whole soil samples were sent to the Australian Genome Research Facility (AGRF, Adelaide, Australia) for DNA extractions, PCR amplification and sequencing. After data cleaning and processing, there were 269 fungal and 3,468 bacterial species (or operational taxonomic units, OTUs) remaining for further analysis.

We selected a set of core OTUs, those present in more than 10 samples, to test how the experimental factors shaped the bacterial and fungal community composition using multispecies generalised linear models (GLMs). GLMs explicitly model the mean-variance relationship characteristic of ecological counts, and is therefore recommended over distance-based methods such as ordination or PERMANOVA (Warton et al., 2012). To answer our third question ("are the microbial communities different between the inoculant sources?") analysis was run using only the pre-experimental samples (before plants where added). To assess whether the soil communities changed over the course of the experiment (question four) models were rerun with all the data and the pre-experimental samples were included as a level of the factor 'species' and compared directly to the soil exposed to each plant species throughout the experiment. If changes were apparent, models were then rerun separately with the pre-experimental samples excluded to make the comparisons between plant species.

Results

Soil properties

Differences in soil physiochemical properties were mostly explained (70.7 %) by PC1 which separated the two bulk soil types (Figure 2). Old-field samples had higher total nitrogen, plant-available (Colwell) phosphorus, total carbon, conductivity and pH ($CaCl_2$) compared with remnant bulk soil samples (Figure 2, Table 1). Along this axis there was also separation between the inoculant sources for the mock-inoculants within each bulk soil (Figure 2). PC2 explained a further 22.8 % of variation and this axis separated samples depending on the inoculant-type (Figure 2). This variation was explained mostly by nitrate-nitrogen which was

higher in the live-inoculants, the opposite to ammonium-nitrogen which was higher in the mock-inoculants (Figure 2). PC2 also separated inoculant sources for the live-inoculants within each bulk soil, again explained by differences in nitrate nitrogen concentrations (Figure 2; Table 1).
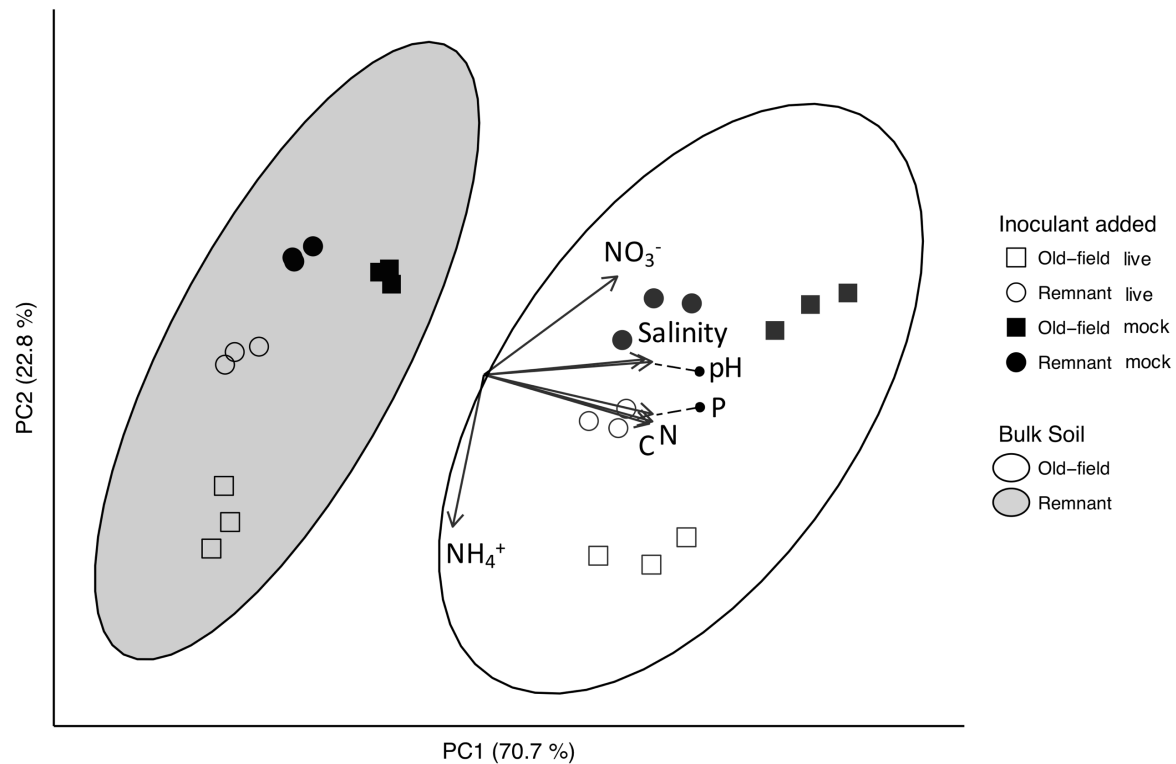

Figure 2. Principal coordinates analysis of soil treatments after two weeks' incubation (n = 40). Total variation explained by principal component (PC) one and two is 93.5 %. Ellipses (black ovals) represent 95 % confidence intervals around the group mean.

Table 1. Mean ± SD soil physiochemical properties of the eight inoculation treatments, mixed as 85 % bulk soil with 15 % inoculum (n = 40). Different letters illustrate statistically significant differences between planned comparisons; uppercase A-B = bulk soil, lowercase a-b = inoculant source, lowercase y-z = inoculant type

Bulk soil	Inoculum *source*	Inoculum *type*	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Plant available (Cowell; mg/kg)	Conductivity (dS/m)	pH (1:5 $CaCl_2$)	Total N (%)	Total C (%)
Old-field	Old-field	Live	42.3 ± 1.2^{Ay}	10.7 ± 1.2^{ay}	29.0 ± 1.0^{Aa}	0.07 ± 0.02^{Ay}	6.0 ± 0.0^{y}	0.27 ± 0.00^{Aa}	3.0 ± 0.0^{A}
		Mock	56.7 ± 2.3^{Az}	2.0 ± 0.0^{z}	30.7 ± 0.6^{Aa}	0.10 ± 0.01^{Az}	6.1 ± 0.1^{az}	0.27 ± 0.00^{Aa}	3.0 ± 0.0^{Aa}
	Remnant	Live	46.7 ± 2.1^{Ay}	5.7 ± 0.6^{by}	24.3 ± 0.6^{Aby}	0.07 ± 00.0^{Ay}	5.9 ± 0.1	0.26 ± 0.00^{Ab}	2.9 ± 0.0^{A}
		Mock	51.7 ± 4.9^{Az}	2.0 ± 0.0^{z}	28.0 ± 1.0^{Abz}	0.08 ± 0.01^{Az}	6.0 ± 0.1^{b}	0.25 ± 0.01^{Ab}	2.9 ± 0.0^{Ab}
Remnant	Old-field	Live	30.7 ± 2.3^{Bay}	10.7 ± 1.2^{ay}	14.0 ± 0.0^{Ba}	0.05 ± 0.01^{By}	5.6 ± 0.1^{y}	0.22 ± 00.0^{Ba}	2.6 ± 0.1^{B}
		Mock	46.3 ± 0.6^{Bz}	2.0 ± 0.0^{z}	12.7 ± 0.6^{Ba}	0.06 ± 0.00^{Bz}	5.8 ± 0.1^{az}	0.22 ± 0.00^{Ba}	2.7 ± 0.0^{Ba}
	Remnant	Live	39.3 ± 2.1^{Bby}	4.7 ± 0.6^{by}	11.0 ± 1.0^{Bb}	0.05 ± 0.01^{By}	5.6 ± 0.1	0.21 ± 0.01^{Bb}	2.6 ± 0.0^{B}
		Mock	47.0 ± 1.7^{Bz}	2.0 ± 0.0^{z}	11.3 ± 0.6^{Bb}	0.05 ± 0.01^{Bz}	5.7 ± 0.1^{b}	0.21 ± 0.00^{Bb}	2.6 ± 0.0^{Bb}

Plant responses

We found some evidence for home-field advantage (question one) with the MGR of the plants. Planned comparisons (see Table 2 for significant interactions) found that regardless of species, a positive MGR was more likely when live-inoculant was added to its home soil (old-field $P < 0.001$, remnant $P = 0.003$, Figure 3). In addition, when plants were grown in remnant bulk soil, *R. auriculatum* had a more positive MGR than *A. barbata,* regardless of inoculant source ($P < 0.001$, Figure 3).

Table 2. Results from the linear model for Microbial Growth Response (MGR). The models included bulk soil (old-field and remnant), inoculant source (old-field or remnant), plant species (*Avena barbata* and *Rytidosperma auriculatum)*. Significant ($P < 0.05$) factors are shown in bold, df = 1 in all cases.

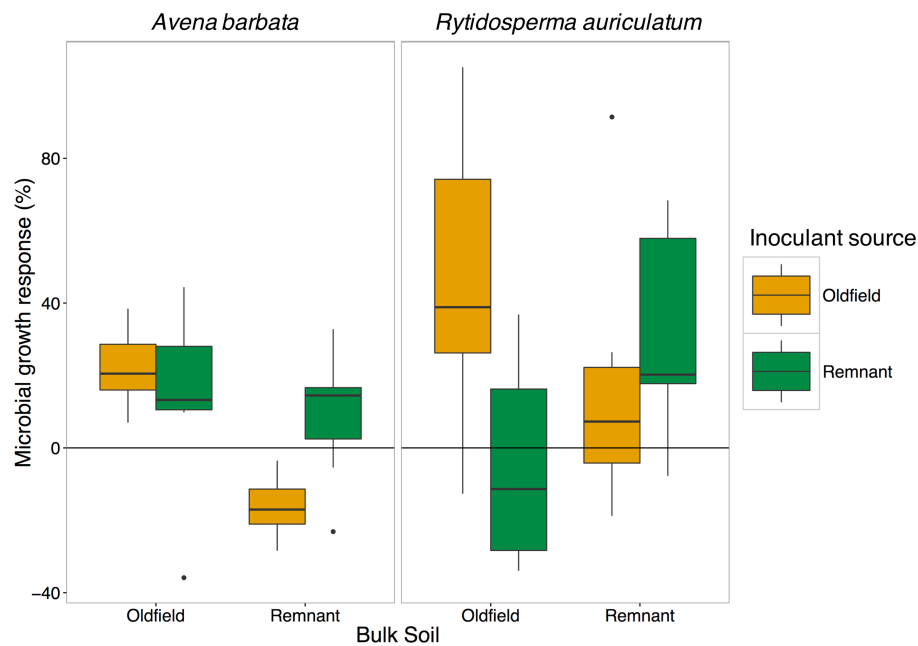| Factor	MGR
Bulk soil (BS)	**0.04**
Inoculant source (IS)	0.76
Plant species (PS)	**<0.01**
BS x IS	**<0.01**
BS x PS	**0.02**
IS x PS	**0.02**
BS x IS x PS	0.06

Figure 3. Microbial Growth Response (MGR) of *Avena barbata* (invasive; graph left) and *Rytidosperma auriculatum* (native; graph right). Plants were grown with either old-field inoculum (mustard) or remnant inoculum (green) added to autoclaved bulk soil from either the old-field or remnant sites (n = 77). See equation 1 for MGR explanation, however briefly, positive values indicate increased growth and negative values indicate reduced growth when soil microbes are present (compared with sterile controls). See Table 2 for ANOVA results.

Microbial community

Using the pre-experimental samples only, our models identified clear differences between the inoculant sources at the beginning of the experiment (question three; see Table 3a for ANOVA results). There were differences in community composition depending on bulk soil and inoculant source, identified from the multivariate GLMs ($P = 0.003$ for bacteria and fungi, Figure 4). When the models were run with all samples, it was clear that the soil communities at the end of the experiment were different to the pre-experimental samples (question four) regardless of which plant species they were exposed to ($P = 0.003$ for bacteria and fungi), according to the multivariate GLMs and the nMDS plots (Table 3b; Figure 4). Overall, the biggest differences in community composition for fungi and bacteria were due to the inoculant source with no points overlapping in multivariate space in the nMDS plots (Figure 4).

Further analysis without the pre-experimental samples (i.e. to compare the effect of plant species) found a significant three-way interaction between the plant species, bulk soil and inoculant source ($P = 0.010$). Planned comparisons found that the composition of the old-field fungal communities began to differ depending on which plant species they were exposed to ($P = 0.030$, Figure 4a). Fungal community composition was also different depending on which bulk soil they were added to for both inoculant sources ($P = 0.020$ for remnant and old-field inoculants), especially when exposed to *A. barbata* ($P = 0.052$, Figure 4a).

For the bacterial communities (without pre-experimental samples) there was also a significant three-way interaction ($P = 0.007$). Further analysis found that community composition was different depending on which plant species they were exposed to for each level of bulk soil and inoculant combination ($P = 0.04 - 0.049$), except for remnant inoculant in old-field bulk soil ($P = 0.056$, Figure 4b). Bacterial communities were also different depending on which bulk soil they were added to ($P = 0.031$), except when old-field inoculant was added to pots with *R. auriculatum* ($P = 0.070$, Figure 4b).
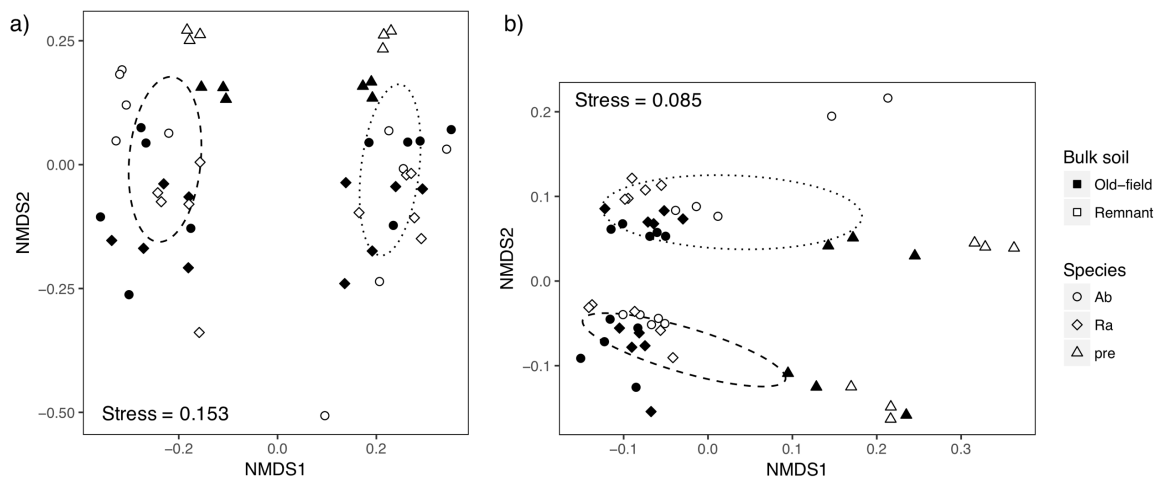

Figure 4. Nonmetric multidimensional scaling (NMDS) ordination of soil a) fungal and b) bacterial communities from live-inoculated experimental samples (n = 52). Inoculant sources are separated by NMDS1 for fungi and NMDS2 for bacteria, where old-field inoculant points are less than zero in both cases. Ellipses (ovals) represent the 95% confidence intervals of the group means for each inoculant source; old-field = dashed and remnant = dotted. Shapes represent the plant species grown in the soil treatments, *Avena barbata* (Ab) and *Rytidosperma auriculatum* (Ra), and the pre-experimental samples (pre).

Table 3. Soil fungal and bacterial community results from multivariate GLM (community composition) including bulk soil (old-field and remnant), inoculant source (old-field and remnant) and species (*Avena barbata, Rytidosperma auriculatum* and pre-experimental samples). Significant (P <0.05) factors are shown in bold, n = 52.

a) Pre-experimental			
Factor	df	Fungi	Bacteria
Bulk soil (BS)	1	**0.01**	**0.01**
Inoculant source (IS)	1	**0.01**	**0.01**
BS x IS	1	**0.03**	**0.03**
b) All samples			
Factor	df	Fungi	Bacteria
Bulk soil (BS)	1	**> 0.01**	**> 0.01**
Inoculant source (IS)	1	**> 0.01**	**> 0.01**
Species (S)	2	**> 0.01**	**> 0.01**
BS x IS	1	**> 0.01**	**> 0.01**
BS x S	2	**0.01**	**0.01**
IS x S	2	**> 0.01**	**> 0.01**
BS x IS x S	2	0.14	**> 0.01**

Discussion

In answer to our first question, we found some support for home-field advantage for plant-soil interactions. In particular, all plants responded more positively to microbes when inoculant was added to its home soil. In addition, when grown in remnant bulk soil the native *R. auriculatum* had a more positive MGR than the exotic *A. barbata* regardless of inoculant origin. Concerning questions two and three, the microbial communities differed significantly between old-field and remnant inoculant and the communities changed in composition over the course of the experiment depending on which bulk soil they were added to and which plant species they were exposed to.

Soil physiochemical properties

The bulk soil types differed significantly in their physiochemical properties, which is consistent with the sites having different land-use histories. Old-fields are known to retain high levels of nutrients long after farming has ceased (Standish et al., 2006; Drenovsky et al., 2010). In this case, a decade after farming had ceased, phosphorus and potassium concentrations were at least twice as high in the treatments with old-field bulk soil than remnant. Given that both bulk soils were treated in the same way these differences should reflect field conditions. However, since they were autoclaved twice, the actual values likely vary from those in the field so caution is needed when making inferences from these results (Warcup, 1957; Skipper & Westermann, 1973). There were also subtle differences between live and mock inoculated soils, most likely due to autoclaving the mock soil. Given that the inoculant only contributed to 15 % of the total soil mixtures and the differences in soil physiochemical properties were small, we concluded that differences in plant growth were more likely due to microbial effects rather than changes in physiochemical properties (Smith & Smith, 1981).

Home-field advantages of plant-soil interactions

Evidence for home-field advantage in the form of greater MGRs when inoculant was added to its home soil, regardless of plant species, demonstrated that the soil biota provides the most benefit to their hosts in their native soils. A longer-term study is needed to determine how these communities change over time in the different soil types as we may have measured during a transition between communities. Home-field advantage of soil biota, as demonstrated here, can have implications for ecological restoration because it highlights the importance of establishing soil conditions suitable for both the desired plant community and their mutualist soil microbial community. The current practice of using soil from a target ecosystem to inoculate a degraded system (Harris et al., 2006) may not have the desired impact if they are not adapted to the local environment (Emam, 2016). More work is needed to understand how the inoculated soil community may compete with the resident soil community and how restoration practices may shift the balance in favour of the former.

Microbial responses

Our results show that the old-field and remnant inoculants contained distinct soil microbial communities. This finding is consistent with other studies where differences in the microbiomes of old-field and remnant areas have been found (Steenwerth et al., 2002; Araujo

et al., 2014; Gellie et al., 2017), and reflects what we know about the land-use histories of these sites.

Fungal and bacterial community composition differed between the pre-experimental samples and those collected at harvest. There were clear differences depending on the bulk soils and plant species present, indicating that these factors influenced the communities over time. The differences observed between the bulk soils were most likely due to the differences in soil physiochemical properties. Varying levels of nutrient availability have been associated with unique soil microbiomes (Ramirez et al., 2010; Fierer et al., 2012; Leff et al., 2015) and in our case, we found a substantial number of unique OTUs in each bulk soil (data not shown see Smith et al. 2018). Out of the OTUs that were affected by bulk soil type, the majority of bacterial OTUs were found in greater abundances in old-field than in remnant bulk soil whereas all fungal OTUs had greater abundances in the remnant bulk soil. This concurs with several studies that have found fungi to be generally more sensitive to increased nutrients and prefer higher C:N ratios (Busse et al., 2009; Fierer et al., 2009).

Differences in microbial communities also appeared to depend on the plant species they were exposed to. Our findings support the theory that suggests native and invasive plants can alter soil microbial communities in different ways (Klironomos 2002; Stinson et al. 2006). For instance, there was an increase in fungal richness when old-field inoculant was exposed to *A. barbata* and there were more unique bacterial and fungal species in the presence of this species (data not shown see Smith et al. 2018). Other studies have attributed an increase in microbial diversity to exotic species and this is one mechanism by which they can dominate a system, particularly if there is an increase in pathogens, which inhibit native plant growth or establishment (Lekberg et al. 2013; Mangla et al. 2008). Of the OTUs found in higher abundances when exposed to *A. barbata* we could not identify any plant pathogens. Often, very little information on the function of OTUs was available or classification was too coarse. This highlights that, while genetic tools show a lot of promise for expanding our knowledge on soil microorganisms, there is a need for better links between description and function of microorganisms before they can be utilized to their full potential. Nevertheless, the results show that the two grass species are associated with distinct microbial communities. More work is needed to determine whether the apparent increase in diversity with the invasive species is

sustained over a longer period or if it is an artefact of the microbial communities shifting from one composition to another.

Conclusions

Home-field advantage played an important role in modulating plant and soil microbial community interactions in this study. However, this relationship is complex, with microbial communities changing in response to the plant species and soil type. Understanding these complicated relationships between plants, microbes and soils has wide practical implications such as whether inoculation of soils with local mutualistic symbionts is beneficial to enhance ecosystem services (Rua et al. 2016). Our results suggest that using remnant soil as an inoculant for old-field restoration may not promote the growth of the desired community, at least over the time period in this study, and that the revegetated plants may be able to promote changes in the microbial community over time anyway (Gellie et al. 2017). While the approach used in this study, i.e. using sterilised bulk soil, is unlikely to match exactly field conditions and processes, this was a necessary step to separate microbial effects from soil physiochemical effects. More work is needed to better understand how the inoculated microbial community interacts and competes with the resident community as soil inoculation becomes more utilised.

References

Araujo ASF, Borges CD, Tsai SM, Cesarz S, and Eisenhauer N (2014) Soil bacterial diversity in degraded and restored lands of Northeast Brazil. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **106**:891-899

Bruckner A, and Heethoff M (2017) A chemo-ecologists' practical guide to compositional data analysis. Chemoecology **27**:33-46

Busse MD, Sanchez FG, Ratcliff AW, Butnor JR, Carter EA, and Powers RF (2009) Soil carbon sequestration and changes in fungal and bacterial biomass following incorporation of forest residues. Soil Biology and Biochemistry **41**:220-227

Cramer VA, Hobbs RJ, and Standish RJ (2008) What's new about old fileds? Land abandonment and ecosystem assembly. Trends in Ecology & Evolution **23**:104-112

Drenovsky RE, Steenwerth KL, Jackson LE, and Scow KM (2010) Land use and climatic factors structure regional patterns in soil microbial communities. Global Ecology and Biogeography **19**:27-39

Emam T (2016) Local soil, but not commercial AMF inoculum, increases native and non-native grass growth at a mine restoration site. Restoration Ecology **24**:35-44

Facelli J, and Pickett STA (1990) Markovian chains and the role of history in succession. Trends in Ecology & Evolution **5**:27-30

Ferrazzano S, and Williamson PS (2013) Benefits of mycorrhizal inoculation in reintroduction of endangered plant species under drought conditions. Journal of Arid Environments **98**:123-125

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, and Knight R (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal **6**:1007-1017

Fierer N, Strickland MS, Liptzin D, Bradford MA, and Cleveland CC (2009) Global patterns in belowground communities. Ecology Letters **12**:1238-1249

Gellie NJC, Mills JG, Breed MF, and Lowe AJ (2017) Revegetation rewilds the soil bacterial microbiome of an old field. Mol Ecol **26**:2895-2904

Harris JA, Hobbs RJ, Higgs E, and Aronson J (2006) Ecological restoration and global climate change. Restoration Ecology **14**:170-176

Leff JW, Jones SE, Prober SM, Barberan A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, Mcculley RL, La Pierre K, Risch AC, Seabloom EW, Schutz M, Steenbock C, Stevens CJ, and Fierer N (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences of the United States of America **112**:10967-10972

Lenz TI, Moyle-Croft JL, and Facelli JM (2003) Direct and indirect effects of exotic annual grasses on species composition of a South Australian grassland. Austral Ecology **28**:23-32

Neuenkamp L, Prober SM, Price JN, Zobel M, and Standish RJ (2018) Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and time. Fungal Ecology

Ramirez KS, Lauber CL, Knight R, Bradford MA, and Fierer N (2010) Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology **91**:3463-3470

Rosser L. (2013) Ecological Restoration Plan for Para Woodlands Reserve. Department of Environment Water and Natural Resources

Skipper HD, and Westermann DT (1973) Comparative effects of propylene oxide, sodium azide, and autoclaving on selected soil properties. Soil Biology and Biochemistry **5**:409-414

Smith FA, and Smith SE (1981) Mycorrhizal infection and growth of Trifolium subterraneum: Use of sterilized soil as a control treatment. New Phytologist **88**:299-309

Smith ME, Facelli JM, and Cavagnaro TR (2018) Interactions between soil properties, soil microbes and plants in remnant-grassland and old-field areas: a reciprocal transplant approach. Plant and Soil **433**:127-145

Standish RJ, Cramer VA, Hobbs RJ, and Kobryn HT (2006) Legacy of land-use evident in soils of Western Australia's wheatbelt. Plant and Soil **280**:189-207

Steenwerth KL, Jackson LE, Calderon FJ, Stromberg MR, and Scow KM (2002) Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology & Biochemistry **34**:1599-1611

Warcup J (1957) Chemical and biological aspects of soil sterilization. Soils Fertil **20**:1-5

Warton DI, Wright ST, and Wang Y (2012) Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution **3**:89-101

Watts-Williams SJ, and Cavagnaro TR (2012) Arbuscular mycorrhizas modify tomato responses to soil zinc and phosphorus addition. Biology and Fertility of Soils **48**:285-294

Wolfe BE, and Klironomos JN (2005) Breaking new ground: Soil communities and exotic plant invasion. Bioscience **55**:477-487