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Partner diversity and identity impacts on plant productivity in *Acacia*–rhizobial interactions

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Summary

1. Genetic variation for functionally important traits is ubiquitous in communities of nitrogen-fixing rhizobia, and while some studies have described significant effects of diversity on the functioning of plant-associated microbial communities, we lack a systematic test of how rhizobial diversity influences plant productivity.

2. The complexity of potential interactions among rhizobia and plants complicates the development of general predictions regarding causal relationships between rhizobial diversity and plant productivity. For example, while rhizobial complementarity may result in positive associations between symbiont diversity and plant productivity, antagonistic competition may reduce rhizobial community function.

3. Using two widespread native Australian *Acacia* species (*A. salicina*, *A. stenophylla*) and experimental rhizobial communities derived from 16 bacterial genotypes naturally associated with these hosts, we examined how the provision of mutualistic benefit varies with rhizobial identity, diversity and phylogenetic relatedness.

4. Analysis of plant performance in relation to rhizobial genotypic richness revealed that the presence of multiple rhizobial genotypes in the rhizosphere was associated with a general decrease in plant productivity compared to growth with single rhizobial genotypes. Importantly, these results appear to be robust in the face of variation in host identity and host diversity (i.e. one or two species mixtures). We also found that rhizobial genotypic identity and host species significantly influenced plant productivity in *Acacia*–rhizobia interactions, both in single- and multistrain inoculations.

5. *Synthesis.* Together, our data show that multiple rhizobia interacting with a single host species creates opportunities for emergent or higher-order effects that extend beyond those that could be simply predicted based upon outcomes of pairwise interactions and that increased mutualist diversity does not necessarily translate into positive effects on plant growth.

Key-words: community, diversity, ecology, function, interaction, mutualism, plant–soil (belowground) interactions

Introduction

Mutualisms between plants and nitrogen-fixing rhizobia play key roles in many terrestrial ecosystems, regulating individual plant fitness and community productivity. Variation in the effectiveness of plant–rhizobial associations is well characterized, such that benefits received by plants are highly variable depending on the genotypes of the interacting plant and rhizobial partners (Devine & Kuykendall 1996; Burdon *et al.* 1999; Heath & Tiffin 2007; Masson-Boivin *et al.* 2009; Drew *et al.* 2011; Thrall *et al.* 2011) and the physical environment within which the interaction takes place (Graham 1992). Indeed, high genetic and functional diversity is seemingly the norm within rhizobial populations (Gibson *et al.* 1975; Drew *et al.* 2011; Bever, Broadhurst & Thrall 2013), and strains of rhizobia that are suboptimal in terms of nitrogen fixation, or fix no nitrogen at all, are common in many soils (Ballard *et al.* 2004; Nandasena *et al.* 2006; Bever, Broadhurst & Thrall 2013). Manipulation of the composition and diversity (Vogelsang, Reynolds & Bever 2006; Jansa, Smith & Smith 2008) of another key group of rhizosphere symbionts, mycorrhizal fungi, demonstrates large effects on plant productivity and composition. However, despite numerous studies showing that plants interact simultaneously with multiple rhizobial genotypes (Gibson *et al.* 1975; Triplett & Sadowsky 1992; Nandasena *et al.* 2006; Rangin *et al.* 2008; Sachs *et al.* 2009;

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Birnbaum *et al.* 2012), there is currently little understanding regarding how *in situ* rhizobial diversity, and resulting multipartite interactions with plant hosts, influences plant productivity.

The complexity of ecological mechanisms that could locally maintain genetic diversity complicates the development of general predictions regarding how rhizobial diversity might influence plant productivity (Vargas & Graham 1989; Jousset *et al.* 2011). Rhizobia possess a range of different life-history strategies (Denison & Kiers 2004) and potentially interact and compete in many different ways in the plant rhizosphere (Triplett & Sadowsky 1992). In addition, hosts are not passive substrates for rhizobial colonization. Rather, the successful establishment of legume–rhizobial symbioses requires cooperation between host and symbiont via multiple rounds of reciprocal signalling and recognition (Masson-Boivin *et al.* 2009; Yang *et al.* 2010), and it is well recognized that establishment of a symbiosis is dependent on both rhizobia and host genotypes (e.g. Heath 2010). Moreover, following establishment of infections, plants can potentially alter rhizobial fitness through sanctions or preferential allocation of resources to particular symbionts (Kiers *et al.* 2003; Sachs *et al.* 2010).

Ecological mechanisms that lead to predictions of increasing plant productivity with increasing rhizobial diversity include 'complementarity' and 'selection' models (Loreau & Hector 2001). Under a complementarity model, simultaneous colonization of roots by distinct rhizobial genotypes leads to increased plant productivity relative to each of the strains alone, and may be expected, if for example, one rhizobial genotype is better at initiating a symbiosis, while a second is better at fixing nitrogen. Such dynamics may occur in interactions involving the N_2-fixing actinomycete *Frankia.* Two major classes of strains, spore producing (Sp+) and non-spore producing (Sp−), have been found to co-occur. Sp+ strains are more infective but less beneficial symbionts than Sp− strains, and it has been suggested that infection by the former may facilitate root penetration by the latter (Lechevalier & Lechevalier 1990). Under a selection model, positive relationships between rhizobial diversity and plant productivity could arise via selection of the most beneficial rhizobia from a pool of variants (Loreau & Hector 2001). Assuming that hosts can efficiently select for the most beneficial rhizobial partner [e.g. via partner choice or post-infection sanctioning], then diverse rhizobial communities should be more productive on average simply because they are more likely to contain strains with a large positive effect on plant productivity, particularly given observed levels of host specificity and the potential for ecological trade-offs among strains (Thrall, Bever & Slattery 2008). Plant productivity under a selection model should therefore be equal to that of the best strain of the mixture alone.

Within rhizobial populations and communities (i.e. the plant root matrix), strong potential for competition exists among individual rhizobia for access to host resources. 'Cheater strains' – rhizobial genotypes that compete by fixing less N_2 while gaining the benefits of N-fixation by other rhizobia – will have low effectiveness and likely low fitness in single-strain infections. However, because N-fixation is a costly trait, less beneficial rhizobia which do not bear this cost should have a competitive advantage over beneficial rhizobia within mixed infections (Bronstein 2001; Denison & Kiers 2004; Zee & Bever 2014). In such situations, cheaters can benefit (Turner & Chao 1999; Bever *et al.* 2009; Barrett *et al.* 2011), either by co-inhabiting nodules with N-fixing strains, or forming their own nodules and benefiting indirectly from nitrogen provided by N-fixers in other nodules. Accumulation of cheating rhizobia will reduce overall plant productivity and potentially generate negative associations between rhizobial diversity and plant performance. Reductions in plant productivity may also arise through other forms of competition between rhizobia. Interference and apparent competition among rhizobia in particular have the potential to strongly influence plant productivity. For example, direct competition via production of bacteriocins and apparent competition mediated by temperate bacteriophage (e.g. Schwinghamer & Brockwell 1978; Joseph, Desai & Desai 1983) provide means by which rhizobia can specifically inhibit growth and nodulation of co-occurring strains (Triplett & Sadowsky 1992). Thus, while competition may promote the maintenance of functional diversity (Hibbing *et al.* 2010), such dynamics have the potential to reduce the effectiveness of diverse rhizobial populations through inhibition of nodule formation by beneficial rhizobia, or facilitation of nodule occupation by suboptimal strains.

The evolutionary history (i.e. genetic relatedness) of interacting rhizobial strains may also play a role in determining the outcomes of co-infection (Jousset *et al.* 2011). There is often a phylogenetic signal associated with niche differentiation, such that genetically divergent taxa are also more likely to be phenotypically and functionally divergent (Blomberg, Garland & Ives 2003). Rhizobia are a taxonomically diverse group of organisms (Masson-Boivin *et al.* 2009), and rhizobial genera behave differently in terms of specificity and N-fixing effectiveness in pairwise interactions with different *Acacia* host species (Bever, Broadhurst & Thrall 2013). Thus, complementary effects may be predicted to be more likely when rhizobial phylogenetic diversity in the rhizosphere is high. Conversely, evolutionary theory predicts that closely related individuals may be more likely to cooperate with one another than distantly related individuals (Griffin, West & Buckling 2004). Cooperation is known to be important in rhizobia, which can induce hosts to generate resources targeted to genetically similar rhizobia (e.g. rhizopines; Zee & Bever 2014). Thus, it may be predicted that facilitation and other specific cooperative behaviours will be more likely to occur among closely related rhizobia. Relatedness may also be an important factor in scenarios involving bacterial competition. It is generally assumed that competition for resources will be stronger among more closely related organisms (Griffin, West & Buckling 2004). However, competition for nodule occupancy is likely to be strong among all potential rhizobial symbionts in the rhizosphere, and bacteria can produce antimicrobials specifically aimed at taxonomically distant

competitors (antibiotics) as well as bacteriocins that target taxonomically closer strains (Riley & Wertz 2002).

The complexity of plant–rhizobial interactions motivates several predictions for plant response to inoculation with multiple rhizobial strains. We would expect a positive response of productivity to diversity if hosts simply select for the best strain in the mixture, as the effectiveness of the mixture would be equal to that of the best strain inoculated alone. It is also possible that plant growth exceeds that of the best strain if the strains interact either in a complementary or facilitative way. Both selection and complementarity can explain the positive dependence of plant growth on diversity of arbuscular mycorrhizal fungi (AMF) (Vogelsang, Reynolds & Bever 2006; Wagg *et al.* 2011). Alternatively, if plants interact with rhizobia in proportion to their initial abundance and rhizobia do not interact within mixtures (e.g. a linear dose–response effect), then the diversity of rhizobia should not influence productivity and plant growth would equal the mean of the productivity of plants colonized by each of the strains alone. Finally, rhizobial diversity could negatively influence plant productivity if cheating bacteria proliferated in mixture or if rhizobial competition interfered with the efficiency of effective strains. At present, there are no manipulative experiments which test these possibilities, though tests of field patterns suggest that increasing diversity of rhizobia can lead to a decline in plant benefit (Bever, Broadhurst & Thrall 2013).

In this study, we explicitly test the relationship between plant productivity and rhizobial diversity using two Australian *Acacia* species, *A. salicina* and *A. stenophylla* as target hosts. These plant species are good models for studying the functional consequences of high symbiont diversity, in that under natural conditions, both harbour highly diverse local communities of rhizobia (Barrett, Broadhurst & Thrall 2012; Bever, Broadhurst & Thrall 2013). For example, strains belonging to all four of the genera *Bradyrhizobium, Sinorhizobium*, *Mesorhizobium* and *Rhizobium* regularly co-occur within single *Acacia* stands (Hoque, Broadhurst & Thrall 2011; Bever, Broadhurst & Thrall 2013). We experimentally assessed the consequences for host performance when plants interact simultaneously with multiple rhizobia, by comparing plant productivity when inoculated with single-strain, multi-strain and multigenera rhizobial cultures. The mean effectiveness of the best strain alone forms our null expectation for plant productivity following inoculation with a mixture of strains. We also assess whether plant–plant interactions alter the effect of rhizobia diversity and composition by growing the two hosts alone and in mixture.

Materials and methods

EXPERIMENTAL DESIGN

We used two widespread Australian *Acacia* spp. (*A. salicina, A. stenophylla*) as focal host species in inoculation experiments. Seeds were collected from adults of each species at nine localities in NSW (Barrett, Broadhurst & Thrall 2012) and then bulked. Prior to germination, seeds were surface sterilized in 1% bleach for 1 min, washed with sterile water 5 times, transferred to beakers of boiling water and left to cool to room temperature overnight. The seeds were then spread over sanitized sand/vermiculite flats, watered daily and left to germinate.

Sixteen rhizobial strains previously isolated from either *A. salicina* or *A. stenophylla* (Hoque, Broadhurst & Thrall 2011) were selected for experimental use. For each strain, taxonomic and experimental assignations, phylogenetic relationships and the host from which they were originally isolated are described in Table 1.

Table 1. Rhizobial strains, phylogenetic relationships, host plants (from which rhizobia were trapped and isolated) and label used in the diversity-functioning experiment. These 16 strains were used to inoculate *Acacia* plants alone and in various mixtures (see Materials and methods for details)

Phylogeny	Genus	Isolation host	Label
44-2 R38	*Rhizobium*	*A. salicina*	R4
44-1 R9	*Rhizobium*	*A. salicina*	R1
4-2 R5	*Rhizobium*	*A. stenophylla*	R2
12-1 R21	*Rhizobium*	*A. stenophylla*	R3
3-3 R30	*Sinorhizobium*	*A. salicina*	S2
37-1 R27	*Sinorhizobium*	*A. salicina*	S4
23-2 R4	*Sinorhizobium*	*A. stenophylla*	S1
44-1 R6	*Sinorhizobium*	*A. stenophylla*	S3
24-2 R18	*Mesorhizobium*	*A. salicina*	M4
34-2 R6	*Mesorhizobium*	*A. salicina*	M3
43-1 R15	*Mesorhizobium*	*A. salicina*	M2
11-2 R27	*Mesorhizobium*	*A. salicina*	M1
23-2 R20	*Bradyrhizobium*	*A. salicina*	B3
10-1 R29	*Bradyrhizobium*	*A. salicina*	B4
13-5 R34	*Bradyrhizobium*	*A. salicina*	B1
24-2 R35	*Bradyrhizobium*	*A. salicina*	B2

All strains were originally trapped from soils sampled from within wild populations of either *A. salicina or A. stenophylla*, as described by Hoque, Broadhurst & Thrall (2011). These strains were chosen to represent the diversity of genera occurring within these soils. All strains were confirmed as genetically unique using markers generated by PCR primers (RPO1) targeting a conserved *nif* promoter, as described by Richardson *et al.* (1995). Otherwise, no *a priori* information regarding the individual effectiveness of these strains was available. More information on the strains used in this study and naturally occurring rhizobial communities associated with *A. stenophylla* and *A. salicina* can be found in recent papers from our laboratory (Hoque, Broadhurst & Thrall 2011; Barrett, Broadhurst & Thrall 2012; Bever, Broadhurst & Thrall 2013).

Rhizobial strains (Table 1: M = *Mesorhizobium*; S = *Sinorhizobium*; B = *Bradyrhizobium*; R = *Rhizobium*) were used to construct a series of inocula that were applied to pots containing either *A. salicina* plants alone; *A. stenophylla* plants alone; or a mixture of *A. stenophylla* and *A. salicina* plants. Bacterial treatments consisted of 40 replicated strain combinations. This included all 16 monocultures and random mixtures (sampled from each pool without replacement) consisting of the following: eight 2-strain mixtures from the same genus [(B1, B4) (B3, B2)(M4, M2) (M1, M3)(R2, R4)(R1, R3)(S1, S3)(S2, S4)]; eight 2-strain mixtures comprising different genera [(R4, M2)(M1, S4)(R2, M3)(S1, B3)(B2, R3)(B4, R1)(B1, S2)(M4, S3)]; four 4-strain mixtures from the same genus [(B1-B4)(M1-M4)(R1-R4)(S1-S4)]; and four 4-strain mixtures comprising different genera [(B1, M3, R4, S1)(B3, M2, R2, S4)(B2, M4, R1, S3)(B4, M1, R4, S2)]. The experimental design was based on the method described by Bell *et al.* (2009). Using this design, species/genus mixtures are chosen at random within the constraint that each species/genus is represented equally at each level of richness. The design creates random mixtures while preventing any single species/genus from unduly influencing the results. In total, not including non-rhizobial controls, the experiment consisted of 120 host by rhizobial treatment combinations. Each treatment combination was replicated 6 times in separate pots, to yield 720 host–rhizobial microcosms. For each species, 16 control plants were grown alongside inoculated treatments to monitor contamination and provide a baseline for plant growth.

Prior to inoculation, individual bacterial strains were grown in 200 mL of yeast–mannitol–broth and incubated with shaking for 5–7 days. Following standardization of optical densities (OD = 0.1: approximately 1×10^7 cells per mL), rhizobial communities were constructed by mixing isolates from each population in equal proportions. For each treatment in the inoculation study, 1 mL of the resultant suspension was added directly to the base of each 2-week-old seedling. Thus, total cell density was held roughly constant, while individual rhizobial proportions varied across treatments. This approach was chosen as previous experimental studies have shown a positive relationship between total rhizobial density and the growth response of *Acacia* plants (e.g. Thrall *et al.* 2007; Bever, Broadhurst & Thrall 2013).

The plant growth experiment was carried out in pots under standard glasshouse conditions at CSIRO in Canberra. Two seedlings were planted into each 15-cm-diameter pot 7 days after germination, and the soil surface was covered with a layer of polyurethane beads to limit splashing among pots and cross-contamination. Inoculated plants were grown in natural light at ambient temperatures. Pots were watered with N-free 1:50 diluted McKnight's solution (McKnight 1949) twice weekly. Plants were harvested 16 weeks after inoculation, and above-ground parts were oven dried and weighed. At the time of harvest, plant roots were separated from the soil and nodulation characteristics also recorded, including: (i) presence/absence of nodules; (ii) and a categorical assessment of nodule biomass based on number and size (ranging from 1 to 5: 1 = small number (< 10) of small nodules (~1–2 mm in diameter); 5 = numerous large N_2 fixing nodules with pink/red centres).

STATISTICAL ANALYSIS OF PLANT GROWTH

Final dry weights of plants were log-transformed and a mean plant weight calculated for each pot. The average plant size in each pot was first analysed using a mixed model in which bacterial diversity, host treatment and their interaction were treated as fixed effects and individual bacterial composition nested within bacterial diversity and their interactions with host treatment treated as a random effect. From this analysis, we identified substantial variation in plant response due to particular rhizobial composition.

Our design manipulated rhizobial genus and species diversity as well as rhizobial composition. We used a regression approach to tease out the relative importance of these factors in plant responses. Because composition and diversity predictors are not necessarily independent (e.g. pots with high proportion of an individual species necessarily is low diversity), we used AIC criteria to identify the best model. However, given our design is best analysed using a mixed model and AIC statistics are difficult to interpret across mixed models (Grueber *et al.* 2011), our first step was to reduce the complexity of the data set by calculating the best linear unbiased predictors (blups) of individual bacterial compositions*host treatment (i.e. the distinct treatment combinations). The best linear unbiased predictor is a method for obtaining point estimates of a random effect in a mixed effect model. These were determined using Proc Mixed in SAS with restricted maximum likelihood variation (REML) estimation.

We then used an information-theoretic approach (Burnham & Anderson 2002) to separate the effects of rhizobial identity and diversity on plant growth. We first fitted a global general linear model using the GLM function in R. This global model included strain presence (n = 16 with 2 levels), strain diversity (3 levels) and generic diversity (3 levels) as main effects. We did not include host identity as a factor (nor any interaction terms) as the initial analyses identified a significant effect of plant treatment on plant growth and the model was already heavily parameterized. To generate a full model set for each diversity-identity combination, we used the 'glmulti' function of the *glmulti* package (Calcagno & de Mazancourt 2010). Each of the models generated was ranked using Akaike's Information Criterion corrected (AICc) for small sample size, and models within 2AICc of the highest ranked model were retained for further analysis. For the retained 14 models, we then used the 'model.avg' function in the *MuMIn* package (Bartoń 2012) to calculate Akaike weights (w_i) [the probability that the model is actually the best fitting model of the candidate models (Burnham & Anderson 2002)]. Because all 14 models received similar support (Table 2), we used model averaging to calculate the relative importance (the sum of w_i across all models that contained the variable) of each parameter and to generate parameter coefficients for the remaining factors. Model averaging was performed by averaging over models in which that predictor appeared and weighting coefficients by the relative importance of that parameter (the natural average method: Burnham & Anderson 2002). To formally evaluate the potential significance of individual predictors, z-tests were conducted to calculate the probability that 95% confidence intervals for Akaike weighted coefficients did not overlap zero.

Table 2. Models predicting average *Acacia* growth. The best model and 13 candidates within 2 AICc units of the best model are presented. The Akaike weights (w_i) represent the relative likelihoods of the individual models being the best model

Model number	N of model parameters	Variables in model*	AICc	Delta	w_i
1	9	bact.div;B1;B3;M3;R1;R3;R4;S1;S2	83.76	0.00	0.12
2	8	bact.div;B1;B3;R1;R3;R4;S1;S2	84.06	0.30	0.11
3	9	bact.div;B1;B3;R1;R3;R4;S1;S2;S3	84.22	0.46	0.10
4	10	bact.div;B1;B3;B4;R1;R3;R4;S1;S2;S3	84.27	0.51	0.10
5	9	bact.div;B1;B3;B4;R1;R3;R4;S1;S2	84.70	0.93	0.08
6	10	bact.div;B1;B3;M2;M3;R1;R3;R4;S1;S2	85.07	1.31	0.06
7	10	bact.div;B1;B2;B3;M3;R1;R3;R4;S1;S2	85.15	1.39	0.06
8	10	bact.div;B1;B3;B4;M3;R1;R3;R4;S1;S2	85.20	1.44	0.06
9	11	bact.div;B1;B2;B3;M2;M3;R1;R3;R4;S1;S2	85.31	1.55	0.06
10	10	bact.div;B1;B3;M3;R1;R3;R4;S1;S2;S3	85.32	1.56	0.06
11	8	bact.div;B1;B3;M3;R1;R4;S1;S2	85.35	1.59	0.05
12	9	bact.div;B1;B3;M2;R1;R3;R4;S1;S2	85.70	1.94	0.05
13	11	bact.div;B1;B2;B3;B4;R1;R3;R4;S1;S2;S3	85.74	1.98	0.05
14	10	bact.div;B1;B2;B3;R1;R3;R4;S1;S2;S3	85.75	1.99	0.05

*The variable bact.div refers to strain richness (i.e. the number of different rhizobial genotypes in a treatment). Letter/number combinations refer to individual rhizobial strains described in Table 1.

We used an essentially identical approach to model differential growth among species.

We further assessed the growth consequences of inoculation with multiple rhizobial strains by measuring the net biodiversity effect, calculated as the difference between the observed yield in mixtures and expected yield from single-strain inoculations (Loreau & Hector 2001). Expected values were calculated for three alternate hypotheses for expected plant growth when exposed to rhizobial mixtures; (i) expected growth based on the best of the single-strain treatment yields for the component rhizobia (maximum effect); (ii) average plant growth with all strains in mixed inoculation treatment alone (mean effect); and (iii) plant growth with the poorest strain in treatment alone (minimum effect). Similar to the approach described above, we then used an information-theoretic approach to separate the effects of rhizobial identity and diversity on each of the biodiversity effects. Specifically, we first fitted a global general linear model using the GLM function in R. This global model included strain presence (n = 16 with 2 levels), host treatment (3 levels), strain diversity (2 levels) and generic diversity (3 levels) as main effects. We then used the 'glmulti' function of the *glmulti* package to evaluate the main effects of all possible explanatory variables. All computed models were ranked using the AICc coefficient. For the top 100 models, the relative importance of each of the main effects was then calculated and parameter coefficients generated.

Results

SINGLE-STRAIN INTERACTIONS AND CONTROLS

The effects of inoculation with the 16 strains alone were highly variable, such that individual strains ranged from being generally ineffective in terms of promoting plant growth (e.g. *Mesorhizobium* strain M1), highly effective with *A. salicina* but much less so with *A. stenophylla* (e.g. *Bradyrhizobium* strain B1), through to highly effective at promoting growth of all host treatments (e.g. *Sinorhizobium* strain S2) (Fig. 1). Rhizobial genera varied in their average plant-growth-promoting abilities, with all *Mesorhizobium* strains proving ineffective at promoting plant growth (Fig. 1). With regard to uninoculated controls, 2 (of 18) *A. salicina* plants were found to have nitrogen-fixing nodules at harvest, while for *A. stenophylla*, only one control plant (of 18) was found to have nodules. In all cases, nodules were small and few in number, occurred on lateral roots, and based on biomass and greening of the plants likely formed late in the experiment. These results indicate that contamination was sparse, had negligible impact on plant performance and suggest that our results are unlikely to be significantly influenced by contaminant rhizobia.

SINGLE- AND MULTISTRAIN INTERACTIONS

In the mixed model analysis, biomass in *A. stenophylla* pots was consistently less than pots containing *A. salicina* or pots with both host species together ($F_{2,\ 64}$ = 4.43, P = 0.02), but the fixed effect of rhizobial diversity and its interaction with host treatment was not significant. However, the rhizobial composition covariances were strongly significant (rhizobial composition*diversity variance component = 0.1741, SE = 0.04768, $P < 0.0001$; rhizobial composition*diversity*host treatment variance component = 0.03650, SE = 0.008635, $P < 0.0001$), indicating there were large effects of rhizobial community composition on plant biomass.

RHIZOBIAL COMPOSITION AND DIVERSITY AS PREDICTORS OF AVERAGE ACACIA GROWTH

To specifically probe the role of rhizobial composition (identity and diversity) in mediating total plant growth, we used generalized linear modelling of blups (calculated for each experimental treatment combination). Main effects of strain presence, strain richness and generic richness were evaluated as predictors of plant dry weights (Fig. 1). Hypothesis testing using AIC and weighted model averaging (Tables 2 and 3) showed that both *Acacia* species responded negatively to the presence of multiple strains of rhizobia, such that compared to single-strain treatments, growth was reduced in two and four strain treatments (Table 3). Plant growth also varied

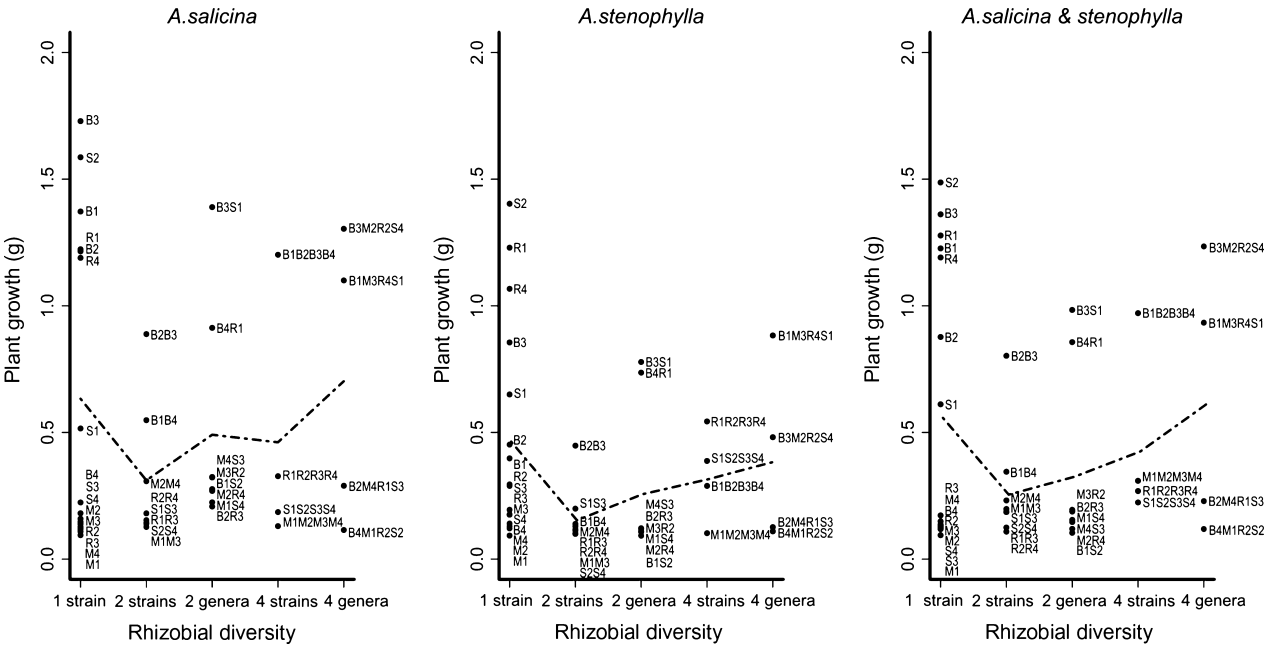

Fig. 1. Plant growth (as modelled by best linear unbiased predictors) in response to inoculation with 16 rhizobial strains constituting 4 rhizobial genera. Each strain was inoculated into pots containing either two *Acacia salicina* seedlings, two *A. stenophylla* seedlings or one seedling of both host species. Individual points are labelled with identity of strains (B1, B2 etc.) which are described in Table 1.

Table 3. Model averaging and hypothesis testing for predictors of average *Acacia* growth response. The average parameter coefficients from the top 14 models (see table 2) are presented. The 'relative importance' coefficient reflects the frequency with which a given parameter is found in the 14 averaged models. In this analysis, both bacterial diversity and the presence/absence of individual strains are very strong predictors of plant growth

Parameter	Parameter estimate	Unconditional SE	Parameter estimate with shrinkage*	Relative Importance	Pr(>|z|)†
Strain richness 2‡	−0.39462	0.07509	−0.395	1	<0.0001
Strain richness 4‡	−0.55584	0.13286	−0.556	1	<0.0001
B3 present§	0.82545	0.11664	0.825	1	<0.0001
R1 present§	0.56887	0.11181	0.569	1	<0.0001
B1 present§	0.29166	0.09941	0.292	1	0.0033
C4 present§	0.27531	0.09862	0.275	1	0.0052
S1 present§	0.24849	0.1012	0.249	1	0.0141
S2 present§	0.2488	0.10912	0.249	1	0.0226
R3 present§	−0.24278	0.11685	−0.229	0.94	0.0378
M3 present§	0.16863	0.10823	0.083	0.48	0.1192
D3 present§	−0.16104	0.11351	−0.055	0.34	0.1560
B4 present§	−0.13334	0.10287	0.037	0.28	0.1949
M2 present§	0.1212	0.11088	0.020	0.17	0.2744
B2 present§	0.11634	0.10843	0.025	0.21	0.2833

*Parameter estimates of zero have been substituted into those models where the given parameter estimate is absent (Burnham and Anderson 2002).
†Probability that 95% confidence intervals for parameter estimates do not overlap zero.
‡Single-strain treatment was the reference category.
§Strain absent is the reference category.

depending on the presence or absence of a particular set of rhizobial strains in the inoculum (Table 3). For example, plants displayed consistently strong positive responses to the presence of strains R1 and B3, while the presence of strain R3 had consistent negative effects on plant growth (Table 3; Fig. 1). Other strains (e.g. B2, M2), while sometimes present as parameters in the top set of candidate models, had seemingly little influence on average *Acacia* growth. Furthermore, while the presence of multiple strains of rhizobia was associated with a decline in *Acacia* growth, generic richness was not present in any of the top models and thus had little influence on plant performance.

To examine biodiversity effects, we first generated pairwise growth comparisons among single- and multiple-strain inoculations. Strain co-inoculation had a consistent negative effect on plant productivity when compared to the growth of plants exposed to the best strain in the mixture alone (i.e. maximum effect), such that the great majority (61 of 72) of plants inoculated with rhizobial mixtures displayed reduced growth relative to plants inoculated with the best strain in the mixture alone (Fig. 2a). Data points that fell above the null line (Table S1 in Supporting Information) were only marginally so (i.e. largely within the limits of error) and were only found at very low levels of productivity (Fig. 2a), and all contained one or more *Mesorhizobium* strains. Co-inoculated plants also tended to grow less than expected based on the mean growth of plants exposed to each strain alone (i.e. mean effect). More than half (46 of 72; Table S1) of data points fell below the null line (Fig. 2b), and the magnitude of negative deviations from the null line was much greater than the positive ones [mean of points above line = + 0.169; mean of points below line = −0.411] (Fig. 2a). However, plants inoculated with multiple strains did tend to perform better than plants that were inoculated with the worst strain in the mixture alone (i.e. minimum effect), such that 55 of 72 (Table S1) points were above the null line (Fig. 2c). Even so, in some instances, the effects of co-inoculation were strongly negative, such that plants inoculated with mixtures of two strains that performed well alone, performed very poorly when those strains were combined (e.g. B1 and S2 on *A. salicina*; Fig. 1). Statistical analyses probing the role of rhizobial identity and diversity in mediating each of the biodiversity effects (maximum, minimum and mean) were conducted using generalized linear modelling and model selection (as per the above analysis). Main effects of host treatment, strain presence, strain richness and generic richness were evaluated as predictors of the three biodiversity effects. Hypothesis testing using AIC and weighted model averaging (top 100 models) identified that biodiversity effects were strongly variable depending on the presence or absence of a particular set of rhizobial strains in the inoculum (Table 4), but that the influence of individual strains varied depending on the hypothesis being tested (i.e. maximum, minimum and mean biodiversity effects). Generic richness and bacterial diversity were identified as important determinants of plant growth in some biodiversity effect models, but overall, these effects were of small magnitude compared to the effects generated by the presence and absence of individual strains (Table 4).

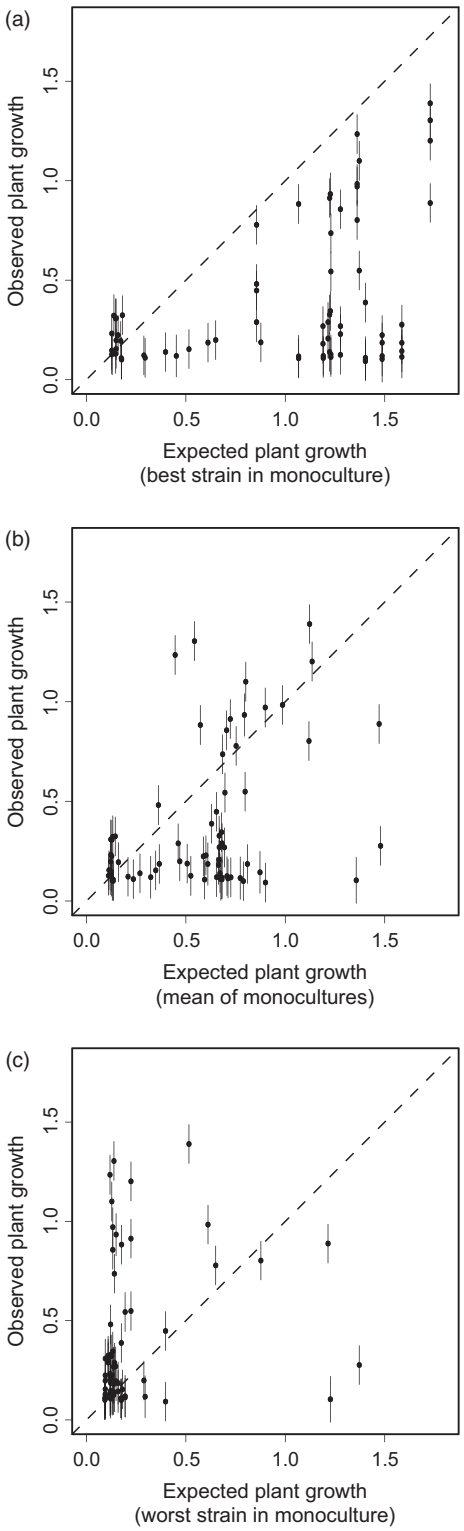

Fig. 2. Observed vs. expected plant growth (grams) of Acacia plants when inoculated with rhizobial mixtures. (a) Expected values are equal to the best strain in the monoculture. (b) Expected values are equal to the mean growth of all strains in monoculture. (c) Expected values are equal to the worst strain in the monoculture.

RHIZOBIAL COMPOSITION AND DIVERSITY AS PREDICTORS OF DIFFERENTIAL GROWTH OF ACACIA SPECIES

We then examined the roles that rhizobial identity and diversity played in mediating differential patterns of growth between *A. salicina* and *A. stenophylla.* In the mixed model analysis, biomass in *A. stenophylla* pots was consistently less than pots containing *A. salicina* or pots with both host species together ($F_{2,\ 64} = 4.43$, $P = 0.02$). Importantly, the strong rhizobial composition covariances indicated large effects of individual rhizobial compositions on these differential patterns of plant biomass (rhizobial composition*diversity*host

Table 4. Model averaging and hypothesis testing for predictors of biodiversity effect. Results are presented for expected growth based on (a) the best of the single-strain treatment yields for the component rhizobia (vs. max); (b) average plant growth with all strains in mixed inoculation treatments alone (vs. mean); and (c) plant growth with the poorest strain in treatment alone (vs. min). The 'relative importance' coefficient reflects the frequency with which a given parameter is found in the averaged models. The average parameter coefficients with relative importance > 0.8 from the top 100 models are presented. In these analyses, the presence/absence of individual strains are very consistent predictors of the different biodiversity effects

Ho	Parameter	Relative Importance	Parameter estimate
vs. max	D2 present‡	1	−1.02
	B4 present‡	0.99	0.35
	C1 present‡	0.98	−0.50
	A2 present‡	0.97	−0.43
	C4 present‡	0.97	−0.41
	Genus richness 2†	0.90	0.16
	Strain richness 4◊	0.83	0.26
	B2 present‡	0.83	−0.26
vs. mean	A3 present‡	1	−0.30
	A4 present‡	1	−0.45
	B3 present‡	1	−0.41
	B4 present‡	1	−0.38
	D1 present‡	1	−0.29
	D2 present‡	1	0.63
	D4 present‡	1	−0.53
	Genus richness 2†	0.98	−0.12
	Genus richness 4†	0.98	−0.12
	A2 present‡	0.84	0.16
vs. min	A4 present‡	1	0.66
	B1 present‡	1	−0.37
	D2 present‡	1	−0.56
	D4 present‡	1	0.63
	B3 present‡	0.95	0.39
	D1 present‡	0.79	0.26

†Single genus treatment was the reference category.
◊Two strain treatment was the reference category.
‡Strain absent is the reference category.

Table 5. Models predicting difference in growth between *A. salicina* and *A. stenophylla* in experimental pots. The five candidate models within 2 AICc units of the best model (for a total of 6) are presented. The Akaike weights (w_i) represent the relative likelihoods of the individual models being the best model

Model number	*n* of model parameters	Variables in model	AICc	Delta	w_i
1	3	B1;B3;S1	31.75	0.00	0.065
2	4	B1;B3;S1;R3	30.64	1.11	0.037
3	4	B1;B3;S1;S2	30.27	1.48	0.031
4	4	B1;B2; B3;S1	29.83	1.92	0.025
5	4	B1;B3;S1; M2	29.81	1.94	0.025
6	4	B1;B3;S1; R4	29.78	1.97	0.024

treatment variance component = 0.03650, SE = 0.008635, $P < 0.0001$; Fig. 1). Therefore, as for analysis of total plant growth, we used generalized linear modelling of mean blup values followed by model selection to assess the main effects of strain identity, strain richness and generic richness on patterns of differential growth between the two *Acacia* species. Because regression analysis showed a strong positive relationship between differential patterns of growth (blup values) when *A. salcina* and *A. stenophylla* were in the same pot, or in different pots ($P < 0.0001$; $R^2 = 0.7429$), we used the mean of these two measures of differential growth as a response variable for further analysis. Hypothesis testing using AIC and weighted model averaging (Tables 5 and 6) showed that the differential growth response of the two *Acacia* species could largely be explained by the presence or absence of three rhizobial strains (B1, B3, S1; Table 6). Other strains (e.g. R3, S2), while sometimes present as parameters in the top set of candidate models, had relatively little influence on differential patterns of *Acacia* growth. Neither strain nor generic richness was present in any of the top models indicating that both species respond similarly to the effects of diversity.

Table 6. Model averaging and hypothesis testing for predictors of differential growth response of *A. salicina* and *A. stenophylla*. The average parameter coefficients from the top 6 models (see table 4) are presented. The 'relative importance' coefficient reflects the frequency with which a given parameter is found in the six averaged models. In this analysis, only the presence or absence of three individual strains can be considered significant predictors of plant growth

Parameter	Parameter estimate	Unconditional SE	Parameter estimate with shrinkage*	Relative Importance	Pr(>|z|)†
B1 present‡	0.25769	0.02957	0.25769	1	0.0005
B3 present‡	0.50020	0.07428	0.50020	1	<0.0001
S1 present‡	−0.15307	0.07501	−0.15307	1	0.0398
R3 present‡	−0.09669	0.08195	−0.01739	0.18	0.2381
S2 present‡	−0.07766	0.07459	−0.01164	0.15	0.2978
B2 present‡	0.06676	0.07900	0.00803	0.12	0.3980
M2 present‡	0.06301	0.07546	0.00750	0.12	0.4037
R4 present‡	−0.05691	0.06934	−0.00668	0.12	0.4117

*Parameter estimates of zero have been substituted into those models where the given parameter estimate is absent (Burnham & Anderson 2002).
†Probaility that 95% confidence intervals for parameter estimates do not overlap zero.
‡Strain absent is the reference category.

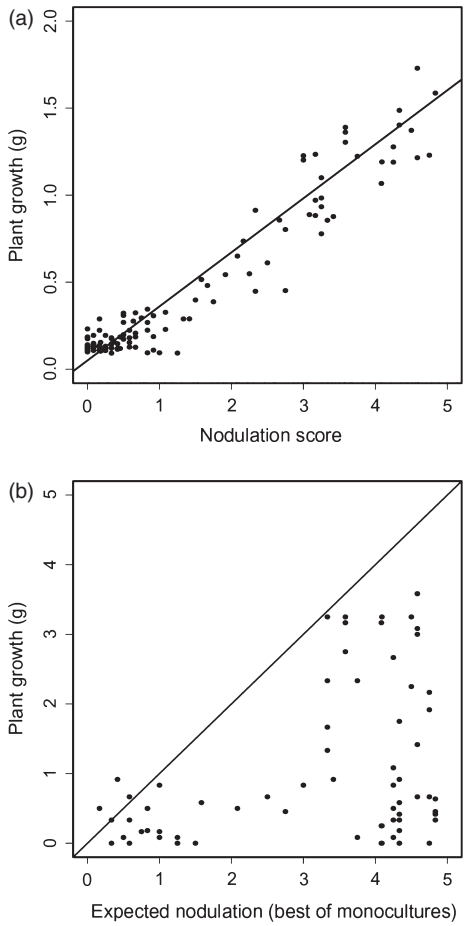

Fig. 3. Categorical estimates of nodule biomass of Acacia plants. (a) nodule biomass vs. plant growth.(b) Observed vs. expected nodulation of *Acacia* plants when inoculated with rhizobial mixtures**.**

PLANT VS. RHIZOBIAL BIOMASS

To examine potential relationships between plant and rhizobial fitness, we examined relationships between nodule and plant above-ground biomass (as potential proxy's for fitness), and effects of multiple inoculation on nodulation. Regression analysis showed strong positive relationships between nodulation and plant growth for both *A. salicina* ($P < 0.0001$; $R^2 = 0.9446$), *A. stenophylla* ($P < 0.0001$; $R^2 = 0.9016$) and mixed host treatments ($P < 0.0001$; $R^2 = 0.9334$), indicating general alignment between plant and rhizobial growth (Fig. 3a). Strain co-occurrence had a general negative impact on nodulation when compared to nodulation of plants exposed to the best strain in the mixture alone, such that the great majority (68 of 72) of plants inoculated with rhizobial mixtures displayed reduced nodulation scores compared to plants inoculated with the best strain in the mixture alone (Fig. 3b).

Discussion

In this study, we investigated how plant productivity varies depending on host identity, host community structure (single vs. mixed species), rhizobial identity and rhizobial diversity. We found that rhizobial diversity and rhizobial identity both significantly influence plant productivity in *Acacia*–rhizobia interactions. Relating plant performance to genotypic richness revealed that, compared to single-strain inoculations, the presence of multiple rhizobial genotypes in the rhizosphere was associated with decreased plant productivity. These results appear to be robust in the face of variation in host identity and host diversity. Together, our results suggest that both rhizobial diversity and identity (i.e. community structure) are important in determining productivity in terrestrial ecosystems, but that increased mutualist diversity does not necessarily have a positive effect on plant growth. In particular, our data show that multiple rhizobia interacting with a single plant creates opportunities for emergent or higher-order effects that extend beyond those that could be anticipated based upon outcomes of pairwise *Acacia*–rhizobia interactions.

Population- or community-level variation in the effectiveness of individual rhizobial genotypes is commonly observed in interactions between rhizobia and acacias (Burdon *et al.* 1999; Thrall, Burdon & Woods 2000; Thrall *et al.* 2007) and also in many other wild host–rhizobial interactions (e.g. Parker 1995; Sachs *et al.* 2009; Heath 2010). Consistent with these previous studies, when plants were inoculated with single rhizobial strains, we found that the outcome of interactions varied markedly depending on the identity of the specific strain, such that some strains promoted plant growth several orders of magnitude beyond that observed in negative controls, while others had no discernible effect on plant growth. Using these strains to subsequently construct artificial communities thus approximates the conditions of genetic and functional diversity observed in natural populations of these two-host species (Hoque, Broadhurst & Thrall 2011; Barrett, Broadhurst & Thrall 2012; Bever, Broadhurst & Thrall 2013).

In no case did we observe what could be interpreted as a significant positive effect of increasing rhizobial diversity. When compared to growth with the best strain in the mixture alone, we observed a continuum of negative responses, ranging from mildly to highly detrimental in terms of consequences for plant growth. This occurred across multiple combinations of rhizobial species and genera. These data thus do not support hypotheses that rhizobial diversity can enhance productivity through mechanisms of either complementarity or selection. However, it should be noted that in our experiments, we held overall density of cells constant in single and mixed inoculations, meaning that density of all strains was proportionally reduced in multistrain treatments, including the density of the best symbiont. Reduced plant performance compared to growth with the best strain might be expected if the capacity of the host to select the best symbiont in the mixture was limited by the density of the best strain in the mixtures. While it is not possible to rule out an effect of strain density on plant growth, linear dose–response effects alone are unlikely to explain our results. The total number of cells used to inoculate seedlings was large (approximately 1×10^7 rhizobial cells at the base of each seedling), ensuring ample opportunity for hosts to form associations with any of

the individual strains in a mixture. Furthermore, plant growth in most cases was not predictable based on the productivity of plants colonized by each of the strains alone (i.e. failing to support the neutrality hypothesis). Rather, in most cases, growth of plants inoculated with mixtures was significantly reduced compared to expectations based on single-strain inoculations. Finally, while in most cases growth in mixtures exceeded growth with the worst strain alone, this was not universally so, such that in some cases, mixtures comprising only effective strains (when alone) largely failed to provide any symbiotic benefit. Thus, we conclude that in general, our data support an antagonism hypothesis; that is, there is a variable but generally negative effect on plant growth associated with the presence of multiple rhizobial strains in the rhizosphere. This is consistent with previous observations from whole soil experiments using field-collected rhizobial communities (Bever, Broadhurst & Thrall 2013).

Our results contrast with many studies of plant–mycorrhizal interactions which report that under some circumstances, plant productivity and mycorrhizal diversity are positively correlated. This body of work suggests that in the right context, multiple mycorrhizal genotypes can enhance host performance and fitness relative to the effects of each alone (Smith, Jakobsen & Smith 2000; Maherali & Klironomos 2007; Jansa, Smith & Smith 2008; Hoeksema *et al.* 2010; Diagne *et al.* 2013), which in some cases likely reflects the capacity of mycorrhizas to access different nutrient pools (Smith, Jakobsen & Smith 2000; Jansa, Smith & Smith 2008). While it is possible that rhizobial complementarity would have been evident under different environmental conditions, it seems more likely that the contrast between rhizobia and mycorrhizas can be attributed to differences in potential for complementarity among these organisms. Whereas mycorrhizas may vary in their ability to access different nutrient pools (e.g. phosphorus *vs.* nitrogen, or different forms of phosphorus), rhizobia are reliably attributed only a single function with regards to nutrient acquisition (i.e. the conversion of dinitrogen to ammonia). Thus, there may be little potential for complementarity among rhizobia. Instead, there is high potential for competition among rhizobia for access to host resources, which in turn generates high potential for rhizobia to engage in interference, antagonistic or cheating-type behaviours.

It has been hypothesized that competition may result in the emergence of cheaters, such that in mixed infections, ineffective strains can potentially cheat both hosts and competing rhizobia by co-inhabiting nodules with N-fixing strains (Bever & Simms 2001; Bronstein 2001; Denison & Kiers 2004). Should such dynamics occur in *Acacia*–rhizobia interactions, declines in plant productivity associated with multiple rhizobial strains may be expected if diverse communities have greater probability of containing less beneficial strains. However, in our experiments, the presence of non-beneficial rhizobia in mixtures was not a necessary requirement to trigger a negative host response to the presence of multiple strains, such that plant growth and nodulation were strongly reduced in treatments where two highly effective strains were combined. In such cases, fitness of all partners must be reduced compared to that observed in pairwise interactions. While this does not preclude the potential for cheating in other treatments containing functionally variable combinations of rhizobia, it does indicate that cheating is unlikely to be a universal explanation for the patterns we have observed.

Antagonistic competition among rhizobial genotypes potentially provides a general explanation for our results. Complementary resource use, competition and subsequent antagonistic interactions are widespread phenomena in microbial systems (Griffin, West & Buckling 2004; Hibbing *et al.* 2010). Considerable research effort has been devoted to trying to understand how the competitive ability of inoculant strains influences the outcome of the symbiosis (Dowling & Broughton 1986; Triplett & Sadowsky 1992; Friesen 2012). For example, it has been shown that production of bacteriocins by rhizobia can specifically inhibit growth and nodulation of co-occurring strains (Triplett & Sadowsky 1992). Similarly, a phenomenon described as 'competitive nodulation blocking' has been described in interactions between *Rhizobium leguminosarum* bv. *viciae* and *Pisum sativum* cv. Afghanistan (Winarno & Lie 1979). In this example, the co-occurrence of rhizobial strains can have strong negative consequences for plant productivity, such that *Rhizobium* strains that are non-compatible with the host produce very high levels of nodulation factor signalling molecules, completely inhibiting nodulation by *Rhizobium* strains that form effective symbioses when alone (Hogg *et al.* 2002). Thus, direct competitive dynamics among rhizobia have potential to reduce performance by inhibiting nodule formation by beneficial rhizobia.

A host partner-choice mechanism (Gubry-Rangin, Garcia & Béna 2010) provides a second general and parsimonious explanation for our results. In particular, in our experiments, the observed decline in nodulation and productivity associated with increased rhizobial diversity may reflect host selective mechanisms that act to circumvent invasion of nodules by multiple (and potentially ineffective) rhizobia. Under this model, *Acacia* plants simultaneously perceiving the presence of genetically distinct rhizobia are less likely to initiate a symbiosis, regardless of rhizobial effectiveness in pairwise interactions. Such a mechanism would have the effect of retarding nodule colonization by multiple rhizobial strains, potentially checking the proliferation of cheating rhizobia within nodules and destabilization of the mutualism. A range of mechanisms have been described by which plants can regulate the initiation and proliferation of nodulation. Specificity in most legume–rhizobial interactions is controlled at least in part by the production and perception of strain-specific nod factors (NF) (Masson-Boivin *et al.* 2009). NFs are perceived at the plant epidermis by legume receptor genes, which, in compatible interactions, trigger nodulation. Perception of multiple NFs by the host NF receptors therefore has potential to act as a negative regulator of nodule initiation or development very early in the nodulation process. Plants also have mechanisms that act to control nodule proliferation at later developmental stages which could potentially be harnessed to prevent the colonization of nodules by multiple strains. For example,

in the process termed 'autoregulation of nodulation' (AON), rhizobia trigger the production of an AON elicitor signal, the perception of which leads to the suppression of nodulation events in the root (Ferguson *et al.* 2010).

A general caveat of experiments performed under controlled conditions relates to the extent to which results can be used to draw inferences regarding outcomes that might be observed under more natural conditions. For example, while our observations of reduced effective nodulation in mixed inocula are consistent with both direct competition and partner-choice mechanisms, neither hypothesis can explain why *Acacia* hosts are able to form effective associations under field conditions, where plants must encounter a diverse array of rhizobial genotypes (e.g. Thrall *et al.* 2005). Under natural conditions, the relative frequencies, densities and distribution of different rhizobial genotypes in the soil are likely to vary spatially throughout the soil matrix. However, in our experiments, we inoculated plants with high, uniform densities of rhizobia (typical for these kinds of experiments). Thus, any fine-scale spatial structure typical of natural soils will be largely absent in the pots in which these plants were grown. A possible role for spatial structure is supported by the findings of Bever *et al.* (2009) who demonstrate that spatial structure in the rhizosphere is critical to the maintenance of beneficial mutualisms between plants and mycorrhizas. Similarly, our experiments were performed using young seedlings which were exposed to a particular array of environmental conditions (e.g. soil used in pots; watering regime; nutrient status etc.). While there is no specific reason to suspect that these factors are likely to qualitatively influence inoculation outcomes, environmental variation is known to be important in the establishment and maintenance of legume–rhizobial symbioses and in influencing outcomes of rhizobial competition in particular (Zahran 1999).

While our data do not speak directly to rhizobial fitness, the results indicate that positive, synergistic interactions (resulting in the delivery of increased levels of N) are unlikely to drive the maintenance of diversity in rhizobial populations. Despite this result, suboptimal rhizobia persist and in some cases dominate soils (e.g. Gibson *et al.* 1975; Drew *et al.* 2011). The question as to what role interactions among rhizobia (e.g. cheating) play in the maintenance of functional diversity (and low quality strains in particular) in rhizobial populations thus remains (Friesen & Mathias 2010). Our data indicate that the fitness (relative to single-strain inoculations) of the rhizobial community as a whole decreases on average in multiple-strain inoculations (i.e. less nodulation in mixtures) and that in some treatments fitness of all rhizobia also decreases (i.e. no nodulation in mixtures comprising only effective strains). Thus, in at least some cases, it seems unlikely that interactions among rhizobia alone promote strain coexistence. However, we have no insight into how the relative fitness of rhizobia that are ineffective in single-strain inoculations might change in multi-strain treatments, particularly when plants still formed some nodules and grew better than the worst strain in the mixture (most cases).

Competitive dynamics among rhizobia are not the only factors that have potential to promote the maintenance of strain diversity and coexistence within soils. Variation in rhizobial effectiveness has in many cases been demonstrated to be context dependent – that is, variable depending on the host species with which a strain associates (Thrall *et al.* 2011), or on the environmental conditions under which the interaction occurs (van Rossum *et al.* 1994; Zahran 1999). We thus hypothesized that increasing rhizobial diversity may enhance plant productivity in plant communities where multiple legume species are present (e.g. Van Der Heijden *et al.* 2006). We tested this hypothesis by measuring plant productivity in response to inoculation with rhizobial treatments in pots containing two plants of *A. salicina*, two plants of *A. stenophylla* and one each of *A. salinina* and *A. stenophylla*. We found no direct evidence to support the hypothesis that rhizobial diversity can enhance productivity in more complex plant communities. Specifically, in our experiments, rhizobial diversity did not result in increased total plant productivity in mixed species pots compared to pots where *A. salicina* or *A. stenophylla* occurred alone. However, our data did show that *A. stenophylla* and *A. salicina* respond differently to the presence of individual rhizobial genotypes in different treatments and thus are consistent with the idea that rhizobial diversity is important when multiple host species are present in the community. Nevertheless, we did not find direct evidence that rhizobial diversity will enhance host diversity or alter plant–plant interactions. As far as we know, our study is the first attempt to decouple the relative importance of rhizobial identity and diversity on the productivity of different host species in a community context. However, it is important to note that the presumably necessary condition of exclusive partner specificity was not met in the single-strain inoculations; in our experiments, *A. salicina* was able to form effective associations with all strains that were effective with *A. stenophylla.* Thus, it is possible that a different combination of host species (with exclusive preference for different rhizobia) would have delivered different outcomes in the two-host-species mixtures. Future experiments testing this hypothesis should take account of both partner identity and diversity, and if possible, select experimental hosts that co-occur in nature, but have discrete preferences for specific rhizobial genotypes.

Conclusion

This study is unique in examining the combined effects of genotypic richness, genetic relatedness and identity in determining the productivity of host–microbial symbioses. There is substantial literature that documents the importance of rhizobial genotype in determining outcomes plant–rhizobial interactions, both in terms of effectiveness in pairwise interactions and competitive ability with other rhizobial isolates. However, few (if any) have specifically examined how diversity *per se* influences productivity, nor the combined effects of variation in identity and diversity. Our results suggest that both identity

and diversity are important for understanding plant responses to associating with rhizobia, that multiple rhizobia interacting with a single plant creates opportunities for emergent effects that extend beyond outcomes that could be anticipated based upon outcomes of pairwise interactions.

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Data accessibility

Data from this study are available from the Dryad Digital Repository (Barrett *et al.* 2014).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Growth of multi-strain treatments compared to expectations based on the mean growth of all strains in the mixture alone, the best strain in the mixture alone, and the worst strain in the mixture alone.