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LETTER

Microbial phylotype composition and diversity predicts plant productivity and plant–soil feedbacks

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Abstract

The relationship between ecological variation and microbial genetic composition is critical to understanding microbial influence on community and ecosystem function. In glasshouse trials using nine native legume species and 40 rhizobial strains, we find that bacterial rRNA phylotype accounts for 68% of amoung isolate variability in symbiotic effectiveness and 79% of host specificity in growth response. We also find that rhizobial phylotype diversity and composition of soils collected from a geographical breadth of sites explains the growth responses of two acacia species. Positive soil microbial feedback between the two acacia hosts was largely driven by changes in diversity of rhizobia. Greater rhizobial diversity accumulated in association with the less responsive host species, *Acacia salicina,* and negatively affected the growth of the more responsive *Acacia stenophylla*. Together, this work demonstrates correspondence of phylotype with microbial function, and demonstrates that the dynamics of rhizobia on host species can feed back on plant population performance.

Keywords

Diversity-productivity, ecosystem function, microbial ecology, molecular ecology, mutualism, plant ecology, plant–microbe interactions, plant–soil feedback, rhizobia, specificity, symbiosis.

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INTRODUCTION

Advances in molecular methods have revealed a tremendous diversity of bacterial taxa in soil, with as many as 10 000 discrete taxa present per gram (Torsvik *et al.* 1990; Roesch *et al.* 2007). This bacterial diversity could be of great functional importance if genetically distinct taxa also have correspondingly different physiologies and ecologies. While differences in habitats, and spatial and temporal patterns support the ecological coherence of higher taxonomic ranks (von Mering *et al.* 2007; Philippot *et al.* 2010), examples confirming differing ecologies of phylogenetically distinct bacterial taxa (phylotypes) within a lineage are limited to a few systems involving intensive studies of tightly circumscribed groups (Palys *et al.* 2000; Johnson *et al.* 2006; Hunt *et al.* 2008). Thus, the generality of correspondence between genetic delineation of species and ecological function remains uncertain (Cohan 2006; Doolittle & Papke 2006; Konstantinidis *et al.* 2006), especially given the numerous examples of lateral transfer of genes for ecologically important traits within major soil microbial groups and across distantly related groups (Ochman *et al.* 2000; Koonin *et al.* 2001; Gogarten *et al.* 2002). This is particularly problematic because the causal link between genetics and ecology is a basic assumption underlying the rapidly growing fields of molecular ecology and environmental microbiology (von Mering *et al.* 2007; Philippot *et al.* 2010).

Soil microorganisms play fundamental roles in biogeochemical cycling and are major drivers of terrestrial ecosystem productivity and diversity (van der Heijden *et al.* 2008; Mangan *et al.* 2010). Experimental studies have identified that soil microbial diversity and composition can have large influences on plant productivity and diversity (Van der Heijden *et al.* 1998; Vogelsang *et al.* 2006). Moreover, the composition of microbial communities has been shown to be dynamic, changing rapidly with plant species (Bever 2002; Mitchell *et al.* 2010). This change in composition can generate feedbacks on plant fitness, and recent work supports a primary role for soil biota in structuring plant communities (Kulmatiski *et al.* 2008; Bever *et al.* 2010; Mangan *et al.* 2010; Johnson *et al.* 2012). While soil-feedback inoculation studies provide phenomenological evidence of the importance of soil microbial change on plant ecology, the microbial drivers of these feedbacks are often obscure, with individual studies illustrating contributing roles of both soil pathogens and soil mutualists (Packer & Clay 2000; Bever 2002).

Here, we investigate the link between microbial genotype and function in the context of interactions between plants and their soil microbial communities, specifically targeting legumes and N_2-fixing symbionts (i.e. rhizobia). This well-known interaction has the advantage that metrics of ecological function can be efficiently scored using assays of host growth. Specifically, average host growth promotion can represent the effect of the rhizobial community on terrestrial productivity and the specificity of host growth promotion underlies the potential impact of the rhizobial community on plant community dynamics (Bever *et al.* 1997; Mangan *et al.* 2010). Moreover, given that the genes coding for N fixation within rhizobia are known to have spread laterally across taxonomic groups (Laguerre *et al.* 2001; Sprent 2001; Finan 2002; MacLean *et al.* 2007), studies of rhizobia provide a conservative test of the relationship between chromosomally defined genotypes and host growth promotion.

In particular, we take advantage of the extensive work on the ecology of the interactions between Australia acacias and their symbiotic rhizobia. Two recent studies of this system have demonstrated specificity of rhizobial effects on plant growth (Thrall *et al.* 2008) and the potential for positive plant–soil feedbacks (Thrall *et al.* 2007b). Here, we integrate information on rhizobial phylotype with phenotypic growth data from these two studies to test the relationship between rhizobial genotype (as assessed by rRNA markers) and ecological function. We first reanalyse the ecological variation measured within a comprehensive glasshouse inoculation study of individual isolates of rhizobia with a diverse set of ecologically important *Acacia* species (Thrall *et al.* 2008). We then integrate genetic analyses of rhizobial community

composition and ecological function into a test of rhizobial mediation of plant–soil feedback between two widely distributed *Acacia* species (Thrall *et al.* 2007b). Using these approaches, we explicitly evaluate the extent to which rhizobial phylotypes and community structure can predict the ecological performance of their legume hosts.

MATERIALS AND METHODS

Experiment 1: rRNA markers as predictors of ecological function

A full factorial replicated glasshouse trial involving nine *Acacia* species and 40 rhizobial isolates was conducted to assess variation in host specificity and rhizobial effectiveness at promoting plant growth (Thrall *et al.* 2008). The rhizobial strains were originally isolated from 17 *Acacia* host species from 22 locations across south-eastern Australia; the strains used in the glasshouse study represented a haphazardly chosen subset of these. The set of host species used in the glasshouse trial included *Acacia brachybotrya, Acacia hakeoides, Acacia ligulata, Acacia mearnsii, Acacia pendula, Acacia pycnantha, Acacia rigens, Acacia salicina* and *Acacia stenophylla*. These were selected to represent a broad range of geographical distributions and ecologies. Experimental details and data are provided in the Electronic Appendix and in Thrall *et al.* (2008).

Characterisation and identification of rhizobial phylotypes

Genomic DNA was extracted from purified single-colony rhizobial isolates, and the SSU was amplified. The SSU PCR product was digested with one of four restriction endonucleases (*Hha*I, *Hinf*I, *Msp*I and *Rsa*I; New England Biolabs) and digested products were separated on agarose gels. Restriction profiles were used to identify genomic species as described by Lafay & Burdon (1998). To determine the generic affiliations of isolates, 16S rDNA was sequenced from representatives of each newly identified phylotype as described in the Electronic Appendix and Hoque *et al.* (2011).

Statistical analysis

Of the 40 isolates used in the glasshouse study, a total of 34 could be identified to phylotype and only these isolates were included in the analysis of phylotype. Log-transformed plant dry weight was analysed using Proc Mixed in SAS (SAS 1990) with plant species, the genus, phylotype and isolate of rhizobia and their interactions with plant species being random effects. Identifying rhizobial isolates and genera and acacia species as random effects allow us to generalise the extent to which taxonomic groupings based on the rRNA gene explain ecological variation in effectiveness of growth promotion of Australian acacias. Variance components were estimated using restricted maximum likelihood.

Experiment 2: correlation of genotypic composition and ecological function in field soils

To examine patterns of genetic variation and adaptation in host and symbiont populations across geographical ranges, studies were conducted on multiple populations of two widespread native Australian *Acacia* spp. (*A. salicina*, *A. stenophylla*) and associated rhizobial bacteria. Both host species have broad distributions across the Murray Darling Basin in eastern Australia. *A. stenophylla* occurs on the western interior of the basin from the River Murray north, whereas *A. salicina* occurs throughout the basin, extending more into the eastern flanking ranges than *A. stenophylla*. In total, 58 sites, including 28 with *A. salicina* and 30 with *A. stenophylla*, were characterised with regard to host abundance, symbiont population sizes, soil chemistry and environmental parameters (Thrall *et al.* 2007b).

Estimates of rhizobial abundance

The most probable number plant infection test was used to enumerate rhizobia capable of nodulating seedlings of *A. salicina* and *A. stenophylla* (Thrall *et al.* 2007b). Measures of rhizobial density using these two species were log transformed, and these values were strongly positively correlated (correlation = 0.80, $P < 0.0001$). We therefore separated these measures into two variables, one being the average of the two measures which approximated overall rhizobial density, and the second being the difference in abundance of nodulation by the two hosts, which represents a measure of the host specificity of association with the resident rhizobia.

Genetic characterisation of rhizobial communities

Across the 58 sites, a total of 1316 isolates (average of 22.7 per site) were obtained and classified into 119 phylotypes (Hoque *et al.* 2011). Of these isolates, 1285 (representing 109 of the phylotypes) could be reliably assigned to genera based on 16S rRNA sequence data (Hoque *et al.* 2011), as summarised in Table S1. Data on phylotype composition and generic affiliation were used to calculate community and species diversity indices.

Acacia growth assays

To examine variation in symbiont effectiveness at promoting plant growth, both *Acacia* species were grown in all 58 soils (data in electronic appendix), plus uninoculated N^+ and N^- controls. At harvest, plant roots were separated from the soil and scored for nodulation characteristics (Thrall *et al.* 2007b).

Statistical analyses

Responses of *A. salicina* and *A. stenophylla* to the individual soil communities were calculated as the average of log-transformed final dry weights. These averages were analysed with a two-way ANOVA (acacia species by soil origin) using Proc GLM in SAS (SAS 1990), with the variation in response to soil samples collected from individual species being the random effect. The sign and magnitude of the soil community interaction coefficient was used to measure soil community feedback (the difference in average growth in home soils from the average growth in each other's soils), which was derived as a critical metric determining soil microbial effects on plant community dynamics (Bever *et al.* 1997). In this measure, growth in pots is assumed to be correlated with fitness in the field [e.g. (Pringle & Bever 2008; Mangan *et al.* 2010)]. Tests of the relationship between *Acacia* growth response and rhizobial phylotype composition were performed using two approaches. First, we constructed a matrix of genetic similarities in rhizobial composition and a separate matrix of the difference in growth response of the individual *Acacia* species. We tested correlations between rhizobial phylotype composition and *Acacia* growth matrices using Mantel tests performed using GenAlEx 6 (Peakall & Smouse 2006).

The second approach to testing the dependence of *Acacia* growth response on the rhizobial soil community was to use regression and ANCOVA. Metrics of rhizobial community composition were constructed using principal components analysis, which included all isolates that occurred at least seven times within the sampling. The top 10 axes of variation (Table S2), which together explained 75% of the total variation, were used as predictors of plant growth, along with

measures of phylotype diversity of each sample [number of rhizobial phylotypes, evenness and diversity (H)], the proportion of *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* in each sample, the average density of rhizobia measured by the two *Acacia* species and the difference in rhizobial density measured by the two *Acacia* species. Rhizobial richness was log transformed prior to analysis to decrease the leverage of a few very diverse samples.

We constructed two sets of regression analyses. The first analysis tested predictors of the average growth response of the two *Acacia* species, and the second tested predictors of the difference in growth response between *A. salicina* and *A. stenophylla*. The later corresponds to the plant-soil interaction term which measures the plant–soil feedback (Bever *et al.* 1997). Regression analyses were performed using Proc Reg in SAS. Within each set of analyses, candidate models were ranked by the Akaike's Information Criterion corrected (AIC_c) for small sample size. The Akaike weights (w_i), which correspond to the likelihood that a given model is the best of those being considered (Burnham & Anderson 2002), were calculated and all models with Akaike weights of at least 5% were included (Tables S3 and S5). For predictors within this set of models, combined estimates of the regression coefficients were obtained by weighting the coefficients by the Akaike weights (Tables S4 and S6). The standard errors of the regression coefficients were calculated from the Akaike-weighted average of individual model coefficients and from variation in coefficients between models (Burnham & Anderson 2002). In these analyses, a coefficient of zero was used for models which did not include a particular predictor. To evaluate the potential importance of individual predictors, we constructed t-tests of overall significance of individual predictors from the Akaike-weighted averaged coefficients and standard errors. Significant relationships were depicted using partial regression plots (Fig. 2b–e) of the most likely model as judged by the Akaike weights.

To test whether the significant rhizobial predictors of acacia growth mediate acacia response to the soils derived from *A. salicina* and *A. stenophylla*, we first evaluated whether these predictors differed between soils of these two origins using ANOVA. The significant predictors that did differ between these two soil origins were then evaluated for their potential to explain the variation in plant response to soils derived from *A. salicina* vs. *A. stenophylla*. We did this by using the significant rhizobial predictors as covariates in ANCOVA of acacia growth response conducted using Proc GLM. Covariates that mediate the soil origin effect on acacia growth will reduce the sums of squares explained by soil origin. We constructed an F test for this mediation from the difference in sums of squared deviations due to soil origin without covariates and the sums of squared deviations with covariates (Tables S7 and S8).

Total rhizobial phylotype diversity was estimated using the mean of the bootstrapped estimates using Chao2 as generated using the program EsimateS (Colwell *et al.* 2012).

RESULTS

Experiment 1: rRNA markers as predictors of ecological function

The 40 different rhizobial strains isolated from natural sites throughout south-eastern Australia (Thrall *et al.* 2008) varied considerably in both their average ability to promote the growth of *Acacia* host plants (covariance estimate = 0.054, $P = 0.0002$) and in the specificity of their growth promotion across the nine *Acacia* host species (covariance estimate = 0.078, $P < 0.0001$). This substantial ecological variation between different isolates of rhizobia represents the majority of variation seen in this study.

We were able to characterise 34 of these isolates using restriction fragment length polymorphisms banding patterns and analysis of 16S rRNA sequences. These 34 isolates fall within 13 different phylotypes across several rhizobial genera (Electronic Appendix).

Phylotype explains a remarkable amount of the variation in the ecological function of these rhizobial isolates. In fact, phylotype accounts for 62% of the variation in average growth promotion of the rhizobial strains ($Z = 1.7$, $P = 0.04$) and 74% of the variation in specificity of growth promotion ($Z = 5.6$, $P = 0.0001$). The four rhizobial genera explained 87% of the species variation in average growth promotion ($Z = 0.9$, *ns*) and 74% of the species variation in specificity of growth promotion ($Z = 2.9$, $P = 0.001$). Most *Acacia* spp. grew better with *Bradyrhizobium* than other genera of rhizobia (Fig. 1). However, *A. stenophylla* grew relatively poorly with phylotypes of *Bradyrhizobium* (Fig. 1b), whereas *A. salicina* was more consistently responsive to inoculation with a broader range of genera or phylotypes of rhizobia (Fig. 1c).

Experiment 2: correlation of genotypic composition and ecological function in field soils

We found that on average, both species of *Acacia* tended to grow larger when inoculated with soils derived from beneath adult plants of *A. stenophylla* than *A. salicina* ($F_{1,56} = 2.89$, $P < 0.1$). This was particularly true for *A. stenophylla,* which grew significantly better in soils derived from its own species compared with those of *A. salicina* ($F_{1,56} = 7.66$, $P = 0.008$, Fig. 2a), as previously reported in Thrall *et al.* (2007b). The differential response of *A. stenophylla* and *A. salicina* to their soils, with *A. stenophylla* growing relatively better with soil inocula derived from conspecific trees, generated a net pairwise positive soil community feedback (as derived in Bever *et al.* 1997).

To test whether this positive plant–soil feedback was mediated by changes in the rhizobial community, we characterised the rhizobial community composition for each of these soil samples, using the information presented in (Hoque *et al.* 2011). We then constructed a matrix of genetic similarities in rhizobial composition and a separate matrix of similarity in growth response of the individual *Acacia* species. We found a significant correlation between the growth of *A. salicina* and *A. stenophylla* and rhizobial community composition (Mantel test with 10 000 permutations, $r_M = 0.11$, $P = 0.02$; $r_M = 0.081$, $P = 0.02$, for *A. salicina* and *A. stenophylla* respectively), affirming that acacias inoculated with soil communities containing similar rhizobial compositions had similar growth responses. This analysis provides a robust test confirming an overall correspondence between rhizobial community structure and acacia growth, but on its own is insufficient to identify rhizobial composition as the primary driver of plant–soil feedbacks.

We therefore tested potential causal relationships mediating the plant–soil feedback using regression approaches. Phylotype richness, diversity and evenness, measures of rhizobial community composition constructed from principal component analysis of the phylotype composition of the soils and estimates of relative rhizobial density from each soil were evaluated as predictors of plant growth. Our analysis showed that the average growth response of the two *Acacia* species to

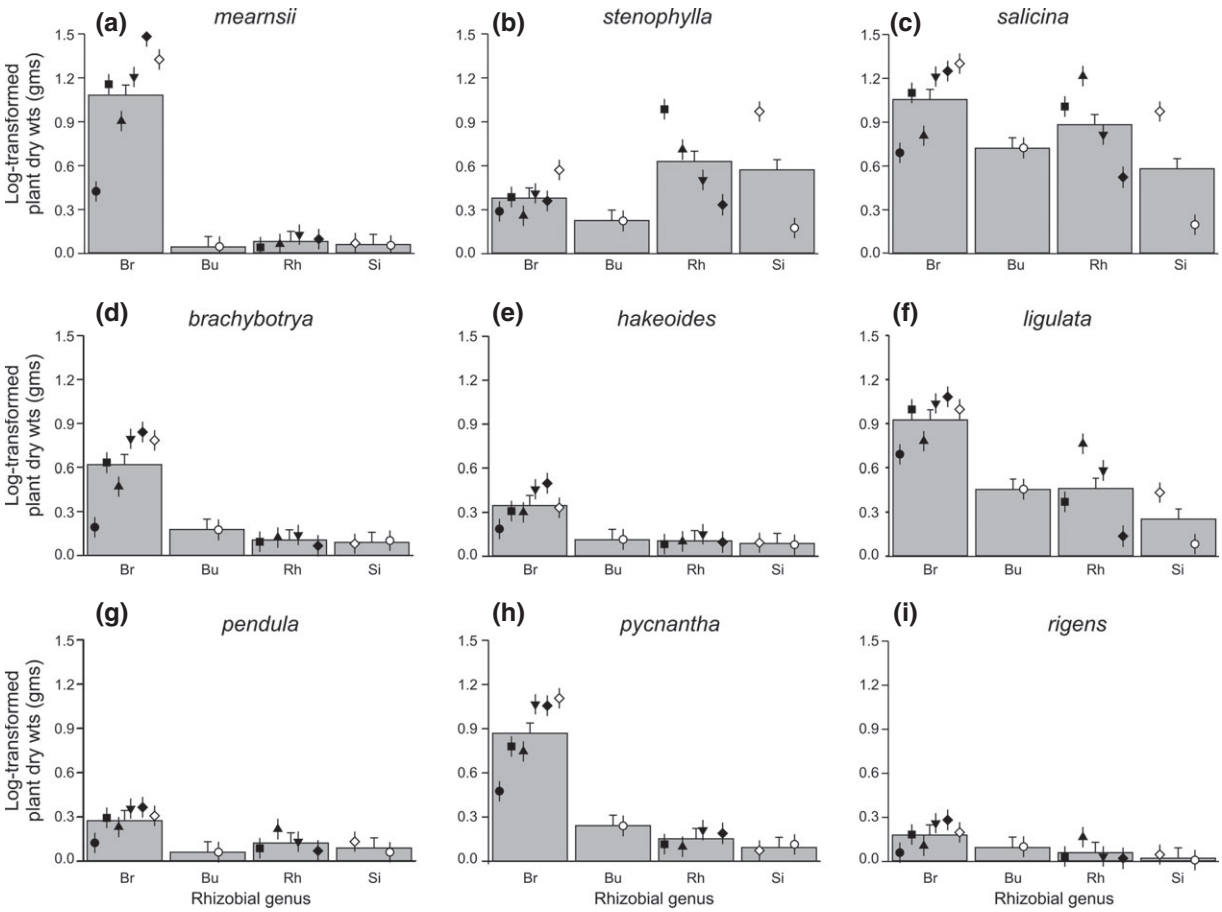

Figure 1 Growth of nine *Acacia* species (a-i) was evaluated in association with 34 different isolates of rhizobia belonging to 13 phylotypes and four genera. Rhizobial phylotypes showed consistent differences in their average growth promotion and the specificity of their growth promotion. While most *Acacia* species grew best with phylotypes of *Bradyrhizobium*, *Acacia stenophylla* (b) grew relatively poorly with phylotypes of *Bradyrhizobium*, and instead performed best with *Rhizobium* and *Sinorhizobium*. *Acacia salicina* was relatively insensitive to different rhizobial phylotypes (c). Symbols represent individual phylotypes and the histograms represent the means of the genera *Bradyrhizobium*, *Burkholderia*, *Rhizobium* and *Sinorhizobium* (= *Ensifer*), which are represented by Br, Bu, Rh and Si respectively.

a given soil inoculum is strongly positively related to measures of overall rhizobial density from that soil ($t = 3.56$, $P = 0.0008$, Fig. 2b, Tables S3, S4). Measures of the phylotype composition of the rhizobial communities were also important predictors, as acacias responded positively to increasing values of the fifth principal component of rhizobial community composition ($t = 2.48$, $P = 0.02$, Fig. 2c, Table S4). Acacia growth also tended to decline with increasing rhizobial species richness ($t = -1.71$, $P = 0.09$, Fig. 2d, Table S4). Of these three predictors, rhizobial density and species richness were dependent on the species of *Acacia* from which the soils originated. Rhizobial density was higher in soils derived from *A. stenophylla* ($F_{1,57} = 2.95$, $P < 0.1$), whereas rhizobial species richness was higher in soils derived from *A. salicina* ($F_{1,57} = 8.25$, $P = 0.006$). Together, these changes in rhizobial density and species richness explained 98% of the decline in average acacia growth in *A. salicina* soils (Fig. 2a), thus mediating the weak effect of soil origin ($F_{1,57} = 2.81$, $P < 0.1$, Fig. 3, Table S7).

Soil community feedback is driven by the difference in growth response of the plant species (Bever *et al.* 1997), and we found that the differential response of the two *Acacia* species across the 58 soils assayed (Fig. 2a) was also determined by measures of rhizobial phylotype composition. Phylotype richness was the strongest predictor, and had a stronger negative effect on the growth of *A. stenophylla* than on that of *A. salicina* ($t = 4.94$, $P < 0.0001$, Figs. 2d, S1). This effect was strikingly robust to the inclusion of other predictors in the model (Tables S5, S6). The difference in soil rhizobial density detected using the two species was also a significant predictor, as *A. stenophylla* grew better in soils in which *A. stenophylla* test plants detected higher densities of nodulating bacteria relative to *A. salicina* ($t = 3.21$, $P = 0.002$, Fig. 2e, Table S6). As rhizobial phylotype richness was significantly higher in soils from *A. salicina* than soils from *A. stenophylla* ($F_{1,57} = 8.25$, $P = 0.006$), this phylotype effect contributes to the reduced growth of *A. stenophylla* when inoculated with soil communities present in *A. salicina* sites (Fig. 3, Table S8). A similar dependence on host origin was found for the differential detection of rhizobial bacteria between the two host species ($F_{1,57} = 9.96$, $P = 0.003$), reflecting a higher density of compatible rhizobia in soils of conspecific acacias. Together, these two predictors explain 99.5% of the difference in growth response between these two species to inoculation with conspecific vs. heterospecific field soils ($F_{1,57} = 7.59$, $P = 0.008$, Fig. 3, Table S8). This result confirms

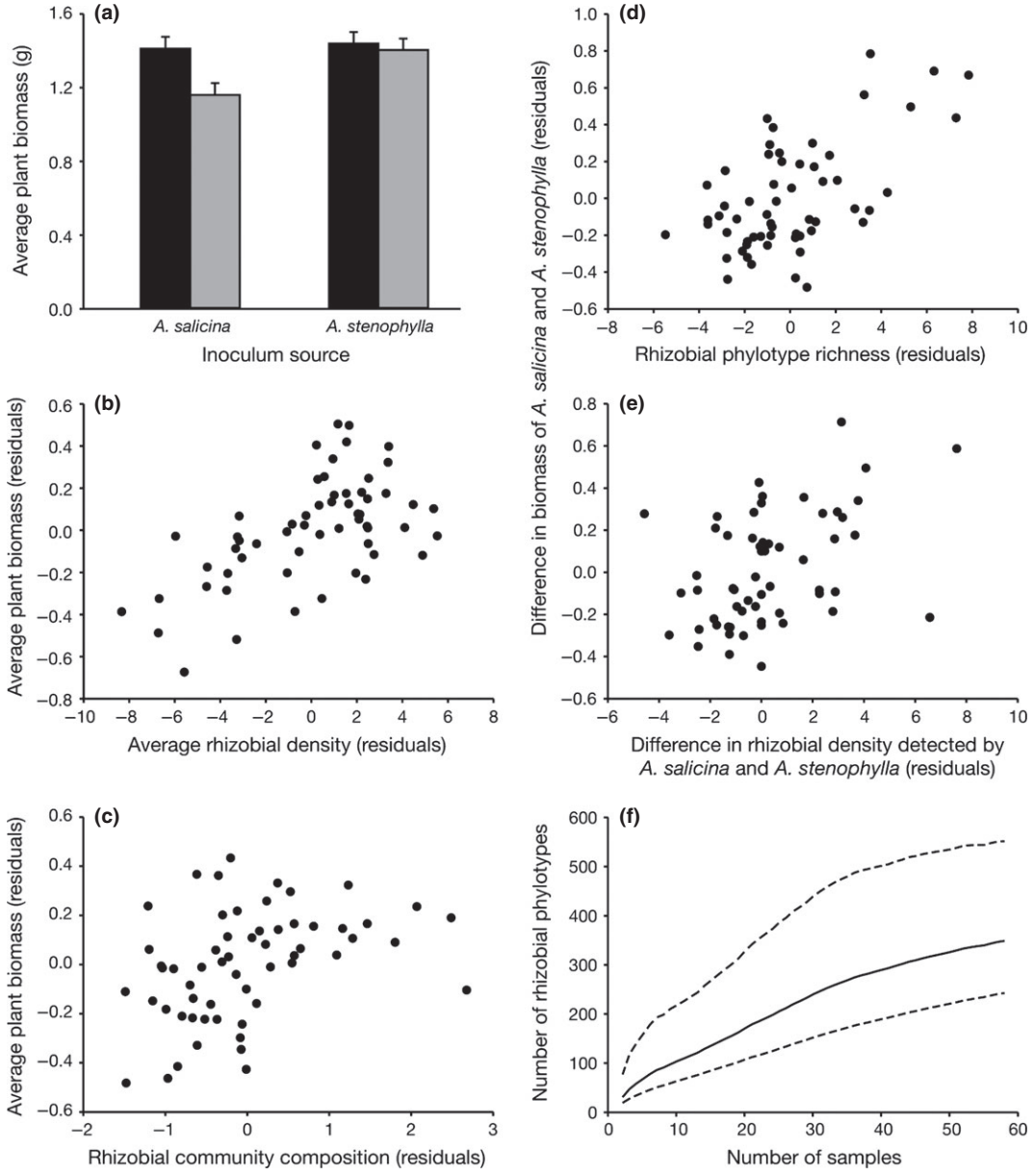

Figure 2 (a) Growth of *Acacia salicina* (black bars) and *Acacia stenophylla* (grey bars) in soils sampled from *A. salicina* and *A. stenophylla*. On average, *A. salicina* and *A. stenophylla* growth increased with increasing rhizobial density (b) and values of the fifth principal component of rhizobial soil community composition (c). The difference in growth of *A. salicina* and *A. stenophylla* increased with phylotype richness of rhizobial communities (d) and with differences in rhizobial density measured by the two plant species (e). (b–e) Partial regression plots, which represent the slope and error after removal of other predictors in the best model. (f) Estimate and 95% CI for total phylotype diversity associated with *A. salicina* and *A. stenophylla* in south-eastern Australia.

that changes in the rhizobial community generated the positive soil feedback observed in this system.

Phylogenetic diversity of Rhizobia on *Acacia salicina* and *stenophylla*

Bootstrapped estimates put the number at nearly 400 (95% confidence limits of 247–610) different rhizobial phylotypes within part of the range of only two host species (Fig. 2f). However, this number is likely to be an underestimate, given the high number of phylotypes that were unique to one site as reflected in the steady rise of the extrapolated total diversity with increasing sample size.

DISCUSSION

We find that rhizobial isolates vary in their effects on plant productivity and in the host specificity of these effects. Together, these results are consistent with rhizobial community structure contributing to both terrestrial productivity and host community dynamics, extending similar findings for mycorrhizal fungi (Van der Heijden *et al.* 1998; Vogelsang *et al.* 2006). Our study is unique in demonstrating concordance between ecological function and rRNA phylotype across a broad range of bacterial isolates, and in demonstrating that changes in rhizobial community composition can drive feedbacks on plant populations.

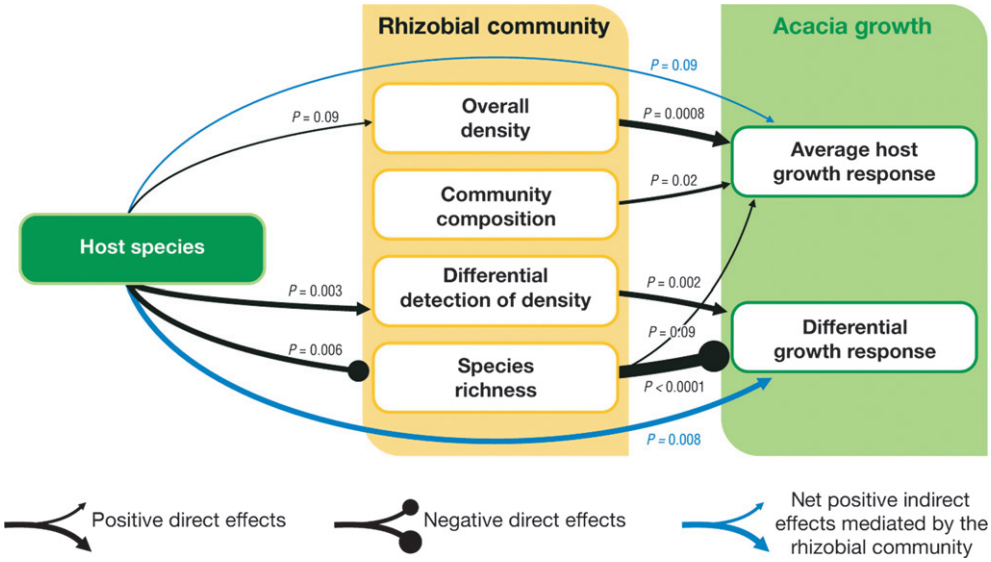

Figure 3 Path diagram showing rhizobial controls on average acacia growth and the difference in response of *Acacia salicina* and *Acacia stenophylla*. Soils from *A. stenophylla* generally have higher overall densities of rhizobia, particularly as measured by *A. stenophylla*, and lower phylotype richness. The increase in overall densities of rhizobia results in an increase in average acacia growth. The differential increase in rhizobia that colonise *A. stenophylla* but not *A. salicina,* and the reduction in rhizobial phylotype richness increase the growth of *A. stenophylla* relative to that of *A. salicina*, resulting in positive soil community feedback.

Relationship of rRNA genotype and ecological function

Our work strongly supports the largely untested hypothesis that rhizobial groups delineated by rRNA gene sequences (i.e. phylotypes) represent ecologically distinct species. In both the factorial manipulation of plant and acacia isolates and an assay of acacia response to field soils, we find that measures of rhizobial composition explain the majority of the observed variation in acacia growth promotion and in the specificity of acacia response. The strong correspondence we observed between rRNA phylotype and rhizobial ecology is particularly surprising, given that genes encoding for their symbiosis with legumes are carried on relatively mobile plasmids or symbiosis islands. As such, rhizobia have been prominent examples of promiscuous exchange of ecologically relevant genes among phylogenetically distant taxa (Laguerre *et al.* 2001; Sprent 2001; Finan 2002; MacLean *et al.* 2007). Evidence of such exchanges has fuelled expectations of facile shifts in ecologically relevant genes and rapid evolutionary adaptation of microbes to their local environment. With this expectation, local mixing and adaptation may obscure any ecological signal of the diversity or composition of genes on bacterial chromosomes. While we cannot say whether horizontal mixing of functional genes alters ecological function of these rhizobia, we can conclude that the level of horizontal mixing in these populations was not so frequent as to obscure a strong signal of the rRNA gene with ecological function. We find that rRNA gene sequence reliably predicts average growth promotion and the specificity of that growth promotion by independently isolated rhizobial isolates. Moreover, the growth promotion of field soils is predicted by the local diversity and composition of rhizobial isolates in those soils as measured from the rRNA gene. This is impressive given the many soil organisms besides rhizobia, from mycorrhizal fungi to soil pathogens (Bever *et al.* 2012), that can impact plant growth response.

Our studies also provide support for functional redundancy of rhizobial species, as, nine of the top 10 axes of variation in the rhizobial community composition did not contribute to an explanation of growth response of *A. salicina* and *A. stenophylla*. We note, however, that our assay of rhizobial function was limited to two dimensions (average and differential effects on these two species), while many other aspects of rhizobial biology could be important in other contexts [e.g. effect on other legume hosts or salinity tolerance (Thrall *et al.* 2008)]. We therefore suggest that given the support we found for groupings based on phylogenetic analyses of rRNA representing functionally distinct bacterial species, there is likely to be a tremendous amount of functional diversity in microbial systems. Even within our limited sampling, we estimate the presence of nearly 400 (95% confidence limits of 247–610) different rhizobial phylotypes within part of the range of only two host species (Fig. 2f). Given the existence of more than 1900 species of native legumes in Australia alone and the breadth of environments in which they occur, we predict a staggering level of ecologically relevant species diversity in symbiotic rhizobia associated with these plant species. Extrapolating to other microbial groups, our results suggest that the extremely high phylogenetic diversity observed in soils likely translates to a correspondingly high level of variation in the broad array of ecological functions they represent (e.g. ammonia oxidisation, denitrification, free-living N_2-fixation).

Rhizobial diversity as a driver of plant–soil feedbacks

The acacia species performed relatively better with soil communities derived from conspecifics, a positive pairwise feedback dynamic that could contribute to physical separation of monomorphic patches of the two species (Bever *et al.* 1997; Molofsky & Bever 2002). While changes in density and composition of beneficial soil symbionts have been observed to generate feedback in other systems (Bever 2002; Dickie *et al.* 2005; Vogelsang & Bever 2009), this is the first

demonstration of positive plant–soil feedback mediated by changes in symbiont diversity. The direction of this effect is surprising, given that *A. stenophylla* growth declines with increasing species richness of rhizobial communities ($P = 0.0005$, Fig. S1), a result that contrasts with results of glasshouse demonstrations of increases in productivity with increasing symbiont diversity (Van der Heijden *et al.* 1998; Vogelsang *et al.* 2006).

The decline in productivity with increasing rhizobial diversity supports general expectations from evolutionary ecology. More diverse communities are expected to have greater likelihood of inclusion of less beneficial symbionts, which can proliferate and decrease the average efficiency of the microbial mutualism (Sachs & Simms 2006; Thrall *et al.* 2007a; Bever *et al.* 2009). While observations of partner choice and host sanctions in legumes (Kiers *et al.* 2003; Simms *et al.* 2006; Heath & Tiffin 2009) suggest that non-beneficial symbionts should decrease over time, weaker sanctions have been observed in less responsive legume hosts (Kiers *et al.* 2007). Consistent with this work, we observed greater rhizobial diversity in soils associated with *A. salicina*, the less selective and less responsive host (Thrall *et al.* 2008). Together, our work suggests that *A. salicina*, by permitting the proliferation of less effective mutualists, indirectly inhibits the success of the highly symbiont-dependent *A. stenophylla*. While more work is required to confirm that the prevalence of non-beneficial rhizobia is correlated with rhizobial diversity, this study potentially represents the first field evidence of ecological consequences of dynamic tensions between host sanctions and the proliferation of cheaters during the evolutionary maintenance of microbial mutualisms (Kiers & Denison 2008; Bever *et al.* 2009).

A growing body of evidence identifies microbial dynamics as important determinants of plant community dynamics and plant community structure (Bever *et al.* 2010; Inderjit & van der Putten 2010; Mangan *et al.* 2010). While plant–soil feedback tests have contributed to our understanding of the role of soil organisms in plant communities, relatively few studies have identified the microbial drivers of these feedbacks (Bever *et al.* 2012). Our work demonstrates that integration of molecular ecology characterisations with phenomenological plant–soil feedback experiments can facilitate the identification of the microbial agents and processes generating feedback on plant populations.

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AUTHORSHIP

PHT designed the individual studies, LMB collected the molecular data and JDB performed the statistical analyses. JDB wrote the first draft of the manuscript, and PHT and LMB contributed substantially to revisions.

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