On the design of productive ecosystems

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Abstract

Humans critically depend on the adequate design of productive ecosystems. With every design comes trade-offs. Agriculture is a clear case in point. We currently produce more than enough food for everyone on the planet but at a severe environmental cost. The degradation of our environment in turn threatens the long-term security of crop production. This negative feedback cycle suggests there is something wrong with the way our current human engineered ecosystems are designed. One striking feature of current industrial designs is that they are incredibly simple: monocultures, founded on reductionist ideas of how ecosystems operate. This thesis explores the challenges and opportunities we face with adding complexity back into the design of ecosystems. The world's first database on interspecific plant-soil feedback experiments is created and used to test theory on temporally lagged plant competition and facilitation. New concepts on the selection of competitive strength across trophic levels and its contrasting effects on population versus community level productivity are elucidated. The need to consider local management of diversified ecosystems in the context of global drivers of ecosystem change is validated.

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You never change things by fighting the existing reality. To change something, build a new model that makes the existing model obsolete.

~R. Buckminster Fuller

1. Introduction

1.1 The design problem

Humans critically depend on the design of productive ecosystems. With every design comes trade-offs. Agriculture is a clear case in point. Currently the world comfortably produces enough food to feed all its inhabitants (Food and Agriculture Organization of the United Nations, 2011; Ng *et al.*, 2014). At the same time agricultural systems cover ~38% of the worlds land surface area (Foley *et al.*, 2011), contribute ~12% to yearly anthropogenic greenhouse gas emissions (Smith *et al.*, 2014), annually consume ~92% of the human water footprint (Hoekstra & Mekonnen, 2012), lead to erosion of ~35 Pg yr^{-1} of soil (Quinton *et al.*, 2010), result in widespread nitrate and phosphate pollution (Vitousek *et al.*, 1997), and drive unprecedented biodiversity loss (Newbold *et al.*, 2015). These environmental losses can in turn feedback and risk yield losses (Pimentel *et al.*, 1995; Gurr *et al.*, 2004; Ponting, 2007; The Royal Society, 2009; Potts *et al.*, 2010; Tai *et al.*, 2014). This negative feedback suggests there is something wrong with the design of human engineered productive ecosystems. Maximizing resource use efficiency within existing monocultures can improve productivity and help to reduce environmentally costly inputs (Mueller *et al.*, 2012; West *et al.*, 2014). However, evidence that biodiversity can be harnessed to improve yields and reduce the magnitude of multiple negative feedbacks concurrently suggests something more radical might need to change in our core design philosophy (Gurr *et al.*, 2004; Drinkwater & Snapp, 2007; Scherr & McNeely, 2007; Gliessman & Rosemeyer, 2010; Vandermeer, 2010).

1.2 Spatial diversification

Agricultural intercropping and industrial synthetic microbial co-cultures are both good examples of how humans can in principle design spatially diverse communities that outperform monocultures (Vandermeer, 1989; De Roy *et al.*, 2013; Iverson *et al.*, 2014; Li *et al.*, 2014; Brooker *et al.*, 2015). Both the identity and the composition of species at a given point in space jointly influence the functional output of a community (Loreau *et al.*, 2001; Hooper *et al.*, 2005; Hector *et al.*, 2011). These joint effects mean that in theory the localized effects of species diversity can be either positive or negative: stemming from positive or negative covariance between competitive dominance and functional performance, or from positive or negative interactions between species that increase with diversity (Tilman *et al.*, 1997; Huston, 1997; Loreau & Hector, 2001). Biodiversity and ecosystem functioning (BEF) experiments conducted over the last 20 years give support for the idea that spatial diversification within a given trophic level can increase productivity by that trophic level (albeit at a decelerating rate) (Hooper *et al.*, 2005; Cardinale *et al.*, 2012). However, experimentally quantifying the effects of spatial diversification on the flow of energy through more complex food webs is still in its infancy, and results of diversification on ecosystem functioning across trophic levels are mixed (Cardinale *et al.*, 2009; Griffin *et al.*, 2013; Bradford *et al.*, 2014; Bardgett & van der Putten, 2014; Lefcheck *et al.*, 2015). Indeed, the importance of both positive and negative BEF effects, is becoming increasingly recognized as researchers consider the idea of multi-functionality in a system (Bradford *et al.*, 2014; Wagg *et al.*, 2014; Lefcheck *et al.*, 2015).

1.3 Temporal diversification

Agricultural rotation planning is a good example of how humans can use temporal diversification to overcome the observed yield declines seen with successive monocultures (Lawes, 1895; Dick, 1992; Raaijmakers *et al.*, 2009; Bennett *et al.*, 2012). Concurrent with the BEF paradigm there emerged a rapid growth in the study of the effects of temporal diversification on productivity. This work was largely fuelled by investigations into the temporal lags in plant competition and facilitation mediated through the soil medium. We now have strong experimental evidence that plants can culture the soil to the benefit or detriment of other plants, either through nutrient depletion, releasing toxic metabolites, or through recruitment of beneficial or pathogenic symbionts in the root zone (Inderjit *et al.*, 2011; Bever *et al.*, 2012; Van der Putten *et al.*, 2013). These 'plant-soil feedbacks' (PSF), in turn are thought to play key roles in ecosystem succession, invasion, and species coexistence (Van Der Putten *et al.*, 1993; Klironomos, 2002; Callaway *et al.*, 2004). There is even evidence that classic plant BEF relationships can depend on PSF like mechanisms (Schnitzer *et al.*, 2011). However, as with BEF research, the majority of PSF work has been hampered by its inability to predict which combinations of plants are likely to be most facilitative or complementary (Van der Putten *et al.*, 2013). Agronomists' few rules of thumb, such as the nutritional benefits of N_2-fixers and the avoidance of species-specific pathogens through interspecific rotations may have received experimental support, but the full potential of PSF in the design of productive ecosystems has largely remained an untapped black box.

1.4 The Holy Grail of ecology

From the design perspective, there is a critical problem with many BEF and PSF type experiments in that they have generally lacked the biophysical detail we need to predict the outcome of combining new sets of species together (Flynn *et al.*, 2011; Van der Putten *et al.*, 2013; Ebeling *et al.*, 2014; Kardol *et al.*, 2015). Species richness *per se* actually tells us little about the characteristics of a given community: what types of species it contains, how they operate, affect and respond to their surroundings, or indeed as we move into the realm of ecosystem services, what 'useful' functions they perform. To possess all such information, and then to be able to predict species interactions and community level performance for a given set of desired functions is however not a small ask. It is probably the Holy Grail of ecology (Lavorel & Garnier, 2002). There are two bits of information that we need to make this possible (1) knowledge of the traits underlying a given function (i.e. N_2 fixation, pathogen control via antibiotics, growth rate for biomass accumulation) and (2) knowledge of the traits that are important for the stable coexistence of species and expression of desired functions in mixtures (i.e. allowing us to remotely control or predict realized abundances). The first of these can be gained *priori* from by simply characterizing phenotypes. The second is much more tricky and requires us to re-examine the idea of the niche (HilleRisLambers *et al.*, 2011; Adler *et al.*, 2013; Turnbull *et al.*, 2013).

1.5 The niche

The niche is an elusive concept, bound to the idea of species coexistence. Original work on competitive exclusion posited, that for two or more species to coexist they have to differ in their resources use (Gause, 1934; Hutchinson, 1957; Hardin, 1960). The niche being defined by resource specialization. However, natural enemies, and spatial or temporal heterogeneity can also cause, or amplify, species specific density dependent growth rates in addition to resource specialization (Janzen, 1970; Pianka, 1974; Tilman, 1982; Chesson, 1985). Although stochastic assembly can be important for species distributions (Hubbell, 2001), species differences do exist (McGill *et al.*, 2006), and the design process is, in its simplist form, deterministic. Contemporary niche theory recognizes all of the different aspects of species differences, with the niche being defined as all the points in biotic and abiotic space that an organism *effects on* and *responds to* (Chesson, 2000a; Chase, 2005). Going from this idea of the niche to quantifying coexistence in a mechanistic way is not so easy. You need to identify which traits underlie a species' self limiting properties and prove that these interspecific trait differences are sufficiently large enough to overcome a whole school of species fitness differences that would otherwise cause dominance (Chesson, 2000a,b; Adler *et al.*, 2013).

1.6 Phylogenetic proxies

The use of evolutionary distance separating interacting species as a proxy for niche partitioning is increasingly used in ecology (Mouquet *et al.*, 2012; Narwani *et al.*, 2015). This use is based on assumptions that (1) closer relatives share similar phenotypic expressions (or 'functional traits') (Cavender-Bares *et al.*,

2004) and (2) that species with similar functional traits will compete more strongly over niche space (Macarther & Levins, 1967). Darwin appreciated that more closely related species were more likely to share natural enemies, and some evidence for this in natural systems (Webb *et al.*, 2006; Gilbert & Webb, 2007) has led to researchers to claim that phylogenetic distance can act as a proxy for temporally lagged interspecific interactions (Brandt *et al.*, 2009; Burns & Strauss, 2011; Liu *et al.*, 2012; Sedio & Ostling, 2013). However, conservatism in traits within a monophyletic clade is not equal to a monotonic relationship between phylogenetic distance and functional distance (Kelly *et al.*, 2014). Mismatches between either (1) phylogenetic distance and functional distances or (2) functional dissimilarity and the relative strength of interspecific to intraspecific competition would cause this relationship to break down.

The idea of using phylogenetic distance as a proxy for niche differences has not been restricted to PSF work and is also becoming prevalent in the BEF literature. In terms of primary production by plants and algae the results are mixed (Cadotte *et al.*, 2012; Narwani *et al.*, 2013; Venail *et al.*, 2015). In bacteria the idea of using phylogenetic distance as a proxy for niche partitioning has also been suggested to hold (Martiny *et al.*; Jousset *et al.*, 2011a,b). However, with bacteria the assumption that phylogenetic distance can be used as a proxy of functional trait distances between populations is potentially even more limited than it is with plants, due to their large accessory genomes, horizontal gene transfer, and rapid rates of evolution (Gravel *et al.*, 2012; Mouquet *et al.*, 2012).

1.7 Functional dissimilarity

The ecological literature is replete with multivariate approaches to defining the niche differences between organisms based on their functional dissimilarity, whether in metabolic profiles, physiological adaptations or life history strategies. The motivation for this approach is that multi-trait composites will be able

capture at least some of the major dominant resource axes used by organisms in niche partitioning (Campbell & Grime, 1992; Westoby, 1998; Kraft *et al.*, 2014; Reich, 2014). However, from the point of view of contemporary niche theory, these trait differences would have to reflect stabilizing differences that dominate over any effects they might play in enhancing fitness differences between organisms. Functional trait dissimilarity within a given trophic level cannot be assumed to *de facto* be a useful predictor of niche partitioning, and allelopathy, unshared mutualisms, and exploitative nutrient uptake are clear examples of this (Bever *et al.*, 2010; Inderjit *et al.*, 2011; Kraft *et al.*, 2014). Targeted combinations of different traits, which maximize facilitation and complementary (i.e. resource use, provisioning of common goods, pathogen dilution) is an important strategy in the design of productive ecosystems (De Roy *et al.*, 2013; Li *et al.*, 2014; Brooker *et al.*, 2015). The question remains as to whether using multivariate trait composites and broad-brush metrics of functional dissimilarity actually add anything to this process.

One place that trait composites might be useful for predicting species interactions is for characterizing directional effects. This is synonymous with the idea of identifying common fitness differences. Mechanistic links between directional and dominant strategy resource economic strategy effects on ecosystem properties have been shown in plants (Cornwell *et al.*, 2008; De Deyn *et al.*, 2008; de Vries *et al.*, 2012, 2013; Ke *et al.*, 2014). If any generality in species responses to these ecosystem properties exist and are dominant then it may be possible to make some sensible predictions. For example, we may expect a general positive plant growth response to a directional fertilization effect, resulting from high quality plant litter inputs or shifts in belowground food web structure. Whether or not these processes can really dominate interspecific interactions is however largely unclear.

1.8 The final frontier

This introduction has explored the generalities in designing productive ecosystems, but has admittedly been biased towards discussing the plant-soil interface and soil microbes. This is intentional. The belowground environment is intricately important to the functioning of the biosphere, yet so poorly described and understood. For example, despite some evidence for plant functional group differences in microbial affiliation, root physiological and biochemical traits, and the microbial "extended phenotype" of root systems are largely unmapped for most plant species (Bardgett & Wardle, 2010; Kattge *et al.*, 2011; Bulgarelli *et al.*, 2013; Philippot *et al.*, 2013; Bardgett & van der Putten, 2014). This is critical because plant associations with belowground biota are essential to plant health and fitness and so represent some of ,the most important putative traits to assess for plant facilitation (Van Der Heijden *et al.*, 2008; Raaijmakers *et al.*, 2009; Bever *et al.*, 2010; Bulgarelli *et al.*, 2013; Bardgett *et al.*, 2014). Moreover, exactly how microbe-microbe interactions are shaped within different plant host contexts or by background plant-plant interactions remains for the large part a mystery (Kinkel *et al.*, 2011, 2014; Bakker *et al.*, 2014). It has been proposed that root environments might shape the evolutionary trajectories of microbial competition, although the validity of these ideas remain largely untested (Kinkel *et al.*, 2011, 2014). If true, this phenomenon could not only help engineer soil suppresiveness, but fill the large unexplained variability in aboveground plant diversity to belowground productivity relationships (Spehn *et al.*, 2000; Milcu *et al.*, 2006; Chung *et al.*, 2007; Eisenhauer *et al.*, 2010). At present however, we currently don't even have any good models to test the spatial and nutritional complexity effects on competition in the rhizosphere environment. Bridging these gaps in our knowledge is both critical and exciting.

1.9 Diversification in the context of global change

In the opening section of this introduction, I argued that agricultural practice can negatively feedback and reduce agricultural productivity. These feedbacks (e.g. soil erosion, pest and disease outbreaks & climate change) can operate over local, landscape and global scales (Pimentel *et al.*, 1995; Gurr *et al.*, 2004; Ponting, 2007; The Royal Society, 2009; Potts *et al.*, 2010; Tai *et al.*, 2014). Feedbacks operating at scales beyond the farm level are problematic because they act indiscriminately, and may affect the productivity of systems that do not contribute to their effects at the local level. An important question therefore remains as to the extent that local management of diversified systems can mitigate these larger scale feedback effects. This question is particularly well addressed using agroforestry systems, which represent some the most biophysically complex mixed cropping production systems in the world today. Shade trees in agroforestry can potentially buffer understory microclimatic conditions, prevent yield instability, control pest outbreaks, maintain soil fertility and stop soil erosion (Beer *et al.*, 1998; DaMatta, 2004; Lin, 2010; Perfecto & Vandermeer, 2015). However, shade trees can also compete directly with understory crops for light, and may provide environments optimally conducive to pathogen proliferation (Cannell, 1985; Vandermeer, 1989; Mouen Bedimo *et al.*, 2010). Understanding dynamic shifts in the advantages and disadvantages of shade tree management under different global change scenarios is therefore critical to the practical management of agroforestry systems. To date, local and global scale drivers of agroecosystem productivity are often only considered in isolation, which has put a limit on our ability to make predictions on the optimal design of diversified systems in realistic contexts.

1.10 Knowledge gaps

It is evident from the above discussion that to design productive ecosystems there are a number of important knowledge gaps to be filled. This thesis will not attempt to fill them all, but will address some key ones necessary to provide an important step forward in our current understanding. As discussed above, an open question remains as to whether plant diversity can shape competition between microbial populations belowground, and filling this gap will be important for modelling the effect of aboveground diversity manipulations on belowground functions, such as productivity and pathogen suppression. In a similar vein, the effect of diversification on competition and the knock on effects of this competition on between trophic level interactions is poorly understood, so developing a better understanding on where positive or negative BEF effects are most important in practical applications is essential. As detailed above, quantitative synthesis on PSF work has to date not explicitly considered the characteristics of species interacting through the soil medium, and filling this gap is important for us to gain prediction over what has been deemed an otherwise largely idiosyncratic set of responses scattered in the literature. Finally, as highlighted in the preceding section, the impact of local management of diversified ecosystems is likely to be highly dependent on global or regional drivers of ecosystem change, and so simultaneously addressing these factors will be critical for production risk assessment in the years ahead.

1.11 Research aims and objectives

In chapter two of this thesis my objective is to understand how plant diversity controls microbial productivity. I will test the effect of plant diversity on the community productivity of a ubiquitous group of soil bacteria, and experimentally partition this into the individual effects of plant diversity on bacterial population productivity and bacterial competition. This chapter is important to the design of productive ecosystems for two reasons. The first is that currently microbial population level interactions are ignored in modelling the effects of plant diversity on belowground productivity, and a large unexplained variance persists plant diversity- soil microbial productivity relationships. The second it that we need empirical tests for recent theoretical predictions that less diverse systems might contain more antagonistic microbial populations, which has important and counterintuitive implications for engineering suppressive soils against plant disease.

In chapter three of this thesis my objective is to understand how bacterial diversity influences the control of fungal plant pathogens. I will orthogonally separate the effects of increasing bacterial genotypic richness and the phylogenetic diversity on the inhibition of fungal growth in abstract experimental microcosms. I will then sequence and analyse the diversity of bacteria in pathogen suppressive and conducive wheat root systems. This chapter is important to the design of productive ecosystems for two reasons. The first is general. To engineer diversity into ecosystems we have to understand both the positive and negative effects diversification will have on functions we are interested in optimising. The second is specific, and has some overlap with that of chapter 2. One of the main problems in current human engineered ecosystems is negative evolutionary and ecological feedbacks that exist with pesticide applications. Understanding how we

might engineer stable biocontrol approaches offers a less environmentally damaging option to farm management practice.

In chapter four of this thesis my specific objective is to understand the extent to which we can predict the effects of temporal diversification of plant species on plant productivity. I will build the world's first database on interspecific plant-soil feedback experiments. I will then build a high-resolution phylogeny for all of the plant species interacting in these experiments and ask if we can predict the outcome of plant-soil feedbacks based on how closely two species are related to each other. This chapter is important to the design of productive ecosystems because we currently have no general theory to predict which combinations of species are likely to be optimal to rotate in a given novel ecosystem design. If a simple piece of knowledge such as the evolutionary distance separating two species could be used to predict the outcome of plant species interactions this would greatly aid the design process.

In chapter five my objective is to build a new layer to the database developed in chapter four, and fill it with data on the resource economics traits of the interacting species in the plant-soil feedback experiments. I will use this new data to test for a directional effect of plant strategy differences on the sign and strength of interspecific plant-soil feedback effects. This chapter is important for the design of productive ecosystems, for same reasons as chapter 4. However, it differs from chapter 4 in that it may allow for a more mechanistic understanding of why certain plant combinations outperform others based on some simple ideas of typologies in resource uptake and nutrient cycling.

In chapter six, my specific objective is to explore the effects of shade management, climate warming and landscape fragmentation on coffee productivity in low input smallholder agroforestry systems. This chapter is important to the design of productive ecosystems because it is essential we

understand the extent to which we can optimise productivity in the diverse ecosystems already in use in the world today. This is true for two reasons. First agroforestry and intercropping systems offer opportunities to gather insights into how we may design novel ecosystems in the future- the 'lessons learned' by tropical farmers employing these techniques should not be underestimated by people interested in changing industrial agricultural practice. Second, because it allows us to test the relevance of local management of functional diversity on productivity in face of global drivers such as climate change, and in the backdrop of real world social and economic constraint posed to farmers themselves.

1.12 Thesis structure

The structure of this thesis fulfils the requirements for a DPhil submitted by papers in Biological Sciences, as recognized by the University of Oxford. These papers are required to be prepared for publication, but do not need to be published at the time of thesis submission. I have begun with an Introduction, and will finish with a General Discussion and Conclusion. The main body of my work includes 5 core research papers, of which one is published, two are in review and two are ready to be submitted.

- Mehrabi, Z., Latz, E., & Jousset, A. Plant diversity selects for belowground complementarity. *In prep*

- Mehrabi Z, McMillan, V., Clarke, I., Hammond-Kosack, K., Preston, G., Hirsch, P., & Mauchline, T. *Pseudomonas* genotypic richness is negatively associated with suppression of the wheat take-all pathogen *In review*

- Mehrabi, Z., & Tuck, S.L. 2014. Relatedness is a poor predictor of plant-soil feedbacks. *New Phytologist.* DOI: 10.1111/nph.13238 *Published*

- Mehrabi, Z., Tuck, SL., Kattge,J., Reich, P.B. Plant-soil feedbacks and the resource economics spectrum *In review*

- Mehrabi, Z., Morel.A., Long, P., Gole, T., Noris, K., Mahli, Y., Willis, K *et al.* Effects of ecosystem change on coffee productivity. *In prep*

1.13 Declaration and contributions

I certify that the majority of work presented in each chapter of this thesis is my own. My supervisors, Professor Katherine Willis and Dr Peter Long, and my examiners, Professors Owen Lewis and Richard Bardgett provided helpful comments on all chapters. Additional individual contributions by co-authors are listed below by chapter title.

∝ Plant diversity selects for belowground complementarity

- Ellen Latz set up and ran the plant BEF experiment, isolated the *Pseudomonas* library, and was involved in project planning.
- Alexandre Jousset provided lab space and facilities, wrote the R code for running the lab robot, and helped me out in the lab.
- All co-authors commented on the manuscript and provided helpful discussions.

∝ *Pseudomonas* genotypic richness is negatively associated with suppression of the wheat take-all pathogen

- Vanessa E. McMillan set up the wheat trials and collected data on yields and take-all disease.
- Tim Mauchline isolated the *Pseudomonas* library and sampled soil for 16s amplicon analysis.
- Ian Clarke ran the processing to assign taxonomic identifiers to amplicon data.
- Penny Hirsch provided lab space and helpful advice on project design.

- All co-authors commented on the manuscript and provided helpful discussions.

∝ Relatedness is a poor predictor of plant-soil feedback

- Sean Tuck contributed the R code for analysis using *metahdep*, and related descriptions of analysis and model checking given in the main text, helped refine argumentation and improved drafts during peer review.
- Two anonymous peer reviewers also gave useful comments that improved the manuscript greatly.

∝ Plant-soil feedbacks and the resource economics spectrum

- Jens Kattge and Peter Reich provided plant trait data
- Parts of the code used in the analysis (i.e. the *metahdep* case) were built on that previously developed by Sean Tuck, who also provided helpful discussions on the project idea
- All authors commented on the manuscript
- Four anonymous peer reviewers gave useful comments that improved the manuscript greatly.

∝ Effects of ecosystem change on coffee productivity

- All authors were involved in the project idea
- Peter Long helped with sampling design and gave advice on

spectroradiometric methods.

- Alex Morel coordinated surveys for mid-season disease, final yields, tree DBH measurements, and setting up of the large met stations.
- All of the co-authors provided helpful discussions during the project and/or comments.

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2. Plant diversity selects for belowground complementarity[1]

Abstract

Soil microbes play key roles in regulating plant fitness and productivity. Plants communities in turn control the way that microbes interact belowground by creating spatially structured and nutritionally specialised environments. Contemporary niche theory predicts that this will set the stage for competition and divergence, with the central expectation that complementarity between microbial populations will be highest in soil cultured by diverse plant communities. Here we experimentally test this idea by establishing a plant biodiversity and ecosystem functioning experiment on a single soil source inoculum, isolating *Pseudomonas* bacterial populations from the root systems of each plant community, and growing these populations alone and in mixtures. We show that bacterial populations at high plant diversity are more complementary than those selected at low plant diversity. To confirm that bacteria at low plant diversity are not simply globally antagonistic we mixed bacterial populations across rhizospheres of different plant species of different rooting types. We show that populations with minimal niche overlap interact less negatively and have higher community productivity than those with overlapping niches. Our results suggest that competition between soil microbes is highest in resource poor environments with few niches, and that complementarity increases with niche space at high plant diversity.

[1]Typescript from Mehrabi, Z., Latz, E., & Jousset, A. Plant diversity selects for belowground complementarity. *In prep.*

2.1 Introduction

Soil microbes are dominantly responsible for mediating the energy flow in terrestrial environments (Wardle *et al.*, 2004; Falkowski *et al.*, 2008; Van Der Heijden *et al.*, 2008; Bardgett *et al.*, 2008; Bardgett & Wardle, 2010; Bardgett & van der Putten, 2014). Plants in turn select the soil microbial community – although exactly how plants influence the spatial-temporal distribution of interactions that take place belowground is still poorly understood (Kinkel *et al.*, 2011, 2014; Bakker *et al.*, 2013, 2014; Schlatter *et al.*, 2014). Filling this gap in our knowledge is critical for predicting how microbial mediated ecosystem services are likely to change with human driven manipulation of the terrestrial biosphere.

Niche theory predicts that environments with greater resource diversity will support more niche diverged populations (Tilman, 1982; Chesson, 2000; Amarasekare, 2003; Chase, 2005). Higher plant diversity is therefore expected to culture microbial populations with less niche overlap, decreasing the competitive interactions between soil microbes (Tilman, 1982; Chesson, 2000; Amarasekare, 2003; Chase, 2005). Our knowledge of microbiology supports this, with microbes from resource poor environments engaging in fierce chemical warfare to directly kill competitors in nutrient territory battles (Riley & Wertz, 2002; Little *et al.*, 2008; Hibbing *et al.*, 2010; Becker *et al.*, 2012; Kinkel *et al.*, 2014). However, interference competition is costly and will be lost as niche overlap declines between populations as plant diversity increases (Amarasekare, 2002). Exploitative competitive traits are inefficient also, but will be maintained if sufficient supplies of resources are available to prevent more frugal types evolving in the population (Tilman 1982; Roller and Schmidt, 2015). The result is that microbial community productivity will be maximal at the intersection between highest levels of population level productivity and lowest levels of niche overlap. Under this scenario, complementarity between microbial populations is expected

to increase with plant species richness. An increase in niche overlap in populations at low plant diversity will in turn lead to intensive competition and a drop in community functioning (Fig 1).

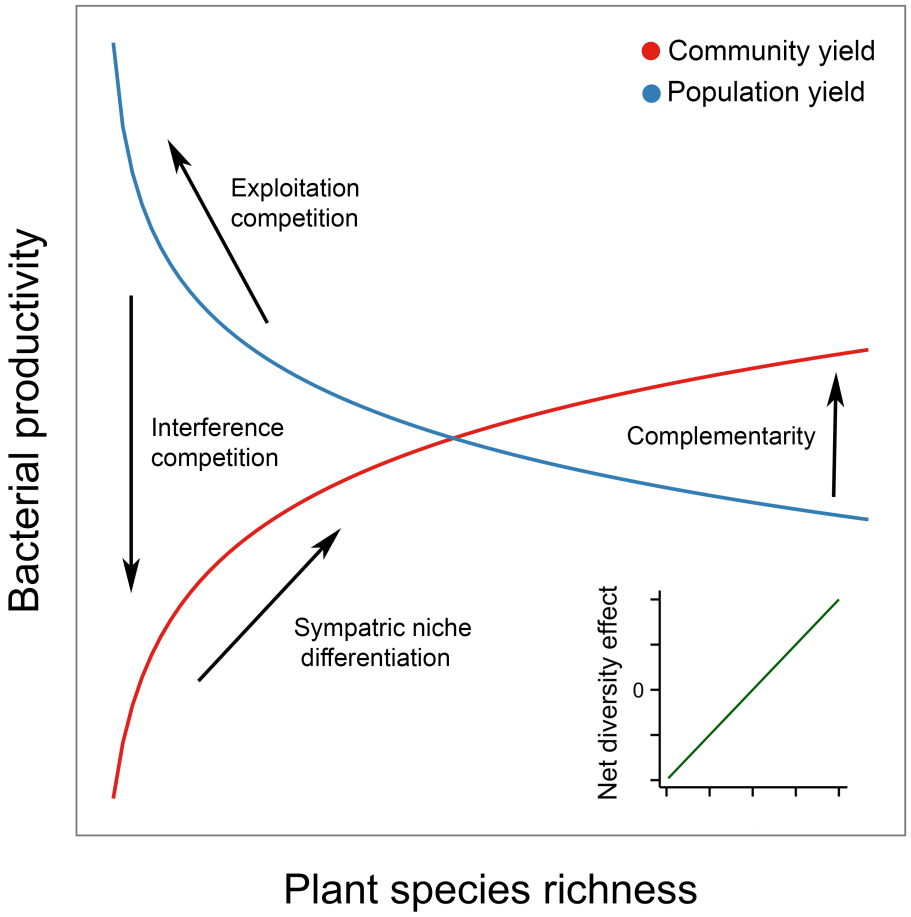


Figure 1. Plant species richness effects on bacterial competition. An increase in plant species richness is expected to culture more sympatrically niche differentiated bacterial populations. Exploitative competition will increase in species poor plant communities and populations will be selected to scramble for resources inefficiently, leading to a higher average population level productivity. However interference competition and lack of niche differentiation between populations in species poor plant communities will lead to a drop in bacterial community productivity below that expected based on potential population output. Community productivity will be maximised where potential population productivity and sympatric niche differentiation are highest. The "Net diversity effect" is the log ratio of average population level productivity and community productivity (0= no difference), and is an index of non-additive interactions, shifting from competitive antagonism at low plant species richness to complementarity at high plant species richness.

Here we tested this prediction by niche differentiating naturally occurring populations of soil bacteria. We cultured the rhizospheres of plant communities in experimental mesocosms of differing plant species richness from a single homogenous soil source inoculum. We then randomly sampled populations of rhizosphere colonising gram-negative *Pseudomonas* (γ-proteobacteria) from each microcosm, and tested their potential population versus realised community productivity (e.g. "Net diversity effects"; Loreau and Hector, 2001). To verify if bacterial populations in low plant species richness treatments were actually niche differentiated, and were not just globally antagonistic, we then mixed populations from the rhizospheres of each plant species monocultures and tested if the difference between population and community productivity decreased with the ecological distance separating the source mesocosms. We chose to use bacteria in our experiments, to directly build on earlier empirical work on which the majority of our theoretical expectations were developed (e.g. Hibbing *et al.*, 2010; Jousset *et al.*, 2011; Foster and Bell, 2012; Kinkel *et al.*, 2012, 2014). We chose *Pseudomonas* in particular as they are often abundant in plant rhizospheres, are very adaptive, geographically widespread, important in a wide range of plant-soil interactions, and easily experimented on (Jessup *et al.*, 2004; Silby *et al.*, 2011; Bulgarelli *et al.*, 2013; Berg *et al.*, 2014; Nikel *et al.*, 2014).

2.2. Materials and Methods

2.2.1 Plant mesocosms

To set up our plant diversity gradient we selected eight plant species out of the species pool of the Jena Experiment (50°55′ N, 11°35′ E) (http://www.the-jena-experiment.de; Roscher *et al.*, 2004), which are representatives of Central European mesophilic grassland *Arrhenatherion* communities traditionally used as hay meadow (grasses: *Bromus erectus*, *Dactylis glomerata*, *Festuca pratense*, *Lolium perenne*; legumes: *Lotus corniculatus, Medicago lupulina, Trifolium pratense, Trifolium repens;* obtained by Appels Wilde Samen GmbH, Darmstadt, Germany). We assembled mesocosms with diversity levels, s, of 1, 2, 4, and 8 species. For each diversity level we created 8/s different mesocosms by randomly selecting species from the species pool without replacement (e.g. if s=2, we randomly assigned the 8 species into 4 mesocosms), for a total of 15 assemblages (Bell *et al.*, 2009). We carried out this process independently four times for 2 and 4 species mesocosms, and twice for monocultures and 8 species richness mesocosms. We also constructed two control mesocosms of 0 plant species richness (total of 44 different mesocosms). A design table for the assignment of species identity and richness treatments to mecocosms is given in Table S1.

We grew our plant communities with bulk soil inoculum collected from bare ground plots at the site of the Jena Experiment (characterised as Eutric Fluvisol; Roscher *et al.*, 2004). Prior to mesocosm establishment soil was thoroughly homogenized and sieved (2 mm) to remove macrofauna, roots and stones and to develop a single starting source microbial inoculum. To maintain a constant humidity mesocosms (10 x 18 cm PVC tubes) were filled with 680g soil mixed with 170g expanded clay (Fibo ExClay Deutschland GmbH, Lamstedt, Germany). To simulate the density in natural grasslands (600 plants m^{-2};

Marquard *et al.*, 2009), we sowed 3 seeds in each of 16 equally spaced seed holes and thinned after germination to reach a total density of 16 plants per mesocosm. Plant communities were grown in a climatic chamber (18 – 22°C; photoperiod 12 h; 150 µmol m^{-2} s^{-1} photon flux density) and were watered, and spatial positions in the chamber randomised, twice a week. To simulate the dynamics of bacterial communities over many plant generations, we used an accelerated cycle design, in which plants were harvested and the microcosms planted again with the same plant communities in 3 week cycles, 5 times (Mazzola *et al.*, 2004; Bergsma-Vlami *et al.*, 2005). Although we did not expect our plant richness gradient to lead to increases in plant productivity *per se* due to the short duration of experimental cycles, we harvested and measured the oven dry weight shoot biomass at the end of each 3 week cycle to check this assumption (Fig S1; the cumulative plant productivity over the 15 weeks did not differ between plant richness levels, R^2= 0.00; P =0.89).

2.2.2. Pseudomonas sampling

At 15 weeks the whole root systems from each mesocosm were harvested. Cultivable bacteria were recovered by horizontally shaking the root systems in 20 ml cold 1/10 phosphate-buffered saline for 0.5 h (Sambrook & Russell, 2001). 0 species richness microcosms followed a similar procedure, except with equivalent mass soil sampled taken in place of root systems (1 gram per mesocosm). Suspensions were 10^6 fold diluted and plated on *Pseudomonas* selective 1/3 King's B agar containing 100 µg ml^{-1} cycloheximide, 13 µg ml^{-1} chloramphenicol and 40 µg ml^{-1} ampicillin (Simon & Ridge, 1974; McSpadden Gardener *et al.*, 2001). After one week of growth at 20°C 6 single separate colonies of the isolates were randomly chosen and frozen at -80°C in 0.85% saline containing 0.25%

glycerine. As in previous works, we used the single isolated colony as the unit for growing our experimental populations, allowing for the randomly sampled isolates from each mesocosm to theoretically all be the same 'species' or 'genotype' (Kinkel *et al.*, 2014). This approach is indispensable for our experiment, as it permitted the biomass rank abundances of recruited populations to differ across treatments, and did not penalize potentially diversified or adapted genotypes with similar core genomes.

2.2.3. Bacterial microcosms

To determine the population and community level productivities for each plant species richness level we assembled microcosms with isolate populations both in monocultures and in substitutive (equal biomass) mixtures including all populations sampled from within each plant mesocosm, in triplicate ([263 isolates + 44 plant mesocosms] * 3 replicates= 921 microcosms) (the original number of isolates sampled was 264, but 1 isolate failed to revive from -80°C). To determine the effect of mixing populations across ecologically identical but geographically isolated root systems we randomly sampled 3 isolates from each of the 16 isolate pools representing 1 plant species richness mesocosms (Table S1: AA-AH & BA-BH), then mixed populations in substitutive manner for each of the 8 species, repeating both the random sampling process three times, in triplicate (3 paired draws*8 species *3 replicates= 72 microcosms). To determine the effect of mixing populations across ecologically distinct root system boundaries we undertook 7 random draws of 3 isolates from 1 plant species richness mesocosms (Table S1: AA-AH), and mixed populations across different plant species identities within that group of mesocosms, in triplicate (7 random draws* 8 plant species* 3 replicates= 168 microcosms). To implement the experiments, all isolates were

grown up overnight at 25°C in King's medium B, with agitation. Late exponential phase bacterial cells were then pelleted by centrifugation, washed twice in 0.85% saline, incubated for 6 h at room temperature to allow terminating division cycles and adjusted to an OD_{600} of 0.1, with 0.85% saline. All 1161 microcosms were set up with 10µL of OD_{600} 0.1 adjusted cell suspensions overlaid with with 90µL of King's medium B in 200 uL capacity 96 well microtiter plates, then incubated at 25°C, with agitation. Mixtures comprised substitutive equal proportions of each 6 populations. Blank controlled OD_{600} at 6,12, 24 & 36 hours was used determine changes in productivity, with 36 hours (during stationary phase) taken as a measure of maximal microcosm productivity. OD scales well to dry weight biomass and stands as a robust integrated metric of productivity (i.e. the product of cell sizes and counts) (Myers *et al.*, 2013; Fiegna *et al.*, 2015).

2.2.4. Statistical analyses

2.2.4.1. Mixtures of sympatric populations

We calculated the "Net diversity effect" (Loreau & Hector, 2001) to explore how negative bacterial interactions changed at each level of plant species richness. This index is simply the log ratio of the observed mesocosm community productivity over the mean of the expected population performance of each population making up that community. It is based on the assumption that if no interactions between populations occur on mixture, then the community level productivity will simply be the sum of the expected production by each of its constituent populations. This interaction index is intuitively bound between -1 and 1; with -1 equal to non-additive negative interactions, +1 equal to non-

additive positive interactions, and 0 equal to complete additivity (i.e. no interactions occurring) (Loreau and Hector, 2001; Foster and Bell, 2012; Fiegna *et al.*, 2015). We built three sets of linear models to test for an effect of plant species richness on bacterial population yields, observed community yields and on the yield gap index (all at OD_{600} at 36 hours). The form of the independent species richness term was graphically determined prior to formal fitting, with linear, quadratic and logged species richness effects fitted for expected, observed and the net diversity effect index, respectively. We fitted linear mixed models for each response, with plant species richness as a fixed effect, and partitions and experimental replicates as random effects in *lme4* (Bates *et al.*, 2014). We then compared these fits to null models with the fixed plant species richness term removed using likelihood ratio tests. All analysis in this manuscript was performed in R 3.1.3 (R Core Team, 2015).

2.2.4.2. Mixtures of allopatric populations

We tested if bacterial populations at low species richness were not simply globally antagonistic and poor at performing in a community. To do this we quantified the 'niche overlap' between bacterial populations based on root trait differences between their parent plant species mesocosms. Species level trait values for *root depth*, *specific root length*, *root tissue density*, *root diameter* and *root length density* were retrieved from the literature, except for *F. pratense* which we replaced with congeneric values of *F. rubra* (Roscher *et al.*, 2004; Fort *et al.*, 2013, 2015). The functional distance separating plant species was computed using a Gower dissimilarity matrix (Gower, 1971; Laliberté *et al.*, 2014). We performed mantel tests, with 10,000 permutations to test for a negative relationship between the historical niche overlap and observed community level functioning and net diversity effect (Mantel, 1967; Goslee & Urban, 2007). All bacterial responses

were calculated as the mean responses for each pair of historical niches (n= 28). To check that these results were not simply occurring from niche differentiation across pot boundaries we tested the differences in mean community productivity and the net diversity effect to change with population mixture across versus within mesocosms of the same plant species identity with paired t-tests (n=8).

2.3 Results

Population yields declined with plant species richness (R^2= 0.16; χ^2_1= 6.3, P =0.012; Fig 2A). Bacterial community yields showed a hump relationship to plant species richness- increasing from the zero to 4 species richness treatments and then declining again at 8 species communities (R^2= 0.10; χ^2_1= 12.52, P =0.002, Fig 2B). Despite the decline in observed community productivity at higher plant species richness, the Net diversity effect became more positive with plant species richness treatments (albeit at a decelerating rate) (R^2= 0.09; χ^2_1= 8.09, P =0.004, Fig 2C). These results show that the maximal bacterial community level productivity at 4-plant species richness occurred at the intersection between maximal population level productivity and minimal inter-population negative interactions (Figs 1-2).

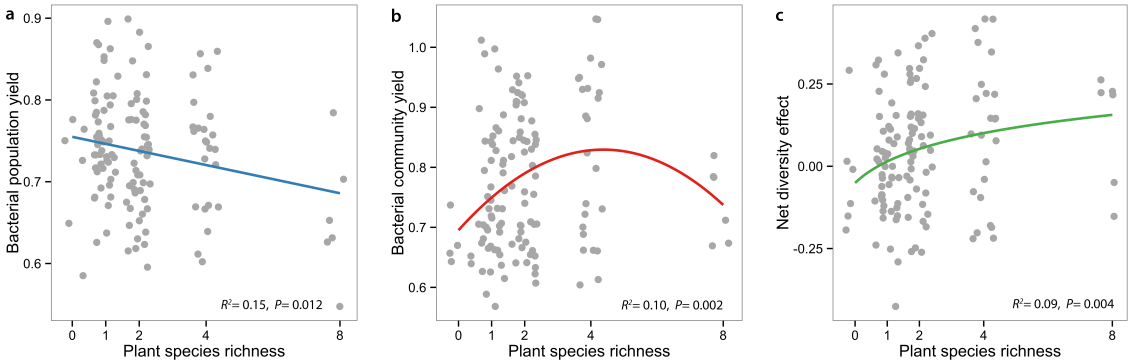


Figure 2. Competition between bacterial populations increases and bacterial community productivity decreases at low plant species richness. a) bacterial population yields decrease with plant species richness b) bacterial community yields show a hump shaped relationship with plant species richness c) negative interactions between populations decrease with plant species richness. Yield values presented are in OD_{600} units. n=132. Jitter is included in the plots for visualisation purposes.

Psuedomonas populations were more complementary when sampled between plant mesocosms that had more functionally distant rooting systems (*Mantel's r*= 0.43, P=0.02) (Fig 3). To test if the simple act of combining populations across spatially isolated mesocosms could also influence interaction strength and community productivity (Kinkel *et al.*, 2014), we also mixed populations across spatially separated plant communities of the same species identity. We failed to find significant increases in the bacterial community productivity when populations were mixed from allopatric versus sympatric pools (Paired t-test: t_8=1.07, P=0.31 Fig S2A). The effect of allopatric versus sympatric population mixture on the interaction index was also idiosyncratic - showing that bacterial populations from the same plant species were not more 'niche differentiated' than sympatric ones (Paired t-test: t_8=0.7, P=0.5; Fig S2B).

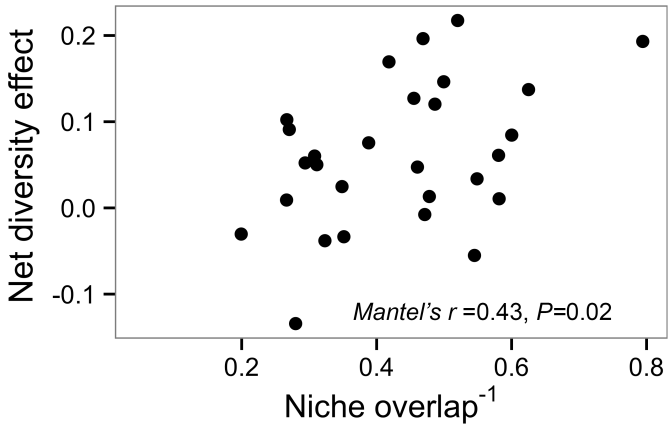


Figure 3. Populations are more complementary when niche differentiated in disparate root systems. Niche overlap is calculated as the Gower dissimilarity between plant species based on root traits (root depth, specific root length, root tissue density, root diameter, root length density). We undertook 7 random draws of 3 isolates from 1 plant species richness mesocosms (*Materials and Methods*), and mixed populations between each pair of plant species, in duplicate (7 random draws* 8 plant species* 3 replicates= 168 microcosms). All bacterial community responses were then calculated as the mean responses for each pair of historical niches (n= 28). Mantel tests were performed with 10,000 permutations. Yield values used to calculate the Net diversity effect are in OD_{600} units.

2.4. Discussion

Soil microbes form complex belowground communities and the interactions within these communities are critical for plant fitness. We show that higher plant diversity can shift belowground microbial interactions from antagonistic to complementary, with negative bacterial interactions decreasing with plant diversity. We found that population productivity declined with plant diversity, in line with the expectation that exploitative traits will decline with competitive interaction strength. Bacterial community productivity showed a hump-backed relationship, peaking at the intersection between maximal population level productivity and minimal negative interactions between populations. We mixed populations across different rooting environments and found that bacterial populations from plant monocultures interacted more complementary with populations that were sampled from distinct belowground rooting environments. Together our results suggest that species rich plant communities culture belowground microbial complementarity, and that this mechanism is likely to be important for mediating belowground processes linked to soil productivity.

We are not aware of any other direct tests of the effect of plant diversity on bacterial competition and community productivity. However, the presence of interference competition has been suggested to increase in plant monocultures as the result of a decrease in resource heterogeneity– with empirical evidence largely coming from observations toxic interactions between pairs of *Streptomycetes* bacteria populations (Kinkel *et al.*, 2011, 2012; Bakker *et al.*, 2013). One place where our results differ from this work is that it has been suggested that *Streptomycetes* may embark on different co-evolutionary trajectories in the same but geographically isolated host systems (Kinkel *et al.*, 2014). In our experiments we found little differences in interactions between sympatric and allopatric populations from the same or similar host environments. However, we think this

discrepancy can be explained by our experimental set up. We established our plant mesocosms in climate-controlled chambers, with homogenous soil, and from a single microbial source inoculum. This allowed us to emphasize plant species richness effects from a variety of potentially other factors that are likely to vary across space and drive separate co-evolutionary trajectories in field environments: such as soil type, assemblage of starting population pools, and abiotic stress (Bakker *et al.*, 2014). We note however, that properly identifying the role of plant diversity on co-evolution would require a response to selection approach using reciprocal transplant experiments, alongside determination of the population genetic structure of source microbial populations at the beginning and the end of the experimental periods (Spratt & Maiden, 1999; Kassen, 2002; Brockhurst & Koskella, 2013).

The commonly reported mechanism for driving belowground microbial productivity at higher plant diversity is increased plant productivity and nutrient quantity (Zak *et al.*, 2003; Chung *et al.*, 2009; Eisenhauer *et al.*, 2010; Lange *et al.*, 2015). Whilst we cannot completely rule out changes in nutrient quantity available to soil microbes from our system, we expect that these effects were likely to be of minimal importance due to the short 3-week successional growth cycle phases of our experiment. The cumulative aboveground plant productivity also did not differ between plant richness treatments in our experiment, suggesting that the classic plant diversity- plant productivity relationship did not have time to establish in our system (Eisenhauer *et al.*, 2010; Cardinale *et al.*, 2011). It therefore seems reasonable to propose that the increase in negative bacterial interactions were driven largely by a reduction in resource heterogeneity as plant species richness declined. As our experimental microcosms collapsed down the spatial resource axes from the plant mesocosms in which populations were cultured, these effects had to result from nutrient based niche differentiation. Moreover, because spatial heterogeneity of root architecture increases with plant diversity, we predict that including a spatial aspect into

model microcosms in future will only act to amplify the differences we reported here. A similar expectation would hold for a closer mimicking of the rhizosphere nutritional complexity (Langenheder *et al.*, 2010; Eisenhauer *et al.*, 2013). Nevertheless, work is still greatly needed, to determine both the realistic spatial distribution of different substitutable carbon sources and nutrients within developing rhizospheres, and the consequences of this heterogeneity on the strength of microbial competition *in situ* (i.e. Ettema and Wardle, 2002; Young and Crawford, 2004).

Whilst our work shows that competition is likely to be an important mediator of microbial productivity at differing levels of plant species richness, we performed our experiments using a single group of rhizosphere colonising bacteria. Changes in microbial productivity with plant diversity are also accompanied by shifts in other taxa (Deyn *et al.*, 2010; de Vries *et al.*, 2012). A critical next step is therefore to determine how other microbial interactions change with plant species richness, and integrate this with the ideas presented here on bacteria. What is clear from the arguments and results we present, is that competitive interactions in the rhizosphere cannot be ignored if we want to successfully model the effects of plant diversity on soil microbial functioning. With the continued widespread shifts in plant diversity across the planet, and the crucial roles soil microbes play in the functioning of the terrestrial biosphere, this point has never seemed more important than it is today.

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Supporting Information

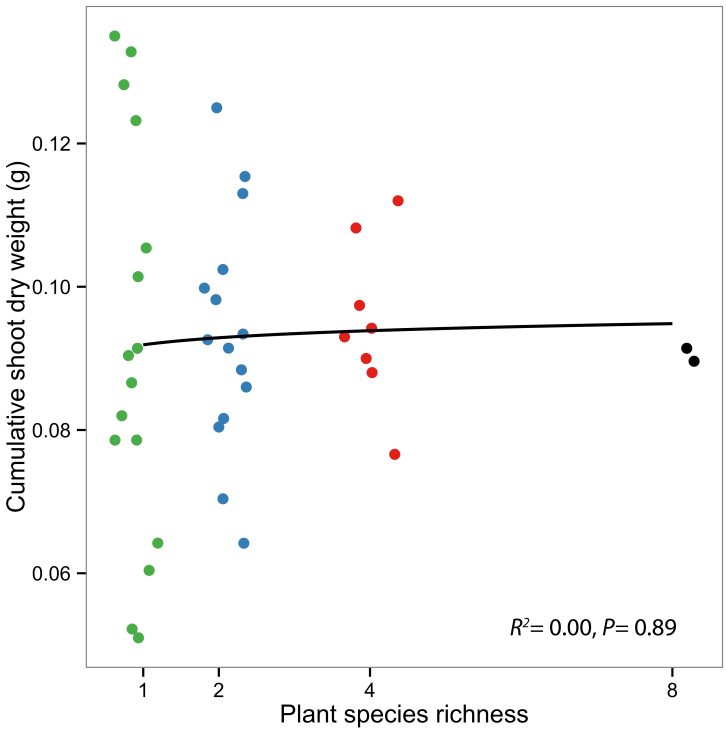


Figure S1. Plant productivity did not differ between the species richness levels in our experiment. Cumulative shoot dry weight is equal to the sum of harvested aboveground biomass over five 3-week cycles of the experiment (total of 15 weeks growth).

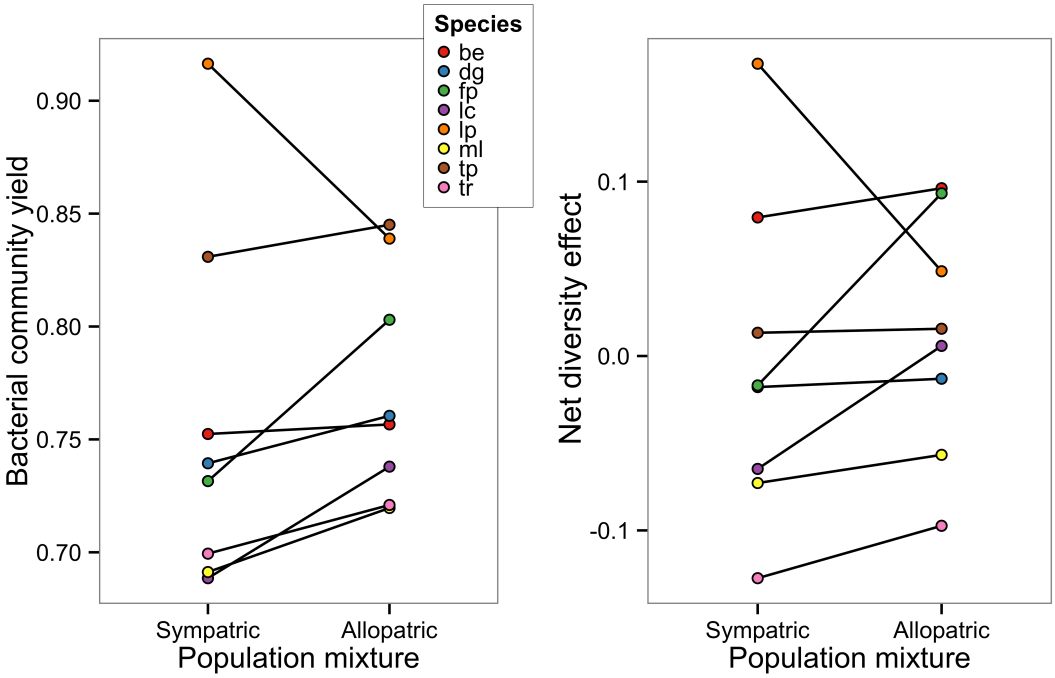


Figure S2. Bacterial populations from the same plant species are not more 'niche differentiated' than sympatric ones. a) bacterial community productivity did not increase when populations were mixed from allopatric versus sympatric pools (Paired t-test: t_8=0.7, P=0.5) b) the effect of allopatric versus sympatric population on the sign of the Net diversity effect index (which controls for the effect of sampling more productive populations on mixture) was also inconclusive (Paired t-test: t_8=0.7, P=0.5). Species codes: *be=Bromus erectus, dg= Dactylis s glomerata, fp= Festuca pratense, lp= Lolium perenne, lc= Lotus corniculatus, ml =Medicago lupulina, tp= Trifolium pratense, tr= Trifolum repens.* Yield values presented are in OD_{600} units.

Table S1. Allocation of plant species to the mesocosms in the plant BEF experiment. Species .codes: *be=Bromus erectus, dg= Dactylis glomerata, fp= Festuca pratense, lp= Lolium perenne, lc= Lotus corniculatus, ml =Medicago lupulina, tp= Trifolium pratense, tr= Trifolum repens.*

		Species identity							
Mesocosm	Species richness	dg	fp	lp	be	tp	lc	tr	ml
AA	1	1	0	0	0	0	0	0	0
AB	1	0	1	0	0	0	0	0	0
AC	1	0	0	1	0	0	0	0	0
AD	1	0	0	0	1	0	0	0	0
AE	1	0	0	0	0	1	0	0	0
AF	1	0	0	0	0	0	1	0	0
AG	1	0	0	0	0	0	0	1	0
AH	1	0	0	0	0	0	0	0	1
AI	2	0	1	0	0	0	1	0	0
AJ	2	0	0	0	0	1	0	1	0
AK	2	1	0	0	1	0	0	0	0
AL	2	0	0	1	0	0	0	0	1
AM	4	1	0	1	1	0	0	1	0
AN	4	0	1	0	0	1	1	0	1
AO	8	1	1	1	1	1	1	1	1
AP	2	0	0	1	1	0	0	0	0
AQ	2	0	0	0	0	1	1	0	0
AR	2	0	0	0	0	0	0	1	1
AS	2	1	1	0	0	0	0	0	0
AT	4	0	1	0	1	1	0	1	0
AU	4	1	0	1	0	0	1	0	1
AV	0	0	0	0	0	0	0	0	0
BA	1	1	0	0	0	0	0	0	0
BB	1	0	1	0	0	0	0	0	0
BC	1	0	0	1	0	0	0	0	0
BD	1	0	0	0	1	0	0	0	0
BE	1	0	0	0	0	1	0	0	0
BF	1	0	0	0	0	0	1	0	0
BG	1	0	0	0	0	0	0	1	0
BH	1	0	0	0	0	0	0	0	1
BI	2	0	0	0	1	1	0	0	0
BJ	2	0	1	0	0	0	0	0	1
BK	2	0	0	1	0	0	1	0	0
BL	2	1	0	0	0	0	0	1	0
BM	4	0	1	0	0	1	0	1	1
BN	4	1	0	1	1	0	1	0	0
BO	8	1	1	1	1	1	1	1	1
BP	2	0	0	0	1	0	0	0	1
BQ	2	0	0	1	0	0	0	1	0
BR	2	1	0	0	0	0	1	0	0
BS	2	0	1	0	0	1	0	0	0
BT	4	0	0	0	0	1	1	1	1
BU	4	1	1	1	1	0	0	0	0
BV	0	0	0	0	0	0	0	0	0

3. *Pseudomonas* genotypic richness is negatively associated with suppression of the wheat take-all pathogen[2]

Abstract

Biodiversity and ecosystem functioning research typically suggests that increasing the species richness of plants, bacteria or fungi will increase productivity. However, local increases in species richness can increase competition within trophic levels, reducing the efficacy of population control of pathogens between trophic levels. Here we experimentally test this idea by assembling *Pseudomonas* spp. bacterial isolates in synthetic communities of differing genotypic richness, relatedness and carbon substrate use overlap. We found that *Pseudomonas* communities at high genotypic richness are poor inhibitors of the take-all fungal pathogen. We also show that bacterial antagonism increases with *Pseudomonas* genotypic richness, and is a good negative predictor of take-all pathogen control. Diversity patterns in field trials closely match the predictions from our simple synthetic models, and take-all disease suppressive soils are characterised by lower *Pseudomonas* genotypic richness on root surfaces of wheat plants. Disease assays on isogenetic wheat lines show that wheat variety specific soil culturing is responsible for differences in soil *Pseudomonas* richness in the field. We

[2]Typescript from Mehrabi Z, McMillan, V., Clarke, I., Hammond-Kosack, K., Preston, G., Hirsch, P., & Mauchline, T. *Pseudomonas* genotypic richness is negatively associated with suppression of the wheat take-all pathogen *In review*

hypothesize that plant driven controls on belowground bacterial diversity could be a key explanatory factor in the control of soil borne pathogens by forcing the degree of competition between potential biocontrol strains and the rest of the bacterial community.

3.1 Introduction

Biodiversity has both positive and negative effects on ecosystem functioning (Hooper *et al.*, 2005; Cardinale *et al.*, 2012). These different effects are known to occur locally from competitive dominance of species that have a high or low functional output and from complimentary or antagonistic interactions that increase with species richness (Huston, 1997; Flynn *et al.*, 2011; Gravel *et al.*, 2012). Positive effects of biodiversity on productivity and resource capture within trophic levels are most commonly reported in the literature, but for practical management of ecosystems it is important we understand the range of conditions under which diversity causes negative effects on functioning. This is particularly relevant in conventional agriculture, where locally antagonistic interactions, such as between weeds and crop plants, have underpinned the rationale for the creation of species-poor production systems. However, lack of research, alongside potential mismatches between ecosystem functions and ecosystem services highlight the large gap in the practical application of current biodiversity and ecosystem functioning research to agriculture (Cardinale *et al.*, 2012; Naeem *et al.*, 2012).

Crop diseases pose some of the greatest challenges to food security of the 21st Century (Oerke, 2006; Crowder *et al.*, 2010; Fisher *et al.*, 2012; Tscharntke *et al.*, 2012). Root-associated soil bacteria offer potential solutions to this problem by

combating pathogens with anti-microbial compounds and improving plant growth (Raaijmakers *et al.*, 2009; Kwak & Weller, 2013). However, a limiting factor to this solution is that soil bacteria are in a constant state of warfare with other bacteria (Riley & Wertz, 2002; Little *et al.*, 2008; Hibbing *et al.*, 2010). Bacteria which could potentially control fungal pathogens can be defeated by other bacteria, representing a long standing challenge to the successful application of biocontrol systems (Xu *et al.*, 2011; Becker *et al.*, 2012). In agricultural crops where fungal pathogens are problematic, reduced yield and economic losses could be increased with bacterial richness through increased bacterial antagonism amongst potential biocontrol genotypes and the rest of the bacterial community. An open question remains as to the extent to which bacterial diversity patterns in fungal pathogen conducive soils match this expectation, and to the degree that other aspects of diversity, such as evolutionary relatedness or niche overlap, might reduce this limit by mediating the intensity of competition between bacteria (Gravel *et al.*, 2011; Jousset *et al.*, 2011a,b).

Take-all disease is caused by a pervasive fungal pathogen (*Gaeumannomyces graminis* var. *tritici*) in wheat (*Tritium aestivum*) and leads to devastating yield losses (Raaijmakers *et al.*, 2009; Kwak & Weller, 2013). Populations of antifungal *Pseudomonas* spp. bacteria have been shown to naturally control take-all, but only after a period of yield losses lasting 4-6 cropping seasons (Raaijmakers *et al.*, 2009; Kwak & Weller, 2013). However, a novel phenomenon was recently discovered in which particular wheat varieties are able to limit the build up of take-all in a single season and create disease suppressive soils prior to an epidemic taking place (McMillan *et al.*, 2011). The putative role of some *Pseudomonas* spp. in take-all control, and their general dominance in the soil bacterial community positions them as a useful focal group taxon to explore this novel phenomenon of reductions in take-all build up, and to test more general ideas of how bacterial diversity in rooting systems might in principle be related to fungal pathogen suppression.

We set up simple *in vitro* experimental model microcosms to test for a directional effect of *Pseudomonas* spp. bacterial genotypic richness on the take-all pathogen suppression. We orthogonally partitioned the effect of bacterial genotypic richness with phylogenetic diversity (based on housekeeping *gyrB* genes) and functional trait diversity (based on carbon use profile utilization) in an attempt to capture the hypothetically positive effects of resource use complementarity in dampening any negative effects of mutual antagonism or competitive dominance resulting from increased genotypic richness (Jousset *et al.*, 2011 a,b). We then conducted a complementary set of competition experiments to test if effects of *Pseudomonas* richness on the inhibition of the take-all pathogen could be explicitly accounted for by antagonism between *Pseudomonas* genotypes (defined here as the compound effects of direct antagonistic toxin production and indirect exploitative resource competition).

We used field trials with wheat to test if the genotypic richness of *Pseudomonas* spp. is correlated to severity of infection by the take-all pathogen and yield loss. We pre-cultured field plots with different wheat varieties to generate treatments for high or low build up of take-all disease, and undertook our field assays in the following season on genetically identical clones of a commercial wheat variety (*T. aestivum* var. Xi19). We tested for generic changes in the background rhizosphere bacterial community structure based on relative abundance of 16S rRNA genes and then sub-sampled culturable isolates to describe the patterns of genotypic richness and phylogenetic diversity across plots at finer resolution.

3.2 Methods

3.2.1 Field trials

The field trial was undertaken on the Rothamsted Research experimental farm UK (N51° 48'27", W0° 21'44") during 2010-2012. A commercial winter wheat variety (Xi19) was sown in the autumn on soils in 2011 that in 2010, had been pre-cultured with either high (Hereward) or low (Cadenza) take-all build up wheat varieties (McMillan *et al.*, 2011). Seeding was at 350 seeds m^{-2} on each of eight 10 m x 3 m plots (4 replicates x 2 soil culture treatments). Take-all disease intensity on wheat was characterized with the take-all intensity index (TAI) (Bateman *et al.*, 2004). To determine the diversity of the dominant members of *Pseudomonas* spp. we randomly sampled 15 roots systems from each plot, pooled the roots, and extracted bacteria from the rhizosphere soil and the root endosphere (after washing roots and surface sterilizing with hypochlorite followed by ethanol). Extracts were dilution plated onto *Pseudomonas* selective agar with CFC antibiotics (Oxoid) and then incubated for 96 h at 24 °C. Fifteen single colonies (or the maximum if fewer) from both the endosphere and rhizosphere of each plot were randomly sub-sampled and DNA from each colony was extracted by cell lysis with MicroLysis+ (Microzone, UK) (Total isolates= 174, 91 from Hereward and 87 from Cadenza plots). This sub-sampling and selection procedure did not attempt to represent the full diversity of these microhabitats, but provide a feasible representation of the diversity of most dominant culturable isolates likely to exert large functional effects in their host environment. Bacterial genotype was determined by genetic fingerprinting of enterobacterial repetitive intergenic consensus sequences (ERIC) (Versalovic *et al.*, 1991). For phylogenetic analysis we PCR amplified, sequenced and aligned a 940 bp fragment of the DNA Gyrase subunit B (*gyrB*) gene for each distinct genotype present in the study (Yamamoto *et al.*, 2000) and perfomed maximum-likelihood based estimation

using RAxML (v.7.0.4), optimized under the GTR-GAMMA substitution model (Stamatakis, 2014). We define phylogenetic diversity (PD) throughout the study as the total phylogenetic branch length of the *gyrB* sequence-determined genotypes present in a given assemblage (Faith, 1992). The sequence data generated in this project have been deposited in the GenBank Nucleotide database (accession numbers *KF059880-KF059937*). The field trial was combine-harvested and fresh grain weights of Xi19 recorded per plot at grain maturity in August 2012. Grain dry matter was determined by oven-drying 80 g sub-samples of the fresh grain for 16 h at 105°C. Grain yields were then adjusted to 85% dry matter and scaled to tonnes ha^{-1}.

3.2.2 Fungal pathogen inhibition

We conducted a biodiversity ecosystem functioning experiment to determine how increasing genotypic, phylogenetic and functional diversity of *Pseudomonas* influenced inhibition of take-all fungus (*Gaeumannomyces graminis* var. *tritici*). Eight isolates representing the phylogenetic breadth of genotypes in our *Pseudomonas* spp. library were grown up in Luria Bertani (LB) broth at 25°C for 12 h, shaken at 200 rpm. Late exponential phase bacteria were then pelleted by centrifugation, washed twice in 1x phosphate buffered saline (PBS), incubated for 6 h at room temperature to allow terminating division cycles and adjusted to an OD_{600} of 1.0, with 1 x PBS. We assembled the genotypes into 49 mixtures in a substitutive design (e.g at constant density) randomly constructing 20 assemblages representing a gradient of PD within each 2 and 4 genotype richness treatments (8 x 1 genotype; 20 x 2; 20 x 4 genotypes; 1 x 8 genotypes = Total 49). To do this first the PD of all possible combinations of 2, or 4 genotypes was computed, and then 20 assemblages with unique PD values were randomly sampled from each pool. This design allowed us to orthogonally test for the role

of phylogenetic diversity vs. genotypic richness in inhibition of the fungus. Eight microliters of each community was spotted 2 cm from the edge of Petri dishes containing potato dextrose agar (PDA), and incubated at 27 °C for 48 hours. We then placed a 5 mm plug from the leading edge of a culture of take-all (grown for 7 days at 24°C) in the center of the dish, and plates were incubated for a further 7 days at 24°C before photographing at standardized heights. All plates were spotted with a negative control of PBS, which had no effect on fungal growth. Photographs were analyzed using Fiji (Schindelin *et al.*, 2012) , and inhibition measured as the total pixels free from fungal growth. Functional traits relevant to niche partitioning were tested for by measuring the differential ability of each isolate to utilise 95 different carbon substrates. Assays were completed using GN2 MicroPlates (Biolog, Inc.), with 100 µL of each OD_{600} adjusted population per well, incubation at 25°C for 72 h and measurement at OD_{590}. We define functional diversity (FD) as: $FD = (\sum_{i=1}^{S}\sum_{j<1}^{S}\sqrt{\sum_{t=1}^{T}(X_{tj} - X_{ti})})/\ S(S-1)/S$ where T is the total number of traits, X_{ti} and X_{tj} are the values of the trait "t" of genotypes "i" and "j" and S is the total number of genotypes in a given assemblage (Walker *et al.*, 1999). The experiment and functional trait assays were repeated in triplicate.

3.2.3 Bacterial antagonism

To assess how different levels of genotypic, phylogenetic or functional diversity influenced antagonistic interactions between bacteria, we conducted a second biodiversity ecosystem functioning experiment. Here we used 45 of the same assemblages used in the take-all pathogen inhibition experiment (8x 1 genotype; 19 x 2 genotype; 19 x 4 genotypes; 1 x 8 genotypes= Total 45; with a reduction to 19 for 2-4 genotypes due to practicalities of 48 wells per replicate) and cultured

(from an initial density of ~10^7 cells ml^{-1}) them in LB broth at 27°C for 18 h, shaken at 200 rpm. We centrifuged each mixture and deposited 150 µl of the cell free supernatant into separate wells of a microtitre plate. We then overlaid 50 µl of OD_{600} adjusted populations of each of the 8 different genotypes, subjecting each genotype to the compound effects of antagonistic toxin production and nutrient depletion by each community in separate wells. Biomass differences were proxied as simply OD_{600} after 6 h incubation at 25°C, shaken at 200 rpm. OD scales well to dry weight biomass and is a useful proxy for integrated productivity (i.e. the product of cell sizes and counts) (Myers *et al.*, 2013; Fiegna *et al.*, 2015). The whole experiment was repeated in triplicate.

3.2.4 Statistical analysis

To determine the differences between take-all disease intensity (logit transformed), log total grain yield and bacterial diversity (genotypic richness/phylogenetic diversity) in our field plots we used Welch's t-test due to low sample sizes. We rarefied phylogenetic diversity measures to account for species differences between plots, using the standalone phylorare() function (Nipperess & Matsen, 2013) in R 3.0 (R Core Development Team, 2013). We also used individual-based rarefaction on genotypic richness in each field plot to account for sampling effort biases that resulted from plots that did not yield 15 single separate colonies (Gotelli & Colwell, 2001) . Analysis of Similarity (Clarke, 1993) based on presence-absence data and Bray-Curtis distances were used to test for differences in bacterial indicator group composition between plots. We used linear models, to orthogonally test for the effect of genotypic richness and phylogenetic diversity on inhibition of take-all and bacterial antagonism in the 2-

4 genotypic richness assemblages (Gravel *et al.*, 2012). Genotypic richness was fitted as a categorical variable first in all the models and phylogenetic (or functional) diversity fitted after as a continuous variable, with first order interactions. As no significant interactions were present in any of the models, we removed the interaction terms and assumed fixed slope estimates for the models presented in the main text. The biodiversity metrics we used as our response were characterized using the net biodiversity effect (Loreau & Hector, 2001): $\log(\Phi_{obs}/\Phi_{exp})$, where, for the fungal inhibition experiment, Φ_{obs} is the overall inhibition of a given assemblage, and Φ_{exp} is the mean inhibition from each genotype present in the assemblage in monoculture; and where, for the bacterial inhibition experiment, Φ_{obs} the overall biomass a genotype produced on medium from a given assemblage, and Φ_{exp} the mean biomass that genotype produced on medium from each genotype present in the assemblage. All models were run and assumptions validated in R (Pena & Slate, 2006).

3.3 Results

3.3.1 Fungal pathogen inhibition

The relationship between our selected genotypes and other well-known biocontrol *Pseudomonas* spp. is given in Fig 1a. Our experimental genotypes expressed differences in *in vitro* take-all pathogen inhibitory activity and intragenotypic vs. intergenotypic bacterial antagonism (Fig 1b). Whilst no clear genotype level trade-off between take-all pathogen inhibitory ability and bacterial antagonism was apparent, the most potent inhibitor of the take-all pathogen (S84: 85% ± 6% SE inhibition of mycelium growth) expressed the classic self limiting trait where intragenotypic antagonism > intergenotypic antagonism (Fig 1b).

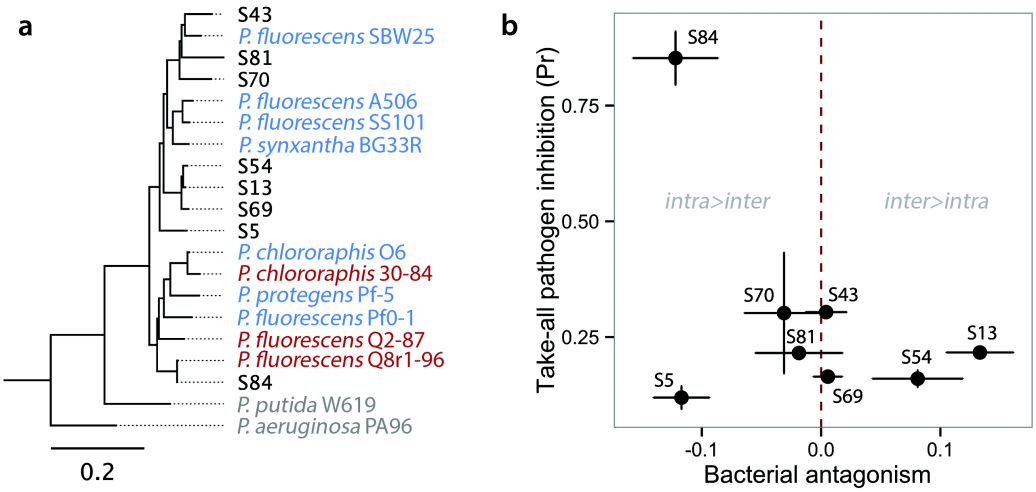


Figure 1. *Pseudomonas* genotypes used in the experiments. a) phylogenetic relationships between the genotypes used in the experiments and other well known biocontrol genotypes based on maximum likelihood tree estimated on a 940 kb fragment of the universal houskeeping gene *gyrB*, using the RAxML algorithm (with GTR-GAMMA substitution). Scale bar represents nucleotide substitutions per site. Blue strains are known biocontrol agents, red strains were previously isolated from wheat root systems and have been implicated in take-all control, gray strains are outgroups (Loper et al., 2012) (b) Take-all pathogen inhibition and bacterial antagonistic ability of the strains. Take-all pathogen inhibition is the area of fungal mycelium growth inhibited by each *Pseudomonas* genotype in Petri dish assays (1= no fungal growth, and 0=complete fungal growth). Bacterial antagonism is the log ratio of strain biomass (OD_{600}) accumulated on spent liquid medium cultured by its own genotype versus mean biomass accumulated on spent medium cultured by all other genotypes. Data show means ± s.e.m. n=3.

To model the effect of *Pseudomonas* spp. diversity on take-all pathogen growth, we created experimental microcosms and presented the take-all fungal pathogen *in vitro* to 49 different synthetic *Pseudomonas* communities of varying phylogenetic diversity, orthogonally nested within different genotypic richness levels. We characterised the response with the net biodiversity effect (Loreau & Hector, 2001): $\log(\Phi_{obs}/\ \Phi_{exp}\ )$, where Φ_{obs} is the overall inhibition by a given assemblage, and Φ_{exp} is the mean inhibition from each genotype present in the assemblage in monoculture. We tested if the ratio of $\log(\Phi_{obs}/\ \Phi_{exp})$ differed between levels of richness and phylogenetic diversity. We found that increasing *Pseudomonas* genotypic richness was associated with a reduced inhibition of the fungal pathogen beyond that expected if antagonism between *Pseudomonas* genotypes did not increase with richness ($F_{1,\ 37} = 10.45$, $P = 0.003$; Fig 2a) (i.e. $\log(\Phi_{obs}/\ \Phi_{exp}\ )$ decreased with increasing richness). We fitted the presence of each genotype before genotypic richness in separate linear models, but none were able to remove its signal, giving no evidence to suggest a single genotype was completely dominating the community's ability to inhibit take-all. We found no influence of *Pseudomonas* phylogenetic diversity on the fungal inhibition net biodiversity effect (R^2= 0.03; $F_{1,\ 37} = 1.4$, $P = 0.23$; S3a).

If functional traits are not conserved, then phylogenetic diversity may be a poor indicator of functional trait diversity and resource partitioning (Mouquet *et al.*, 2012). In attempt to circumvent this, we calculated functional trait diversity measures for each *Pseudomonas* assemblage based on the ability of each participant genotype to metabolise 95 different resources *in vitro*. We found no correlation between pairwise carbon resource use distance and phylogenetic distances based on *gyrB* with our experimental genotypes (Mantel test, P =0.74;

Fig S2). We fitted functional diversity in carbon resource use traits in place of phylogenetic diversity in our main model presented above, and found it was also a poor predictor of the fungal inhibition net biodiversity effect (R^2= 0.02; $F_{1, 37}$ = 0.7, P = 0.38; Fig S4a).

3.3.2 Bacterial antagonism

We tested the idea that antagonistic interactions between bacterial genotypes increase with richness by conducting a second microcosm experiment. We measured how much biomass each genotype accumulated when grown on medium that had been cultured by the assemblages used in the *in vitro* take-all fungal inhibition experiment (Methods). This assay accounts for the compound antagonistic effects of toxin production and nutrient depletion on bacterial growth which both potentially operate when increasing richness (Jousset *et al.*, 2011b; Becker *et al.*, 2012). Here we define Φ_{obs} as the overall biomass a genotype produced on medium from a given assemblage, and Φ_{exp} as the mean biomass that genotype produced on medium from each genotype present in the assemblage. As before, we tested if the ratio of $\log(\Phi_{obs}/\ \Phi_{exp})$ differed between levels richness, phylogenetic or functional diversity. We found that *Pseudomonas* biomass decreased with increasing genotypic richness beyond what would be expected if antagonism between bacterial genotypes did not increase with richness ($F_{1, 35}$= 5.6, P = 0.024; Fig 2b) (i.e. $\log(\Phi_{obs}/\ \Phi_{exp})$ decreased with increasing richness).

Again, we fitted the presence of each genotype before the genotypic richness term in separate linear models, but as before dominant selection effects by any one genotype were not observed. We were unable to detect any clear signal on the

effect of phylogenetic (R^2= 0.00; $F_{1, 35}$ = 0.03, P = 0.85; Fig S3b), or functional diversity based on carbon utilization traits (R^2= 0.01; $F_{1, 35}$ = 0.33, P = 0.57, Fig S4b) on the increases in antagonism between bacteria in mixtures within richness levels. If antagonism between *Pseudomonas* genotypes was an important factor driving fungal inhibition we would have the expectation that the net biodiversity effects observed in our fungal inhibition experiment could be explained to some degree by the variation in the net biodiversity effects in the *Pseudomonas* inhibition experiment. And indeed, we found that the relative increase in bacterial inhibition in bacterial mixtures from monocultures was a positively related to the relative reduction in inhibition of the take-all pathogen by those same bacterial communities (R^2= 0.29; $F_{1, 35}$= 19.56, P< 0.0001; Fig 2c).

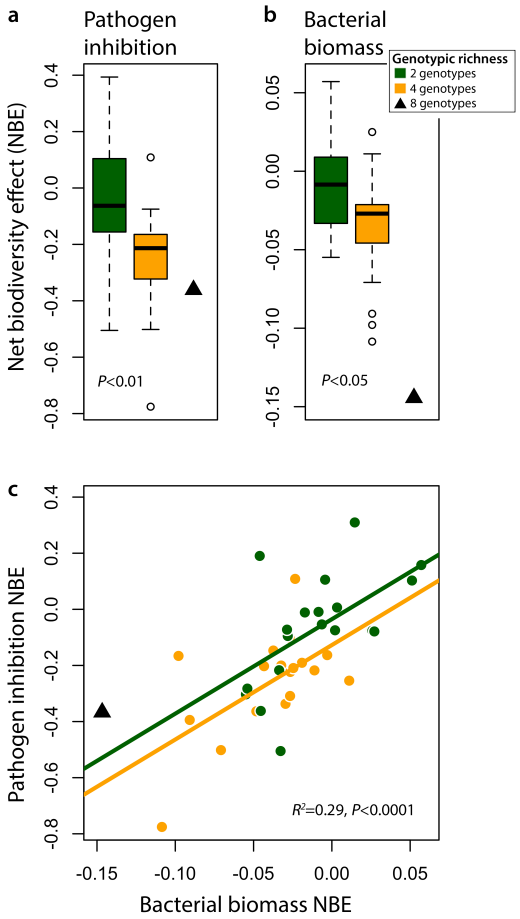


Figure 2. Negative effects of bacterial genotypic richness on fungal pathogen inhibition result from increasing intergenotypic antagonism between soil bacteria (a) net negative effects of increasing *Pseudomonas* genotypic richness on inhibition of wheat take-all fungal pathogen (n=20) (b) net negative effects of increasing *Pseudomonas* genotypic richness on *Pseudomonas* biomass (OD_{600}) via intergenotypic bacterial antagonism (n=19) (c) relationship between net effects of Pseudomonas intergenotypic antagonism and fungal pathogen inhibition at 2 and 4 genotypic richness treatments (n=19). R^2 calculated from the variance in pathogen inhibition NBE explained by bacterial biomass NBE, independent of species richness effects (Total $R^2 = 0.48$, $P < 0.001$). All comparisons are made with Analysis of Covariance (see METHODS for details). All points are the mean of three experimental replicates. The values of net biodiversity effects of 8 genotypes are included for visual comparison. See main text for details on how Net Biodiversity Effects (NBE) responses were calculated.

3.3.3 Field trials

In our field trials we found that the take-all severity on wheat roots of isogenic *T. aestivum* var. Xi19 was dependent on the wheat variety used in soil pre-culture treatments (Welch's t-test, $t_{3.91}$ =11.64, P<0.001; Fig 3a). This difference in disease load was related to large differences in overall grain yields of wheat (31% ± 4% SE [~3t ha^{-1}] higher yields in plots with low take-all disease) (Welch's t-test, $t_{4.24}$ =9.26, P=0.0006; Fig.3b). To investigate whether this difference in disease load and yield was associated with *Pseudomonas* spp. we sampled *Pseudomonas* communities in the endosphere (inside) and rhizosphere (outside) of the roots of the isogenic wheat plants, and then compared genotypic richness and phylogenetic diversity across plots. We found that the plots with highest take-all disease loads, and lowest yields contained the highest genotypic richness of *Pseudomonas* in the rhizosphere (Welch's t-test, $t_{5.98}$ =2.63, P=0.039; Fig 3c). We didn't detect differences in rhizosphere *Pseudomonas* community composition *per se* between plots with high or low disease load (Analysis of Similarity, Bray-Curtis, R=0.19, P=0.08). We found no differences in phylogenetic diversity of *Pseudomonas* in the rhizosphere of wheat roots between low or high take-all plots (Welch's t-test, $t_{4.96}$ =0.40, P=0.70; Fig S5). Endosphere *Pseudomonas* genotypic richness or phylogenetic diversity also showed no significant relationships with take-all intensity or yield (Fig 3, Fig S5). On average 1.7% of 16S rRNA reads were identified as *Pseudomonas* across the study plots, highlighting their importance in the rhizosphere community. However, *Pseudomonas* relative abundances and generic microbial community composition based on 16S rRNA sequences did not differ between treatments (Fig S6, Supplementary Notes 1).

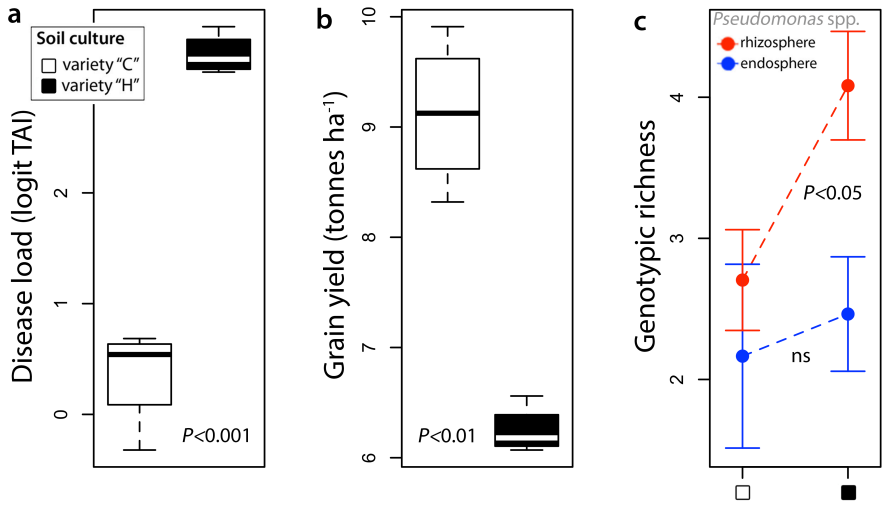


Figure 3. Increased bacterial richness is associated with increased fungal disease load (TAI) and wheat yield losses in the field (a) wheat variety choice in the first year of cropping directly influences the intensity of take-all disease in the following isogenic wheat crop (cv. Xi19); (b) this has a marked effect on grain yield of the following wheat crop; (c) these negative effects of the fungal pathogen are associated with an increased rarefied genotypic richness of *Pseudomonas* spp. in the rhizospheres of wheat plants (data show means ± s.e.m). (174 isolates in total; Cadenza plots= 87, Hereward plots= 91). All comparisons are made with Welch's t-test. ns=non significant. C= Cadenza; H= Hereward; TAI = take all intensity index (see METHODS for details). In all cases n=4.

3.4 Discussion

Here we show that antagonistic interactions within a group of soil bacteria at high genotypic richness can in principle reduce the ability of those bacteria to inhibit growth of a pervasive fungal pathogen of wheat. We used experimental model microcosms to partition the effects of different components of diversity and found genotypic richness increased bacterial antagonism and decreased pathogen control. We found no support for the idea that functional diversity in resource use traits, or phylogenetic diversity, within bacterial communities can help to reduce bacterial antagonistic driven reductions in fungal pathogen control. Our simple synthetic models were matched closely by field observations showing that take-all disease is associated with high *Pseudomonas* spp. richness in wheat rooting zones. We hypothesize that plant driven controls on belowground bacterial diversity could be a key explanatory factor in the control of soil borne pathogens by forcing the degree of competition between potential biocontrol strains and the rest of the bacterial community (e.g. Fig 4).

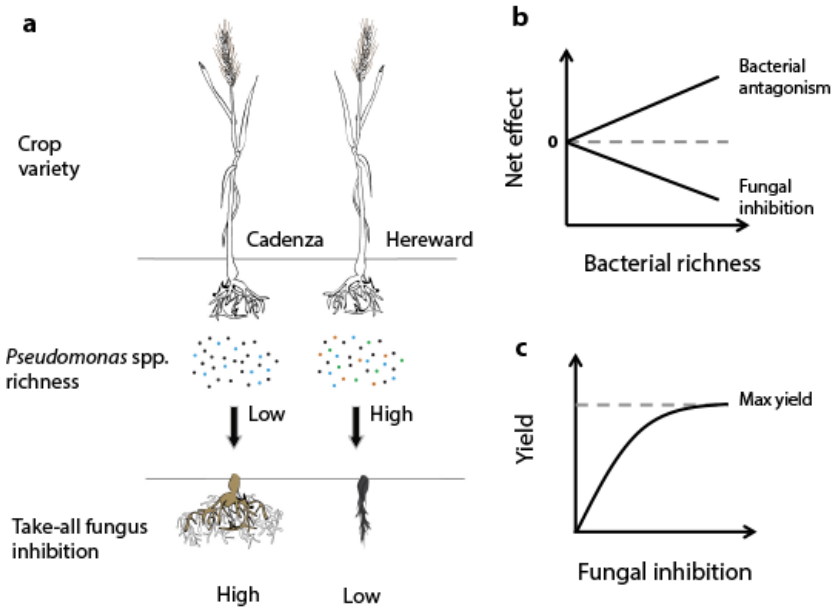


Figure 4. Hypothetical effects of bacterial richness on fungal pathogen inhibition at the plant-soil interface (a) different wheat varieties culture high or low richness of soil bacteria (*Pseudomonas* spp.) which leads to low or high suppression of fungal pathogens in the soil (take-all fungus), respectively. (b) as bacterial richness increases, antagonism between bacterial genotypes increases which causes a decrease in ability of the bacterial community to combat fungal pathogens (c) an increase in fungal pathogen inhibition decreases disease incidence and leads to increase in crop yield.

Our *in vitro* modelling studies were designed to orthogonally test for the effects of bacterial phylogenetic and resource use diversity versus genotypic richness on fungal pathogen suppression. We found no effects of phylogenetic and functional trait diversity based on carbon resources, but consistent negative effects of genotypic richness, on bacterial antagonism and pathogen suppression. Previous studies have suggested that negative effects of bacterial genotypic richness might dominate any positive effects of phylogenetic or resource use trait diversity on bacterial growth as a result of resource scarcity (Jousset *et al.*, 2011a,b; Eisenhauer *et al.*, 2013). This would suggest that any neutral effects of phylogenetic or resource use trait diversity in our field observations and *in vitro* experiments may have resulted from limited opportunity for niche partitioning in those environments (Jousset *et al.*, 2011a,b; Eisenhauer *et al.*, 2013). However, characterizing functional traits in genotypes grown in isolation may have poor predictive capacity on species interactions if trait values are plastic and change in the presence of different species in mixtures. Future studies might investigate the influence of this and the importance of other traits such as toxin and secondary metabolite production on co-operation and the bacterial richness vs. pathogen suppression relationship. Finally, we stress that core genome phylogenies may be poor predictors of functional trait dissimilarity and niche partitioning (Pavoine *et al.*, 2013; Narwani *et al.*, 2013) because functional traits may be selected by convergent evolution or horizontal transfer of accessory genome components (Mouquet *et al.*, 2012). Nevertheless, as genome sequencing costs decline, linking dissimilarity in specific gene sequences to variation in ecological traits and functions will become more feasible.

The negative effects of genotypic richness we observed in our model experiments do run contrary to the commonly reported phenomenon that diversity has a positive effect on ecosystem functioning (Hooper *et al.*, 2005; Griffin *et al.*, 2013). In support of our findings, other laboratory experiments have previously shown bacterial antagonism to cause ecosystem functioning to collapse

at high richness (Jousset *et al.*, 2011b; Becker *et al.*, 2012), but see Jousset *et al.*, (2014). We did not find evidence that a single dominant genotype was completely driving the negative effects we observed, but did not measure the separate yield of different genotypes in bacterial mixtures and so were unable to determine if any isolates were completely run to extinction. It is possible that antagonistic interactions, the presence of competitively dominant but functionally poor (i.e. of a desired functional output) species, or the non-additive effects of exploitative and interference competition operate in other taxonomic groups. Nevertheless, we suggest it may be of limited use to look for general statements of positive vs. negative biodiversity effects. Cautioning against a dichotomy seems important because, the direction of the biodiversity ecosystem functioning relationship can depend on both the function and the trophic level of interest.

Although previous studies have shown the transfer of negative complementarity effects in *Pseudomonas* communities from the Petri dish biofilms to plant roots surfaces (Becker *et al.*, 2012), the forced interactions, and potential for increased diffusion of antibacterial toxins and metabolites through agar and liquid media in our study may have increased the effect size of competition in our model microcosms. It may also be likely that ecological history effects (e.g. root or individual plant clone) could have influenced the steepness of BEF relationship we observed in our *in vitro* work conducted on randomly selected isolates (Gravel *et al.*, 2011). More fine scaled sampling, or sampling across spatial gradients in future could be useful for identifying the impact of isolate sampling location on the form of the genotypic richness and pathogen suppression relationship.

It is important to emphasize our field results were unable to conclude if the negative relationship between *Pseudomonas* spp. genotypic richness and take-all in the field was causal, and we are only able to say that our *in vitro* models give one possible explanation for how this interaction might take place. Sequential

sampling of bacterial isolates to map the dynamic interactions between the microbiota of wheat varieties and take-all disease, alongside microbiome transplant experiments and further *in planta* manipulations would help validate this idea. Similarly, although we assayed the most virulent strain of a field isolated take-all pathogen in our model microcosms; we did not assay the effect of different take-all pathogen strains or consider the intraspecific population diversity of the take-all pathogen in our study. Moreover, whilst our field collections were useful for representing the diversity of the dominant members of the culturable *Pseudomonas* on root surfaces at a treatment level, the isolates were randomly sampled from pools of multiple root systems and plant clones, not a typical infection zones on a single root system. Future high resolution sampling, or imaging in real time of bacterial- fungal interactions on the root system would help greatly to see exactly how microbes are actually interacting in spatially structured systems.

Our findings argue for an integrated view that appreciates the importance of both the negative and positive effects of biodiversity in driving specific ecosystem functions within and across trophic levels. Notably we have focused on the diversity of a single bacterial group, containing putative biocontrol strains highly relevant to our focal fungal pathogen. An analysis of generic groupings of 16S rRNA sequences in the soil at each of our trial plots showed that other bacterial groups are also highly relatively abundant, some of which may play important roles in plant fitness. Testing how co-cultures of larger numbers of isolates from different bacterial genera respond to diversity manipulations, and perform one or more ecosystem functions, remains an exciting avenue of research. In particular, future work could identify the applicability of our findings to functions involved in both concurrent disease control and nutrient supply, across multiple bacterial indicator groups and trophic levels. A sharpened focus on these services may help to significantly reduce environmentally and economically costly inputs into global food systems.

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Supplementary Information

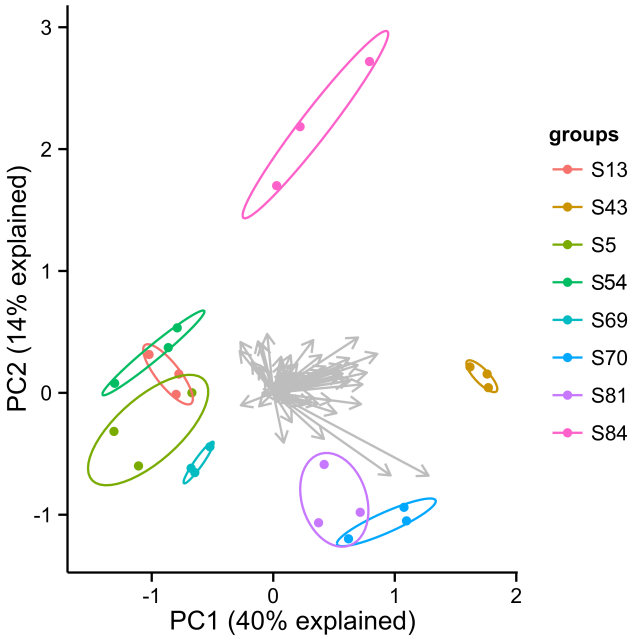


Fig S1. Carbon resource use profile differences between experimental *Pseudomonas* spp. Assays were completed using GN2 MicroPlates (Biolog, Inc.), with 100 µL of each OD_{600} adjusted population per well, incubation at 25°C for 72 h and measurement at OD_{590}., in triplicate. Trait values are depicted with a threshold of OD_{590} >0.05. The 8 strains are separated along two major principle components (PC1 and PC2) accounting for 54% of the total variation in carbon use metabolism. Ellipse probability =0.69

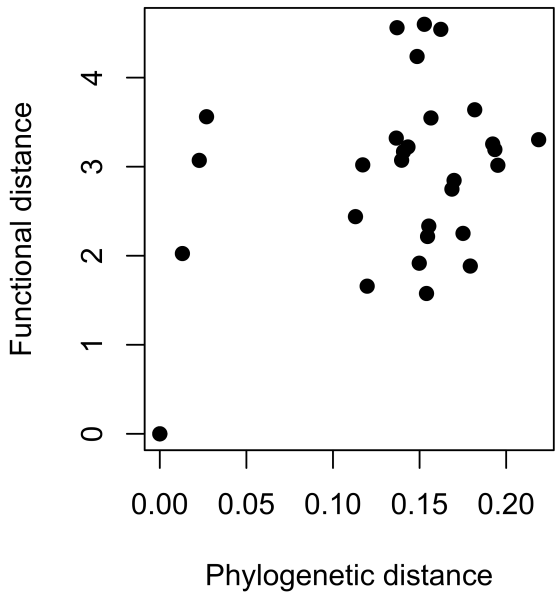


Fig S2. Phylogenetic distance is a poor predictor of the functional distance in carbon use metabolism between experimental *Pseudomonas* genotypes. Phylogenetic distance is based on 940kb *gyrB* fragment phylogeny estimate (Fig 1) and functional distance is based on the euclidean distance between strains for 95 carbon resource traits assayed in Biologg GN2 plates (Fig S1). A Mantel test on the distance matrices was insignificant (P=0.74).

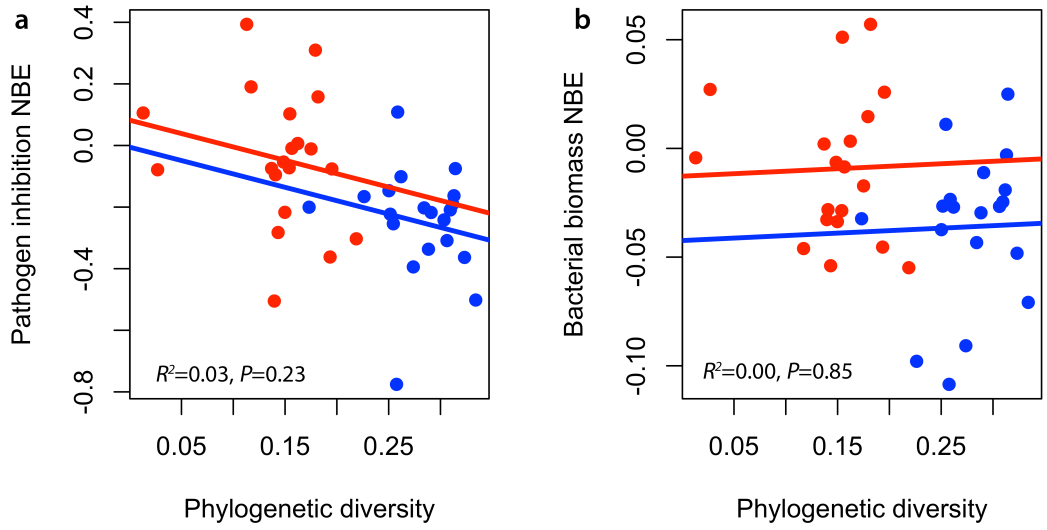


Fig S3 Phylogenetic diversity and net biodiversity effects (NBE) a) Phylogenetic diversity did not influence pathogen inhibition NBE b) Phylogenetic diversity did not influence bacterial biomass NBE. Genotypic richness (catogorical) was fit first and phylogenetic diversity (continuous) second in all models to partition the independent effects of genotypic richness (intercept differences) from phylogenetic diversity (slopes). See main text for calculation of net biodiversity response variables. All analysis was performed in the mean of three experimental replicates (to account for psuedoreplication). R^2 values are calculated from the variance explained by phylogenetic diversity, independent of species richness. Red= 2 genotype assemblages; Blue= 4 genotype assemblages.

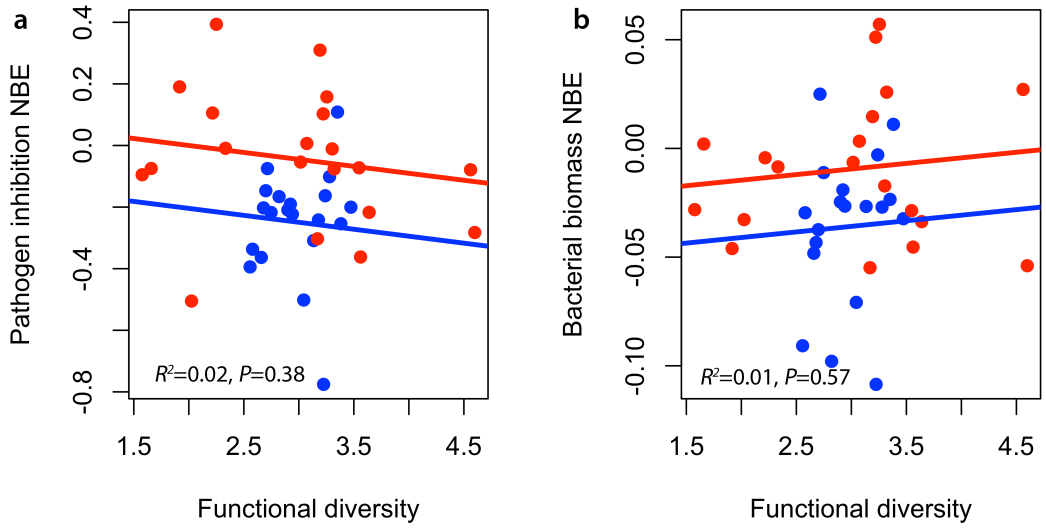


Fig S4 Functional diversity and net biodiversity effects (NBE) a) Functional diversity in carbon resource metabolism (FD) did not influence pathogen inhibition NBE b) FD did not significantly influence negative bacterial biomass NBE. Genotypic richness (catogorical) was fit first and functional diversity (continuous) second in all models to partition the independent effects of genotypic richness (intercept differences) from phylogenetic diversity (slopes). See main text for calculation of net biodiversity response variables. All analysis was performed in the mean of three experimental replicates (to account for psuedoreplication). R^2 values are calculated from the variance explained by functional diversity, independent of species richness. Red= 2 genotype assemblages; Blue= 4 genotype assemblages.

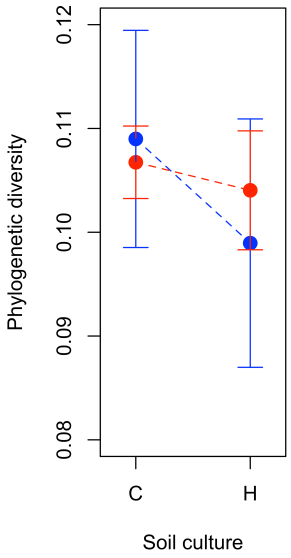


Fig S5 Bacterial phylogenetic diversity and fungal disease load in the field. Mean (±s.e.m) differences in rarefied phylogenetic diversity of *Pseudomonas spp.* between plots with high (H) and low (C) intensity of take-all fungal disease. Blue= endosphere, Red=rhizosphere. All comparisons are non significant (P> 0.05) with Welches t-test. See main text for details. n=4

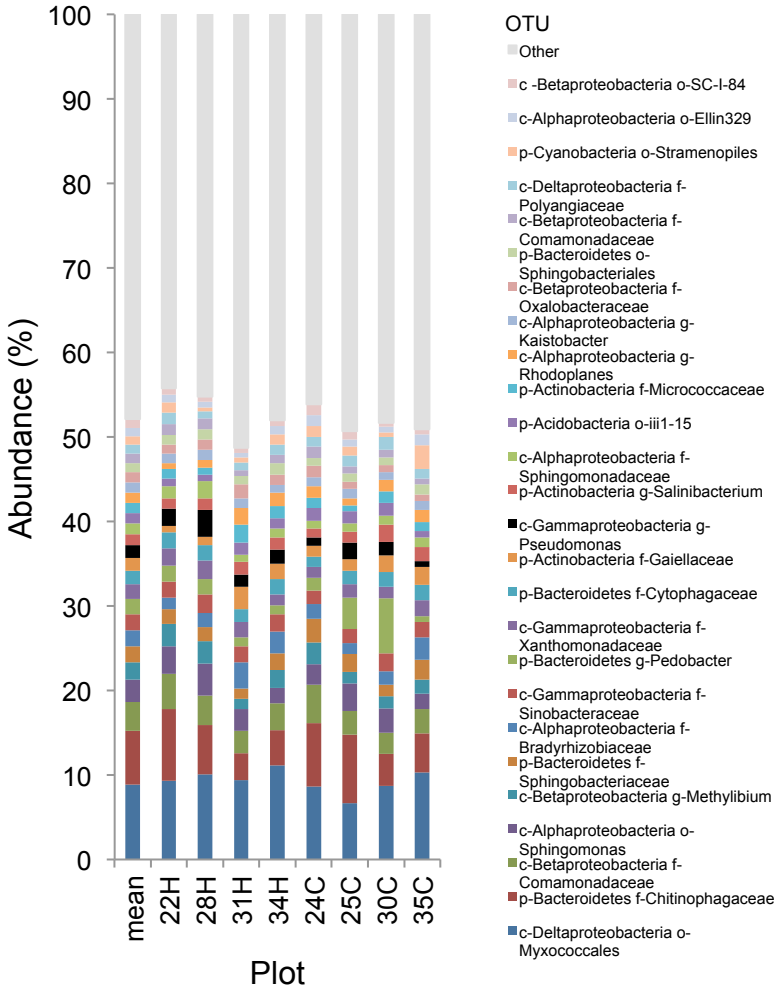


Fig. S6. Comparison of bacterial genera identified by 16S rRNA gene illumina amplicon sequencing in the rhizosphere soil of wheat. This figure shows that *Pseudomonas* spp (in black) are highly prevalent in each of our 8 plots used in the field study. xxH= Hereward, xxC= Cadenza, Mean= mean abundances of all 8 plots. Only operational taxonomic units (OTUs) represented at >1% of total identified amplicons are shown. (see Supplementary Notes 1)

Supplementary Notes 1

These notes describe the methods underlying Fig S6 and highlight the results of microbial community analysis based on 16S rRNA generic groupings. On the 2nd July 2012, 5 x 10cm soil cores were sampled in a W formation in each of the study plots and pooled into 8 samples (one per plot). Bacterial and archaeal 16S rRNA genes were amplified from rhizosphere soil DNA samples with barcoded universal prokaryotic primers (515F/R806) targeting the V4 region, subjected to Illumina[ff] sequencing and analysed using the QIIME 1.8 pipeline (utilizing greengenes for taxa classification and the UCLUST algorithm for OTU clustering), as described by (Caporaso *et al.*, 2010, 2011). After amplicon sequencing 37,788 reads were sub-sampled from each of 8 samples (a total of 302,304 reads). Reads were assigned to bacterial genera (where possible) with 97% sequence identity. We did not observe differences in soil community composition between plot treatments based on this amplicon data (ANOSIM: R=0.06 and P=0.37). We found that an average of 1.7% of reads were identified as *Pseudomonas* across the study plots. Lack of sequence variation within the relatively conserved segment of the 16S rRNA gene limit inference to above the level of genus, and so further hypotheses regarding changes in *Pseudomonas* richness, as explored in the main text, cannot be addressed with this amplicon data.

4. Relatedness is a poor predictor of negative plant-soil feedbacks[3]

Abstract

Understanding the mechanisms underlying negative plant-soil feedbacks remains a critical challenge in plant ecology. If closely related species are more similar, then phylogeny could be used as a predictor for plant species interactions, simplifying our understanding of how plant-soil feedbacks structure plant communities, underlie invasive species dynamics, or reduce agricultural productivity. Here we test the utility of phylogeny for predicting plant-soil feedbacks by undertaking a hierarchical Bayesian meta-analysis on all available pairwise plant-soil feedback experiments conducted over the last two decades, including 133 plant species in 329 pairwise interactions. We found that the sign and magnitude of plant-soil feedback effects were not explained by the phylogenetic distance separating interacting species. This result was consistent across different life forms, life cycles, provenances, and phylogenetic scales. Our analysis shows that, contrary to widespread assumption, relatedness is a poor predictor of plant-soil feedback effects.

[3]Typescript from Mehrabi, Z., & Tuck, S.L. 2014. Relatedness is a poor predictor of plant-soil feedbacks. *New Phytologist.* DOI: 10.1111/nph.13238

4.1 Introduction

Negative plant-soil feedbacks occur when conspecific adults culture soil to the relative disadvantage of their own offspring (Bever, 1994; Mangan *et al.*, 2010; van der Putten *et al.*, 2013). This process can maintain diversity by generating frequency-dependent population growth, and plays key roles in agriculture and invasion ecology (Packer & Clay, 2000; Klironomos, 2002; Callaway *et al.*, 2004; Petermann *et al.*, 2008; Mangan *et al.*, 2010). Two widely proposed mechanisms by which negative plant-soil feedbacks operate are through accumulation of host-specific soil borne pathogens and nutrient resource depletion (Kulmatiski *et al.*, 2008; Mangan *et al.*, 2010; Burns and Strauss, 2011; van der Putten *et al.*, 2013; Flory & Clay, 2013). Evidence that closely related plant species are more likely to share natural enemies or resources (Darwin, 1859; Webb *et al.*, 2006; Gilbert & Webb, 2007) has led to a belief that that plants will perform less well on soils cultured by more closely related species (Brandt *et al.*, 2009; Burns and Strauss, 2011; Liu *et al.*, 2012; Sedio & Ostling, 2013; Callaway *et al.,* 2013) (Fig 1). However, discontinuities between evolutionary history, plant functional traits, and niche partitioning do not support the generalisability of this hypothesis (Mouquet *et al.*, 2012; Best *et al.*, 2013; Pavoine *et al.*, 2013; Narwani *et al.*, 2013; Kelly *et al.*, 2014). If traits responsible for resource use or host susceptibility to natural enemies are not widely conserved, or if the sum of plant-soil interactions remain neutral, relatedness may in fact be a poor predictor of negative plant-soil feedback effects.

More than two decades of plant-soil feedback research have generated sufficient number of studies to allow us to test the utility of evolutionary history for predicting negative plant-soil feedback effects. Here we present a formal meta-analysis of 329 experiments which compared the growth response of plant species to soil cultured by conspecifics relative to heterospecifics (133 plant species in 276 unique pairwise interactions). For each pairwise species interaction, we calculated

Hedges' *d* standardized mean difference, where a negative effect size corresponds to a positive plant response to heterospecific-cultured soil relative to conspecific-cultured soil (i.e. a signal of negative plant-soil feedbacks). We then extracted the phylogenetic distances between species pairs in each experiment from a chronogram built using publicly available data. We meta-regressed our calculated effect sizes on the phylogenetic distance estimates by fitting a hierarchical Bayes linear model that accounts for sampling dependence (e.g. same conspecific control soils across multiple experiments), and hierarchical dependencies (e.g. multiple experiments within the same study) in the dataset (Stevens & Taylor, 2008). We estimated an overall slope with 95% credible intervals to test whether phylogenetic distance between species pairs could predict negative plant-soil feedbacks, and whether this relationship differed among different subsets of our dataset: plant life forms, life cycles, and native-exotic interactions.

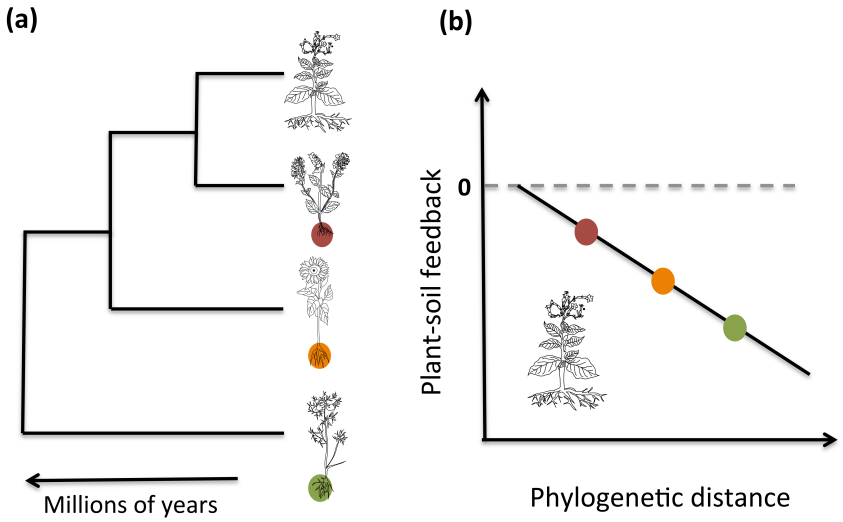


Fig 1. The proposed relationship between phylogeny and plant-soil feedback effects. (a) shows the phylogenetic relationships between species; (b) shows the expected outcome of the interaction between a focal species and the soils of its relatives. Negative plant-soil feedbacks represent the increased performance of species on soils cultured by heterospecifics relative to conspecifics.

4.2 Materials and Methods

4.2.1 Study selection

We searched all published work on ISI Web of Knowledge for the last 20 years (1993-2013) with 3 topic-level searches: soil* feedback* phylogeny; soil* feedback* experiment*; and plant* feedback* soil*, and screened these alongside additional references and unpublished works for studies that recorded pairwise plant-soil feedback biomass responses. A PRISMA statement, showing this flow of information through our study selection process, can be found in the Supporting Information (Supporting Information Notes S1). All studies in our final selection had data on means and SD that were readily accessible from figures or through author correspondence.

4.2.2 Phylogeny estimation

Genbank accessions for 5 gene regions (ITS, *rbcL*, *matK*, *ndhF*, *trnL-F*) were retrieved for the species in the feedback experiments and individually aligned using MAFFT (v.7.205) with the l-ins-i algorithm (Katoh & Standley, 2013). Alignments were concatenated into a supermatrix and maximum-likelihood based estimation of the phylogeny performed using RAxML (v.7.0.4), optimized for each gene region under the GTR-GAMMA substitution model (Stamatakis, 2006). We restricted our search by using a constraint tree built from systematic treatments of the recognized tribes, families, orders and higher-level clades of the species in our study (Wojciechowski *et al.,* 2006; Potter *et al.,* 2007; Panero & Funk, 2008; Couvreur *et al.,* 2010; Bendiksby *et al.,* 2001; Soltis *et al.,* 2011; Grass Phylogeny Working Group II, 2012). We congruified (Eastman *et al.,* 2013) our maximum likelihood estimate with the most resolved angiosperm chronogram currently available (Zanne *et al.,* 2013; Tank *et al.*, 2013), and used penalized likelihood rate smoothing to create a time scaled phylogeny of the species in our

study (Smith & O'Meara, 2012). Phylogenetic distances between species in the 329 pairwise interactions were extracted using the *cophenetic.phylo* function in the R package *ape* (Paradis *et al.*, 2004). Full details of the phylogeny estimation are presented in Supplementary Information (Methods S1).

4.2.3 Hierarchical Bayesian meta-analysis

To determine the influence of phylogeny on plant-soil feedbacks we fitted a hierarchical Bayesian model accounting for sampling and hierarchical dependencies in our data. Our model in matrix form is:

$$d = \mathrm{Xb} + \mathrm{d} + \mathrm{e} \qquad (1)$$

where d is the vector of effect size estimates from all the experiments; X, a design matrix with our covariates, b, a vector of parameters; d, a vector of hierarchical errors; and e a vector of sampling errors. It assumes the distributions:

$$\mathrm{d} \sim N(0, \mathrm{D})$$

$$\mathrm{D} = \mathrm{t}^2\mathrm{I} + \mathrm{zM}$$

$$\mathrm{e} \sim N(0, V) \qquad (2)$$

Where V is the sampling variance-covariance matrix, with known sampling variances and covariances. D represents the variance and covariance among random deviations of Xb from the effect sizes being estimated. D is a block-diagonal matrix, with hierarchical variance t^2 on the diagonal (I) and blocks (M) of hierarchical covariance z on the off-diagonal for pairs of hierarchically dependent effect sizes. We estimated the slope for phylogenetic distance with 95% credible intervals for this estimate, calculated by multiplying the posterior standard error of the coefficients by the 95% quantile of a *t*-distribution with *N-k*

degrees of freedom. Our analysis was carried out using R 3.1.0 (R Core Development Team, 2013) with the R package *metahdep* (Stevens & Nicholas, 2009). Further details of the methods and our study design, including the full dataset and R code used in the paper, can be found in our Supporting Information (Methods S1; Notes S2; Table S1

4.3 Results and Discussion

Our analyses show that phylogenetic distance between species pairs is a very poor predictor of plant-soil feedback effects (-0.00035; 95% CI: lower = -0.00077, upper = 0.00007, n = 329; Fig 2a). Whilst there was a moderately negative plant-soil feedback effect observed on average (-0.330; 95% CI: lower = -0.503, upper = -0.156, n = 329), variation in magnitude of this effect could not be explained by phylogenetic distance between species pairs in plant-soil feedback interactions. As life history is linked to evolution rates and niche conservatism (Petit & Hampe, 2006; Smith & Beaulieu, 2009), we may expect heterogeneity in the response such that a phylogenetic signal in negative plant-soil feedbacks is easier to detect in longer-lived species. However, despite differences in mean feedback effects within these subgroups (i.e. intercept estimates), the lack of relationship with phylogenetic distance (i.e. slope estimates) was consistent among plant life forms (grasses, herbs, shrubs and trees) and life cycles (annual, biennial and perennial) (Table 1). It is widely argued that a phylogenetic signal exists in invasiveness (Strauss *et al.*, 2006; Sol *et al.*, 2014), such that we may expect distantly related species to establish and invade in ecosystems through natural enemy release. However, when we analyzed whether plant-soil feedback effects created in native-exotic interactions were related to phylogenetic distance between the species pairs, we found no evidence to suggest this was the case (Table 1). Finally, it has been suggested that the effect of phylogeny may be dominant between close relatives, such that relatedness might predict plant-soil feedbacks between species

interacting within taxonomic divisions such as genera or tribes within plant families (e.g. Burns and Strauss, 2011; Callaway *et al.,* 2013). However, we analyzed plant-soil feedbacks from species interacting across a range of phylogenetic distances within two major plant families represented in our dataset (33 interactions within Asteraceae and 65 within Poaceae), and found the relationship between phylogenetic distance and plant-soil feedback interactions within these families was no different from that observed in the aggregate dataset (Poaceae: 0.0010; 95% CI: lower = -0.0079, upper = 0.0100, n = 65; Asteraceae: 0.0058; 95% CI: lower = -0.0524, upper = 0.0640, n = 27; Figs 2b-c). In summary, these results give little support for the notion that phylogeny can be used as a general predictor of plant-soil feedback effects.

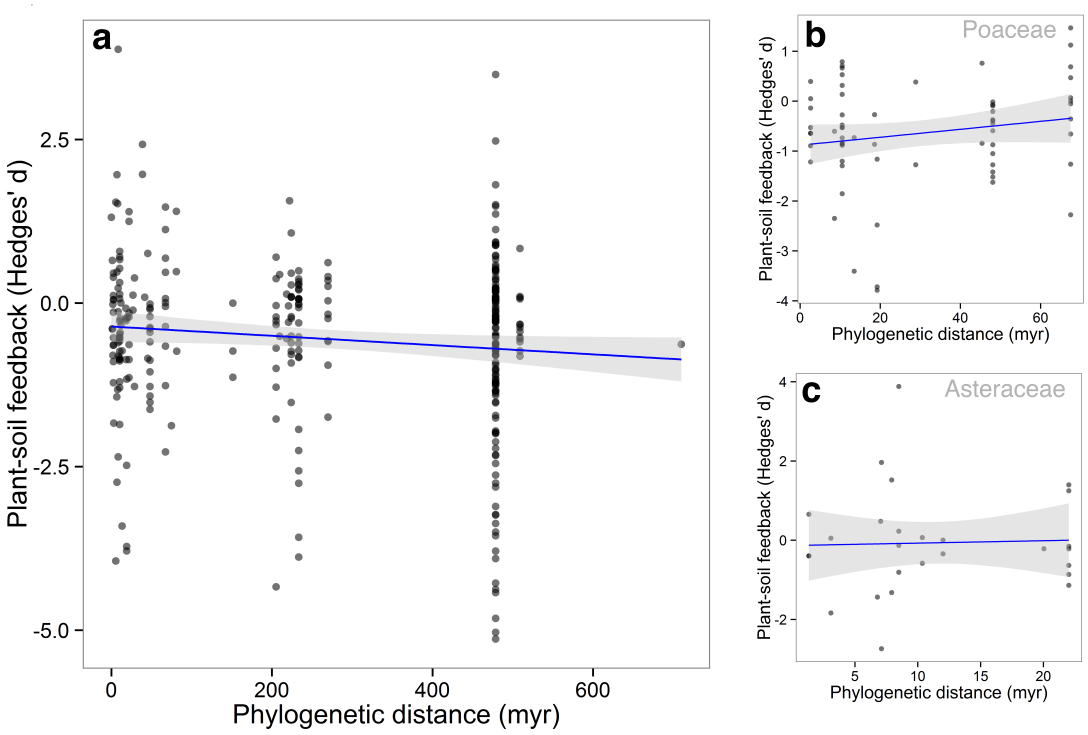


Fig 2. (a) Relatedness is a poor predictor of negative plant-soil feedbacks. Meta-regression of 329 experimental plant-soil feedback effects against phylogenetic distance between interacting species pairs (total species = 133). There was also no relationship between relatedness and plant-soil feedbacks for closely related species interacting within two major families in our data set, (b) Poaceae and (c) Asteraceae. Unlike the estimates shown in the text, the plotted regression slope and 95% intervals assume a fixed model with no hierarchical dependence.

In order to check if our results were sensitive to disproportionate representation of species or phylogenetic distance levels across studies and experiments, we performed two subset analyses, re-analysing a trimmed dataset representing species in only one hierarchical grouping, and assessing the effect of unbalanced data across the range of phylogenetic distances. In order to undertake the first subset analysis with the unbiased representation of species, we trimmed the dataset to ensure species were not replicated in multiple studies, overcoming the non-independence of multiple effect sizes for a species among studies. We selected studies for this analysis that maximised the number of total number effect sizes analysed whilst maintaining the trimming criteria. The results from this subset analysis were consistent with our initial findings: phylogenetic distance between species pairs had no effect on the magnitude of plant-soil interactions (-0.00036; 95% CI: lower = -0.00079, upper = 0.00006, n = 287). The second subset analysis, for unbiased representation of phylogenetic distance, was conducted by running 100 simulations, where an effect size for each observed value on the phylogenetic distance gradient was randomly sampled, and taking the mean and standard error for this sample of slope estimates. The results from this subset analysis were also consistent with our findings that the magnitude of plant-soil feedback observed is not dependent on phylogenetic distance (mean slope = -0.00019 ± 0.00005 SE). We checked for bias in our models using a funnel plot combined with trim and fill assessment, and a cumulative meta-analysis approach, which evaluates how the slope of the relationship between phylogenetic distance and plant-soil feedback was influenced by experiments with high sampling variance (i.e. within-study error). The model checking and subset analyses we have conducted found little evidence for publication bias in our dataset and showed that our results were robust to biases that may exist (Supporting Information Methods S1).

Table 1. The effect of phylogeny on negative plant-soil feedbacks. Intercept and slope estimates from the hierarchical Bayesian meta-analytic model. 95% credible intervals for each estimate are calculated by multiplying the posterior standard error of the coefficients by the 95% quantile of a *t*-distribution with *N-k* degrees of freedom. None of the slopes showed significant effects of phylogenetic distance.

	Intercept ±95% CI's	Slope ±95% CI's	n
Life form			
Grass	-0.32 ±0.283	-0.0005 ±0.0007	119
Herb	-0.30 ±0.410	-0.0004 ±0.0011	133
Shrub	-0.21 ±0.554	-0.0003 ±0.0013	27
Tree	0.11 ±0.582	-0.0004 ±0.0011	50
Life cycle			
Annual	-0.224 ±0.375	-0.0003 ±0.0009	114
Biennial	-1.029 ±2.021	0.0007 ±0.0050	8
Perennial	-0.236 ±0.403	-0.0004 ±0.0010	207
Provenance			
Native	-0.207 ±0.601	-0.0005 ±0.002	296
Exotic	-0.853 ±0.582	0.0017 ±0.002	33

There is currently much debate on the use of phylogenetic data in ecology (Mayfield & Levine, 2010; Best *et al.*, 2013; Pavoine *et al.*, 2013; Narwani *et al.*, 2013; Kelly *et al.*, 2014). The growing recognition that dissimilarity in functional traits is not related in a monotonic way to phylogenetic distance suggests that phylogeny cannot be used as a general proxy for predicting the outcome of species interactions (Best *et al.*, 2013; Pavoine *et al.*, 2013; Kelly *et al.*, 2014). The poor ability of phylogenetic distance to predict plant-soil feedbacks indicates that the traits underlying this phenomenon may be no different. Future experimental work might better focus on interactions among species at fine phylogenetic scales, and identify if and where specific traits important for plant-soil feedbacks saturate on the phylogenetic distance vs. trait dissimilarity curve (e.g. Kelly *et al.*, 2014). Our meta-analysis however gives strong evidence against the general use of phylogeny for predicting plant-soil feedbacks and their associated effects on plant community assembly, plant species invasions, and agricultural soil sickness.

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Supporting Information: Methods S1

Study selection

The study selection criterion was that a study presents data on the response of species grown in monoculture on soil cultured from a conspecific versus soil cultured from a heterospecific species. The response on each soil could either be biomass, survival, growth or germination. All published work on ISI Web of Knowledge for the last 20 years (Jan 1993- May 2013) were searched using 3 topic-level searches: soil* feedback* phylogeny; soil* feedback* experiment*; and plant* feedback* soil*. This returned 3560 studies, which were screened for suitability by reviewing the titles and abstracts, along with additional 68 records identified through reviews and unpublished material. After removing duplicates, 147 studies passed the initial screening and were downloaded for main text review. Out of this final selection process 41 studies were found to fit study criteria, including one study we chose to retain that reported responses of species grown on soils cultured by its own plant family versus soil cultured by a different plant family. However, not all authors provided data for these studies, and overall studies did not provide enough data to investigate responses other than biomass. As a result, our final dataset included 28 studies, comprising a sample size of 331 experiments. The analyzable number of these experiments was 329 (see supplementary R. code for details).

A PRISMA statement flowchart, showing this flow of information through our study selection process, can be found in the Supporting Information (Notes S1). All studies in our final selection had data on means and SD that were readily accessible from figures or through author correspondence. Data collection was coded after Kulmatiski *et al.* (2008), but adapted to include information on plant-soil interactions between each specific plant species pair.

Calculation of phylogenetic distance

Genbank accessions for 5 gene regions (ITS, *rbcL*, *matK*, *ndhF*, *trnL-trnF*) were retrieved for the species in the feedback experiments. These five sites represent a range of both slow and fast evolving regions used for resolving phylogenetic relationships at a range of evolutionary scales. We supplemented 9 species in our study for which genetic data was not available (*Northea hornei, Pennisetum centrasiaticum, Falcataria moluccana, Ratibida pinnata, Juncus lesueurii, Juncus lesueurii, Solidago californica, Panicum sphaerocarpon, Centaurea maculosa*) with congenerics with coverage (*Northea seychellana, Cenchrus purpureus, Falcataria toona, Juncus effusus, Ratibida columnifera, Solidago canadensis, Panicum capillare, Centaurea jacea*). This substitution only reduced the phylogenetic resolution in the pairwise feedback interactions between species on one occasion (e.g. *Juncus lesueurii* vs. *Juncus lesueurii)*, and so was not problematic for our analysis. As one study in our dataset undertook a fully factorial experiment comparing six species responses on five family soils (Mehrabi, 2011), we utilized the species in the experiment as representative of the family level interactions (as all interactions in this study were across families, this did not influence the phylogenetic distance estimates and these data were not included in our analysis for testing signals within families, see Results and Discussion, Main Text). The final species coverage for gene regions were ITS (83.7%) *rbcL* (76%) *matK* (76%) *ndhF* (35.6%) and *trnL-F* (75%). Each gene was individually aligned using MAFFT (v.7.205) using the l-ins-i algorithm (Katoh & Standley, 2013). Alignments were manually checked and then concatenated into a 7009bp long supermatrix, containing a total of 60.2% missing data. Maximum-likelihood based estimation of the phylogeny was performed using RAxML (v.7.0.4), optimized for each gene region under the general time reversible model of nucleotide substitution with gamma distributed rate heterogeneity (GTR-GAMMA) on partitioned gene regions (Stamatakis, 2006). We restricted our search by using a

constraint tree built from systematic treatments of the recognized tribes, families, orders and higher-level clades of the species in our study (Wojciechowski *et al.,* 2006; Potter *et al.,* 2007; Panero & Funk, 2008; Couvreur *et al.,* 2010; Bendiksby *et al.,* 2001; Soltis *et al.,* 2011; Grass Phylogeny Working Group II, 2012). We congruified (Eastman *et al.,* 2013) our maximum likelihood estimate with the most resolved angiosperm chronogram currently available (Zanne *et al.,* 2013; Tank *et al.*, 2013), and identified 28 concordant nodes well distributed across the tree (Fig S1). For congruification we used the NCBI Taxonomy database as a reference, and manually updated a taxonomy table representing each species in the study. We then used penalized likelihood rate smoothing (optimized smoothing parameter of 0.1) to create a time scaled phylogeny of the species in our study (Smith & O'Meara, 2012). Phylogenetic distance between each species pair of the 329 pairwise interactions were extracted using the *cophenetic.phylo* function in the R package *ape* (Paradis *et al.*, 2004). We checked the robustness of our results to different time scaled phylogenetic distance estimates. To do this we utilized the widely used chronogram of Bell *et al.* 2010 (with log-normal priors) as a reference tree for congruification, identified 36 congruent nodes, and time scaled our maximum likelihood estimate using penalized likelihood with node ages estimated based on upper and lower 95% HPDs for each congruent node. We then estimated the phylogenetic distances between species pairs as before. We re-ran our meta-regression models on both alternative estimates of phylogenetic distances. We found that the chronogram we employed as a time reference did not influence our results. As a result, we present the results from phylogenetic distances estimated using the most recent chronogram in the main text (e.g. Tank *et al.* 2013).

A cumulative distribution plot of the evolutionary distances between species interacting in our study is given in Fig S2, and a chronogram used for calculating those distances is given in Fig S1. These plots show the phylogenetic distances between species interacting in the experiments in our meta-analysis covered a wide range of scales, including recent divergences (e.g. ~16% of the plant-soil

feedback responses in our dataset were associated with estimated divergences of <5myrs, and ~24% with estimated divergences of <15myrs). From a taxonomic perspective, of the total plant soil feedbacks in the study, 4% were intrageneric, ~24% were intratribal and ~34% were intrafamilial.

Analysis of effect sizes

Our effect size is Hedges *d* standardised mean difference. This quantifies the plant-soil feedback effect by comparing the means of a conspecific-culture control and a heterospecific-culture treatment, standardised by the pooled standard deviation. Hedges *d* includes a small sample-size bias correction factor. A negative effect size corresponds to a positive plant response to heterospecific-cultured soil relative to conspecific-cultured soil (i.e. a signal of negative plant-soil feedback effects). The main aim of our study was not to assess the overall plant-soil feedback effect and variability in this effect among sub-groups, but instead to estimate how this effect varies with change in phylogenetic distance between the focal plant and soil-culture plant. We fitted a meta-regression to the data, estimating a slope for phylogenetic distance, to investigate whether the proposed relationship between phylogenetic distance and negative plant-soil feedback effect exists.

Our analysis was carried out using R 3.1.0 (R Core Development Team, 2013) with the R package *metahdep* (Stevens & Nicholas, 2009). The models were fitted to the data using the function *metahdep.HBLM*. We fitted these hierarchical Bayes linear models to account for sampling and hierarchical dependencies among the data. Sampling dependency exists due to multiple effect sizes using the same control group (conspecific-cultured soil). Hierarchical dependency exists among multiple effect sizes within each separate study. Defining these levels of non-independence in the data meant that a large group of extreme effect sizes within

one study would be down-weighted, preventing them from having a dominant effect on the overall result. Our model in matrix form is:

$$d = \mathrm{Xb} + \mathrm{d} + \mathrm{e} \qquad (1)$$

where d is the vector of effect size estimates from all the experiments; X, a design matrix with our covariates, b, a vector of parameters; d, a vector of hierarchical errors; and e a vector of sampling errors. It assumes the distributions:

$$\mathrm{d} \sim N(0, \mathrm{D})$$
$$\mathrm{D} = \mathrm{t}^2\mathrm{I} + \mathrm{zM}$$
$$\mathrm{e} \sim N(0, V) \qquad (2)$$

Where V is the sampling variance-covariance matrix, with known sampling variances and covariances. D represents the variance and covariance among random deviations of Xb from the effect sizes being estimated. D is a block-diagonal matrix, with hierarchical variance t^2 on the diagonal (I) and blocks (M) of hierarchical covariance z on the off-diagonal for pairs of hierarchically dependent effect sizes (Stevens and Taylor 2008). The heterogeneity between effect sizes, represented by t^2, estimates the proportion of among-studies variance that is true variance, as opposed to within-study sampling error. This heterogeneity measure was used to estimate I^2, the proportion of total variance that is due to true variance among effect sizes ($I^2 = 0.55$) (Higgins and Thompson, 2002).

We estimated the slope for phylogenetic distance with 95% credible intervals for this estimate, calculated by multiplying the posterior standard error of the coefficients by the 95% quantile of a t-distribution with N-k degrees of freedom. We estimated an overall slope to quantify the relationship between phylogenetic

distance and the magnitude of plant-soil feedback and then estimated slopes for individual sub-groups to assess whether this relationship varied within different families, and across different plant life forms, life cycles, and native-exotic interactions.

Model checking

Whilst accounting for multiple levels of non-independence in our data, we were not able to account for non-independence of multiple effect sizes pertaining to the same species across studies, as opposed to within a single study. The sampling dependence of multiple within-study effect sizes that were based on the same control was accounted for, but we were unable to account for the across-study phylogenetic dependence across studies in our model framework. Instead, we performed a subset analysis, re-estimating the relationship between phylogenetic distance and plant-soil feedback after removing any species duplication across studies. This provided a conservative approach to show how robust our results were to this species duplication.

We also assessed the presence and impact of publication bias. We used a funnel plot to assess the asymmetry in our dataset, which could suggest a greater tendency for significantly positive results to be published. To produce the funnel plot from our hierarchical model, we plotted the model residuals against precision (inverse sampling error) to account for the non-independence in the data that was dealt with in the model (Nakagawa and Santos, 2012). The model residuals calculated were conditional on the random effects (Henderson 1975). The funnel plot shows there is little evidence of publication bias that could cause skew in the distribution of residuals (Fig S3). A trim and fill analysis was used to more formally show that any bias that was present did not have any effect on the results interpretation (31 studies added, 0.0075 increase in effect size, $z = 0.2171$, $p = 0.8281$).

Additionally, to assess the impact of any observed bias on our results, we conducted a cumulative meta-analysis, whereby studies are cumulatively added to the analysis in the order of increasing sampling variance (Fig S4). This qualitatively shows how quickly the mean estimate stabilizes with the addition of evidence and whether the final estimate with all data included is strongly affected by the less variable studies. The cumulative meta-analysis shows that the slope estimate stabilizes quickly with increasing sample size, and that the slope estimate becomes slightly more negative with the final exclusion of effect sizes with the highest sampling variance (Fig S4).

Despite accounting for sampling and hierarchical dependencies in our dataset, as described in the methods section, a potential source of non-independence still existed among these data. In some cases, a focal species (i.e. control) occurred within multiple studies. The dependency among multiple effect sizes for a single focal species within a given study were accounted for as sampling dependency in our model. But this did not account for any dependency that may exist among multiple effect sizes for a single focal species across more than one study. To assess how sensitive our results were to this, we conducted a subset analysis, which was reported in the main text. We also reported a second subset analysis that accounted for disproportionate representation across the range of phylogenetic distance values. The methods for these subset analyses are reported in detail in the paper. Overall, these model checking procedures show that we can be confident in our estimate of the relationship between phylogenetic distance and negative plant-soil feedbacks.

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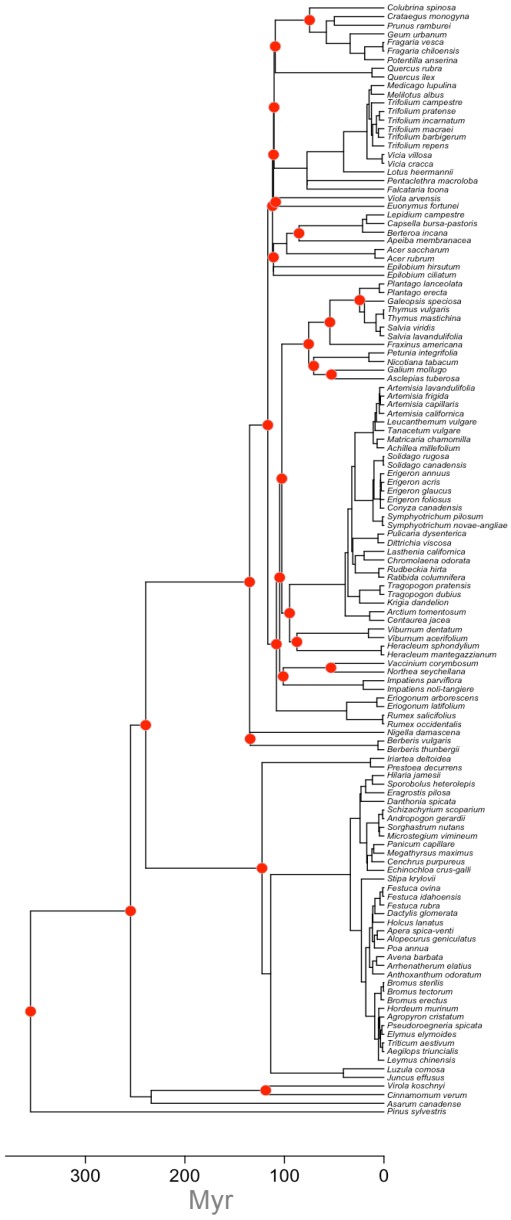


Fig S1 Dated maximum likelihood estimate of the phylogenetic relationships between the species used in the meta-analysis. Red dots represent the congruent nodes with the Tank *et al.* 2013 chronogram used for time scaling the estimate.

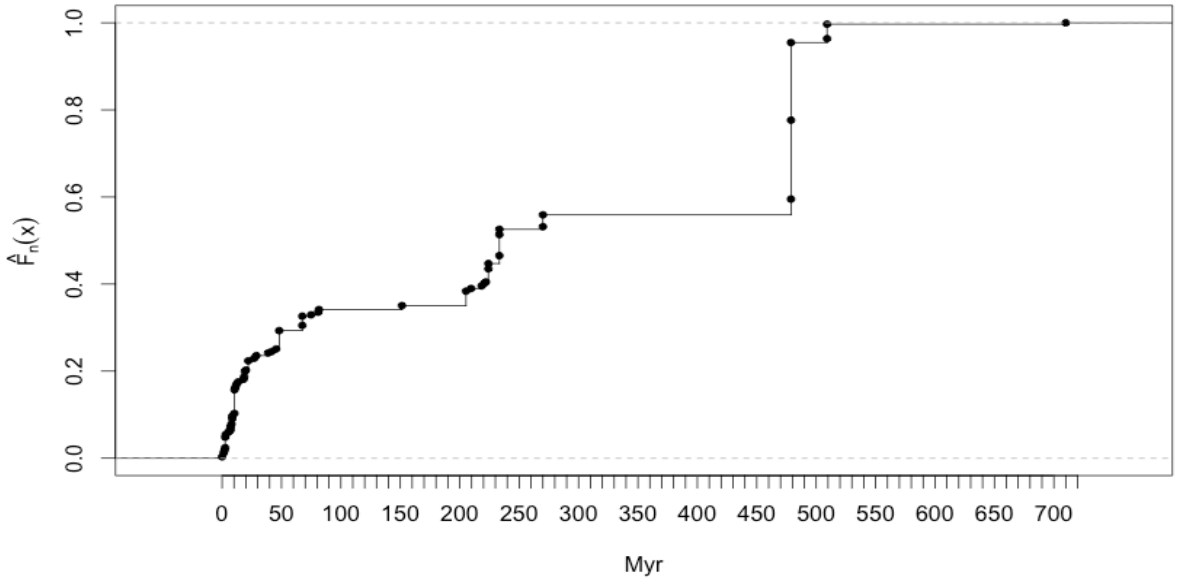


Fig S2 Cumulative distribution of the phylogenetic distances between species interacting in our study.

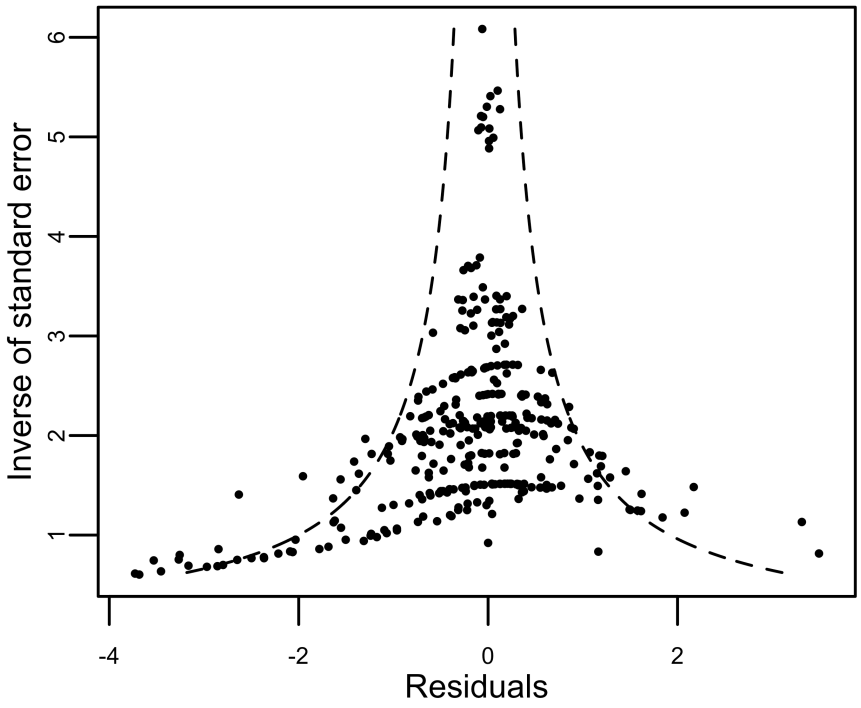


Fig S3. Funnel plot showing model residuals vs precision (inverse sampling variance).

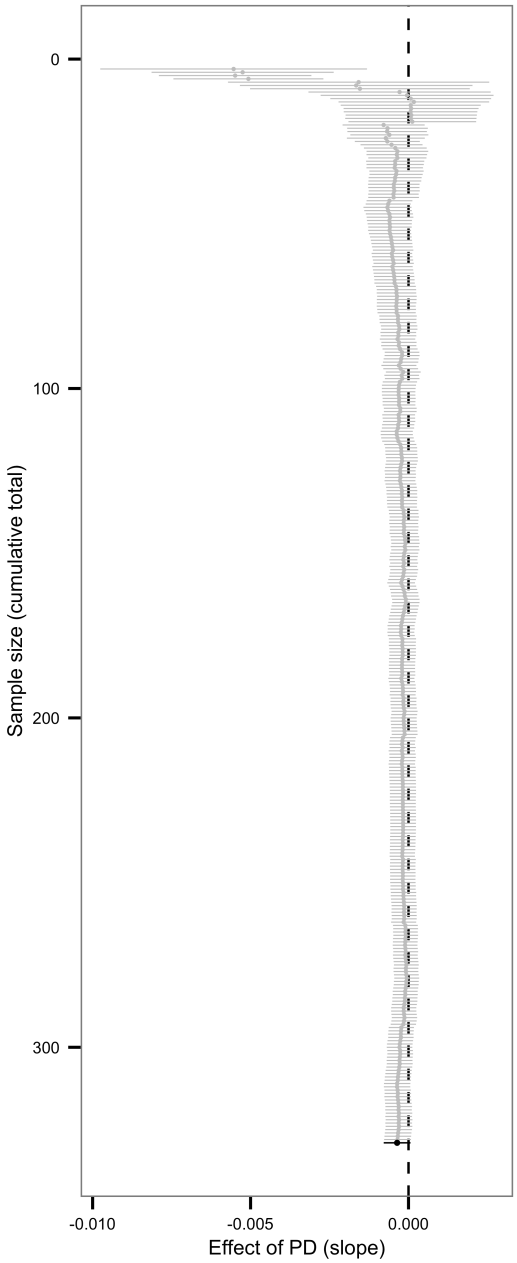


Fig S4. Forest plot of a cumulative meta-analysis of data sorted by increasing sampling variance.

Dated maximum likelihood estimate of the phylogenetic relationships between the species used in the meta-analysis:

(Pinus˙sylvestris:355.000003,((Asarum˙canadense:233.854429,(Cinnamomum˙verum:118.800003,Virola˙koschnyi:118.800003):115.054426):20.710305,(((((Juncus˙effusus:40.741861,Luzula˙comosa:40.741861):72.997038,(((((Leymus˙chinensis:5.603769,((((Aegilops˙triuncialis:1.470434,Triticum˙aestivum:1.470434):2.019767,(Elymus˙elymoides:2.102293,Pseudoroegneria˙spicata:2.102293):1.387909):0.793814,Agropyron˙cristatum:4.284015):0.954575,Hordeum˙murinum:5.238590):0.365179):3.679287,(Bromus˙erectus:2.913028,(Bromus˙tectorum:0.511798,Bromus˙sterilis:0.511798):2.401230):6.370028):8.975635,((Anthoxanthum˙odoratum:11.291884,(Arrhenatherum˙elatius:7.351406,Avena˙barbata:7.351406):3.940477):3.133919,((Poa˙annua:9.613753,(Alopecurus˙geniculatus:6.742626,Apera˙spica-venti:6.742626):2.871127):2.018881,(Holcus˙lanatus:11.211033,(Dactylis˙glomerata:9.124505,(Festuca˙rubra:3.921101,(Festuca˙idahoensis:1.238518,Festuca˙ovina:1.238518):2.682583):5.203404):2.086528):0.421601):2.793169):3.832888):4.446605,Stipa˙krylovii:22.705296):11.051309,(((Echinochloa˙crus-galli:16.700300,(Cenchrus˙purpureus:12.125506,(Megathyrsus˙maximus:10.236142,Panicum˙capillare:10.236142):1.889365):4.574793):0.578962,((Microstegium˙vimineum:4.574397,Sorghastrum˙nutans:4.574397):0.674239,(Andropogon˙gerardii:1.311339,Schizachyrium˙scoparium:1.311339):3.937297):12.030626):6.761972,(Danthonia˙spicata:23.077463,(Eragrostis˙pilosa:18.392257,(Sporobolus˙heterolepis:11.622029,Hilaria˙jamesii:11.622029):6.770228):4.685206):0.963771):9.715371):79.982293):8.806196,(Prestoea˙decurrens:13.523993,Iriartea˙deltoidea:13.523993):109.021101):116.847923,(((Berberis˙thunbergii:6.028313,Berberis˙vulgaris:6.028313):128.371690,Nigella˙damascena:134.400003):0.600000,(((((Rumex˙occidentalis:1.288728,Rumex˙salicifolius:1.288728):36.161516,(Eriogonum˙latifolium:7.110080,Eriogonum˙arborescens:7.110080):30.340164):70.623823,(((Impatiens˙noli-tangiere:20.802140,Impatiens˙parviflora:20.802140):80.397862,(Northea˙seychellana:53.298816,Vaccinium˙corymbosum:53.298816):47.901186):3.608026,((((Heracleum˙mantegazzianum:2.763919,Heracleum˙sphondylium:2.763919):84.681567,(Viburnum˙acerifolium:15.619121,Viburnum˙dentatum:15.619121):71.826365):7.380879,((Centaurea˙jacea:14.298099,Arctium˙tomentosum:14.298099):24.685550,((Krigia˙dandelion:24.412945,(Tragopogon˙dubius:3.963624,Tragopogon˙pratensis:3.963624):20.449321):11.777234,((((Ratibida˙columnifera:5.180634,Rudbeckia˙hirta:5.180634):23.690645,(Chromolaena˙odorata:19.940654,Lasthenia˙californica:19.940654):8.930626):2.457600,(Dittrichia˙viscosa:6.005039,Pulicaria˙dysenterica:6.005039):25.323840):1.011602,(((Symphyotrichum˙novae-angliae:1.542719,Symphyotrichum˙pilosum:1.542719):9.469874,((Conyza˙canadensis:3.524354,(Erigeron˙foliosus:3.523909,((Erigeron˙glaucus:2.808987,Erigeron˙acris:2.808987):0.585645,Erigeron˙annuus:3.394632):0.129277):0.000445):6.499826,(Solidago˙canadensis:0.666282,Solidago˙rugosa:0.666282):9.357898):0.988413):18.163328,((Achillea˙millefolium:6.864792,Matricaria˙chamomilla:6.864792):3.929091,(Tanacetum˙vulgare:8.943351,(Leucanthemum˙vulgare:7.488468,(Artemisia˙californica:4.706823,((Artemisia˙capillaris:3.557142,Artemisia˙frigida:3.557142):0.687609,Artemisia˙lavandulifolia:4.244751):0.462072):2.781645):1.454883):1.850532):18.382038):3.164560):3.849697):2.793470):55.842716):7.818208,(((Asclepias˙tuberosa:52.768692,Galium˙mollugo:52.768692):17.801215,(Nicotiana˙tabacum:15.145322,Petunia˙integrifolia:15.145322):55.424585):5.111158,(Fraxinus˙americana:54.300002,((((Salvia˙lavandulifolia:3.669413,Salvia˙viridis:3.669413):4.315772,(Thymus˙mastichina:0.374960,Thymus˙vulgaris:0.374960):7.610225):11.620476,Galeopsis˙speciosa:19.605661):4.803086,(Plantago˙erecta:4.689277,Plantago˙lanceolata:4.689277):19.719470):29.891255):21.381063):26.963508):2.163455):3.266039):8.615715,(((Epilobium˙ciliatum:111.013533,Epilobium˙hirsutum:111.013533):0.000965,((Acer˙rubrum:9.053574,Acer˙saccharum:9.053574):88.605415,(Apeiba˙membranacea:85.352841,(Berteroa˙incana:21.515929,(Capsella˙bursa-pastoris:17.393603,Lepidium˙campestre:17.393603):4.122326):63.836912):12.306149):13.355508):1.033116,((Euonymus˙fortunei:108.812514,Viola˙arvensis:108.812514):2.135315,(((Falcataria˙toona:77.326307,Pentaclethra˙macroloba:77.326307):0.001301,(Lotus˙heermannii:40.472457,((Vicia˙cracca:1.859407,Vicia˙villosa:1.859407):15.205797,(((Trifolium˙repens:11.437674,((Trifolium˙barbigerum:5.594074,Trifolium˙macraei:5.594074):1.977904,(Trifolium˙incarnatum:4.513454,Trifolium˙pratense:4.513454):3.058525):3.865696):1.137381,Trifolium˙campestre:12.575056):2.397932,(Melilotus˙albus:12.770785,Medicago˙lupulina:12.770785):2.202203):2.092216):23.407253):36.855151):33.057681,((Quercus˙ilex:12.030752,Quercus˙rubra:12.030752):97.206891,((((Potentilla˙anserina:19.378535,(Fragaria˙chiloensis:1.032365,Fragaria˙vesca:1.032365):18.346170):14.611685,Geum˙urbanum:33.990220):23.926438,(Prunus˙ramburei:49.857547,Crataegus˙monogyna:49.857547):8.059111):16.808578,Colubrina˙spinosa:74.725236):34.512407):1.147647):0.562539):1.099785):4.642168):18.310221):104.393014):15.171717):100.435269);

Maximum likelihood estimate of the phylogenetic relationships between the species used in the meta-analysis:

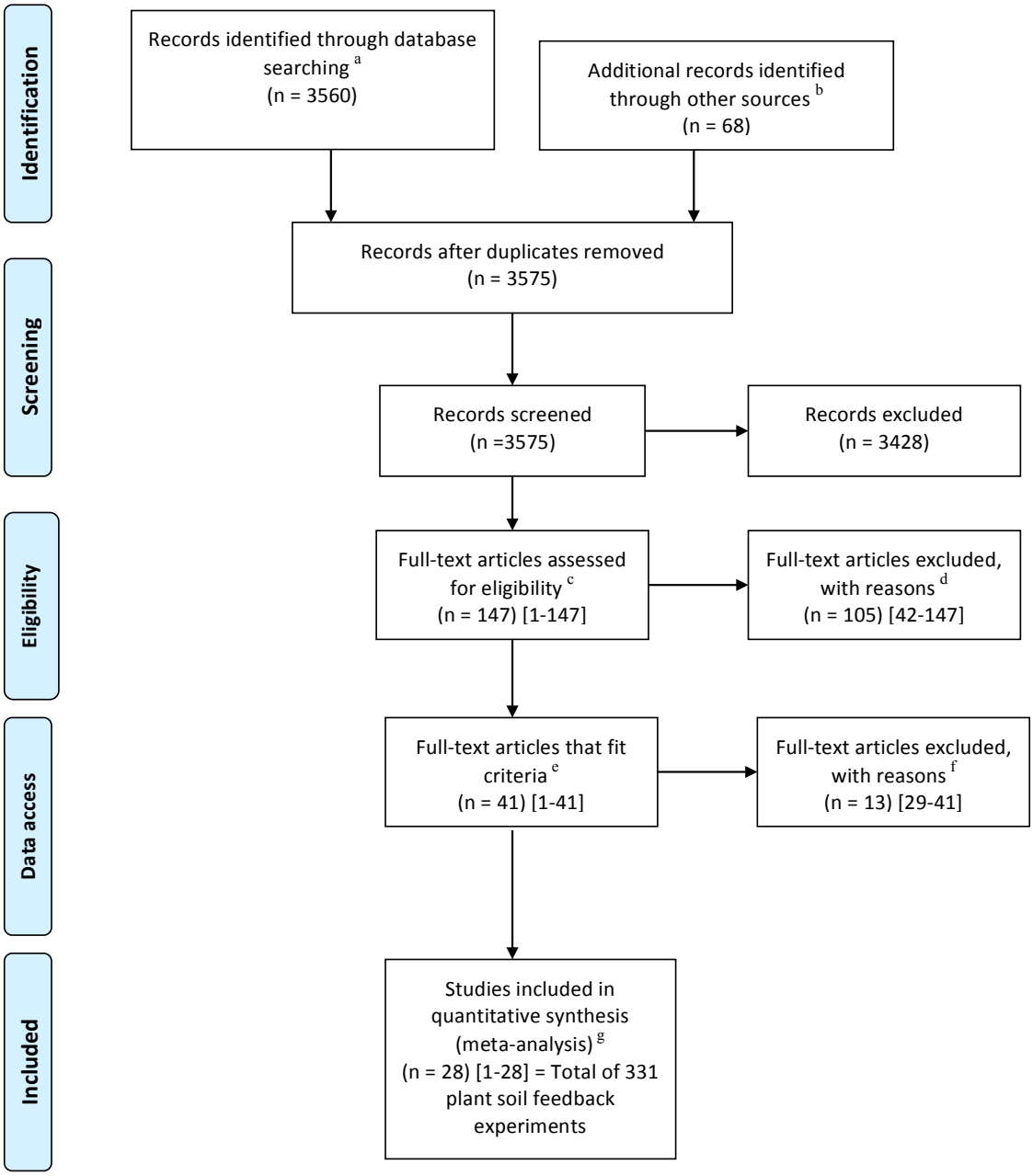
(Pinus˙sylvestris:0.32213026542687833720,((Asarum˙canadense:0.12977924918884586880,(Cinnamomum˙verum:0.10628208641283407032,Virola˙koschnyi:0.09253775929944123291):0.00222575607904970142):0.0078253584836
1852818,(((((Juncus˙effusus:0.06586515227387866600,Luzula˙comosa:0.14556941143005497352):0.21297147990144668639,(((((Leymus˙chinensis:0.00526753078346379761,((((Aegilops˙triuncialis:0.00584827736168993736,Triticum˙aestivum:0.00244035015143775902):0.00698979284609705428,(Elymus˙elymoides:0.00612400283362341327,Pseudoroegneria˙spicata:0.00157436582838840137):0.00211657523670111865):0.00224293221731844666,Agropyron˙cristatum:0.00649230899988716137):0.00210066289714281066,Hordeum˙murinum:0.01346214466906143874):0.00095738420259035696):0.00533201479708476228,(Bromus˙erectus:0.00414025361620720418,(Bromus˙tectorum:0.00000121759078656250,Bromus˙sterilis:0.00277839703847255299):0.00780180317241016552):0.01591920173246611642):0.01622324014374460985,((Anthoxanthum˙odoratum:0.03509116574158567536,(Arrhenatherum˙elatius:0.01650086165773596153,Avena˙barbata:0.01781057305203816860):0.00921475997599908309):0.00967168854313531917,((Poa˙annua:0.03183408122328515444,(Alopecurus˙geniculatus:0.01197588456533552233,Apera˙spica-venti:0.01342410173893746408):0.00425794467309277558):0.00495763757585125728,(Holcus˙lanatus:0.023038431356166625555,(Dactylis˙glomerata:0.03174220400162158090,(Festuca˙rubra:0.01141441338879397444,(Festuca˙idahoensis:0.00273050261805742045,Festuca˙ovina:0.00253300083107704582):0.00498928188654905978):0.01233855538535651164):0.00712813979136584560):0.00129973770434853547):0.00886356521068877926):0.01490069106130058850):0.01527017092482388198,Stipa˙krylovii:0.01776184205868321792):0.02379330408604292402,(((Echinochloa˙crus-galli:0.02489133942686000411,(Cenchrus˙purpureus:0.02872582884821733157,(Megathyrsus˙maximus:0.02064164195721852890,Panicum˙capillare:0.02017718414854716494):0.00363250913541478433):0.01009296364627991840):0.00095966279653845207,((Microstegium˙vimineum:0.01293676843344941825,Sorghastrum˙nutans:0.00739084986837351514):0.00157988037679002396,(Andropogon˙gerardii:0.00218322586450078130,Schizachyrium˙scoparium:0.00279728202210897687):0.00675512864622056577):0.02368372141913311843):0.01057262791745271387,(Danthonia˙spicata:0.05757949495051500194,(Eragrostis˙pilosa:0.05116209215386953446,(Sporobolus˙heterolepis:0.02554981359771279353,Hilaria˙jamesii:0.03422184251822954493):0.01877127201699509834):0.01486489848509208941):0.00320411600466089137):0.02613549823833080654):0.21600417636069910166):0.02818751848388810141,(Prestoea˙decurrens:0.00966177064300136404,Iriartea˙deltoidea:0.00992928456333385528):0.06590996608008292679):0.09566088147122847607,(((Berberis˙thunbergii:0.01006741846832449015,Berberis˙vulgaris:0.00301906612664218192):0.13355985815828849694,Nigella˙damascena:0.13553454074539725283):0.04404175987723878832,((((Rumex˙occidentalis:0.00517703459295458177,Rumex˙salicifolius:0.00000121759078656250):0.07632544873607428892,(Eriogonum˙latifolium:0.02140202139950753493,Eriogonum˙arborescens:0.00638477630436974034):0.06108981478707040808):0.15521597515138549350,(((Impatiens˙noli-tangiere:0.03463034214709165975,Impatiens˙parviflora:0.03804839327588512010):0.14245285209005612015,(Nothea˙seychellana:0.05158891265134109977,Vaccinium˙corymbosum:0.15701904258481777510):0.00651228315040650822):0.02994923340572357845,((((Heracleum˙mantegazzianum:0.00427201702770924497,Heracleum˙sphondylium:0.00373497880178923431):0.12846305834988860917,(Viburnum˙acerifolium:0.01784179328690416871,Viburnum˙dentatum:0.00729244851824300665):0.04638587398748107021):0.00475667148885318154,((Centaurea˙jacea:0.01572566019636864251,Arctium˙tomentosum:0.01711731446063117709):0.02308405922514601694,((Krigia˙dandelion:0.03840180959631080138,(Tragopogon˙dubius:0.00443410162609179665,Tragopogon˙pratensis:0.00731234614942662640):0.02909838841551821292):0.01709199946977944806,((((Ratibida˙columnifera:0.01155571374285673143,Rudbeckia˙hirta:0.00411613347777488835):0.03101486287808044931,(Chromolaena˙odorata:0.05949347056187207633,Lasthenia˙californica:0.04239407225368479332):0.02748599404708530758):0.00618167354472999673,(Dittrichia˙viscosa:0.01010980854636265268,Pulicaria˙dysenterica:0.00740155157547509653):0.03265891750747551558):0.00209787739865353916,(((Symphyotrichum˙novae-angliae:0.00248263582855599504,Symphyotrichum˙pilosum:0.00173576092040357718):0.01005534086599297293,((Conyza˙canadensis:0.00421351706832305637,(Erigeron˙foliosus:0.01018128261578297999,((Erigeron˙glaucus:0.00787651879389207436,Erigeron˙acris:0.00442574831872430147):0.00145987386830988991,Erigeron˙annuus:0.00779935570796799673):0.00037525140792042270):0.00000121759078656250):0.02116456474964482604,(Solidago˙canadensis:0.00156573101018169045,Solidago˙rugosa:0.00032246088300514811):0.01042379721708582181):0.00255059292935409625):0.03494356168538760576,((Achillea˙millefolium:0.00830567188825046682,Matricaria˙chamomilla:0.00643110107968558001):0.00221062803092605172,(Tanacetum˙vulgare:0.00682594783189597286,(Leucanthemum˙vulgare:0.01445392519691918350,(Artemisia˙californica:0.01143397989749915002,((Artemisia˙capillaris:0.01096394088332167463,Artemisia˙frigida:0.008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lavandulifolia:0.00995112919896242246,Salvia˙viridis:0.01580674650968375353):0.01752072476565291054,(Thymus˙mastichina:0.00000121759078656250,Thymus˙vulgaris:0.00226152573367727292):0.02367305158845313742):0.04901971349840823777,Galeopsis˙speciosa:0.07738321144354361192):0.02489366139990721941,(Plantago˙erecta:0.03133953620881742286,Plantago˙lanceolata:0.01989373279157692284):0.15472494211856788193):0.03864305293609453240):0.03995876159265958349):0.03127775041134420869):0.00996282847692414771):0.01658307713452714105):0.00670314581176742127,(((Epilobium˙ciliatum:0.19784067141940361867,Epilobium˙hirsutum:0.198437904396908088802):0.00000121759078656250,((Acer˙rubrum:0.01204990955628624898,Acer˙saccharum:0.01410805316704323055):0.12132520028883241048,(Apeiba˙membranacea:0.09371268372945443237,(Berteroa˙incana:0.05406273453960236397,(Capsella˙bursa-pastoris:0.05308994425691842406,Lepidium˙campestre:0.02452313587576922249):0.00992817085114541288):0.18186599245491508192):0.01170026422741633765):0.00964779426079350952):0.01375474494090014356,((Euonymus˙fortunei:0.14762068778740003450,Viola˙arvensis:0.20354780971984476334):0.01858317722666714272,(((Falcataria˙toona:0.15070839398308125134,Pentaclethra˙macroloba:0.06349209283492236733):0.00000121759078656250,(Lotus˙heermannii:0.08718496972463185557,((Vicia˙cracca:0.00335913640009771189,Vicia˙villosa:0.00402881742835618176):0.03025394594397185985,(((Trifolium˙repens:0.01597959368532626476,((Trifolium˙barbigerum:0.01240175997640783484,Trifolium˙macraei:0.01528879295002154651):0.00554070167174680085,(Trifolium˙incarnatum:0.00787687711949547516,Trifolium˙pratense:0.01312419018731169652):0.00770740316659787768):0.01227249635864337274):0.00283226887719888534,Trifolium˙campestre:0.02881535259290526549):0.00648441117259541608,(Melilotus˙albus:0.01945699329626629850,Medicago˙lupulina:0.02676902996887487937):0.00364688209468837015):0.00489211821347563820):0.05438031693535800432):0.09065159030589445277):0.06691658971707929726,((Quercus˙ilex:0.02121509266570396207,Quercus˙rubra:0.00972811568300206361):0.11614727691895858352,((((Potentilla˙anserina:0.03991122109523816130,(Fragaria˙chiloensis:0.00140779171299708458,Fragaria˙vesca:0.00189504401685503779):0.02773006027370326920):0.02705509239814734429,Geum˙urbanum:0.06562137405034371151):0.04880502759925360590,(Prunus˙ramburei:0.05917347306037082366,Crataegus˙monogyna:0.08066819090647728285):0.01082514393903179564):0.02962739479638018189,Colubrina˙spinosa:0.14384695176590930887):0.01840986144625579926):0.00251096089310159833):0.01307240038453201698):0.00698079014232880039):0.02731669681736727251):0.05203763978165305143):0.03891278893992729249):0.00000121759078656250):0.35847901717545627154);

Supporting Information Notes S1

PRISMA statement

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement



PRISMA Footnotes

[a] All published work on ISI Web of Science for the last 20 years (January 1993-May 2013) were searched using 3 topic-level searches: soil* feedback* phylogeny; soil* feedback* experiment*; and plant* feedback* soil*.

[b] All plant soil feedback reviews published in the last 10 years were downloaded and references screened. Principle investigators of all main plant-soil feedback labs around the world were personally emailed and asked to contribute unpublished work or work in press.

[c] The eligibility study selection criterion was that data present the response of species grown in monoculture on soil cultured from a conspecific versus soil cultured from a heterospecific species. The response on each soil can be biomass, survival, growth or germination. [d] Any studies that did not fit this criteria were dismissed, except one study [28] which presented responses of species grown on soils cultured by its own plant family versus soil cultured by a different plant family.

[e] If data on means and SD that was readily accessible from figures this was retrieved. If data was not readily accessible authors were contacted directly to contribute their raw data. Data collection was coded to include information on plant-soil interactions between each specific plant species pair.

[f] 12 studies authors did not respond, or responded but in the end did not share the data. One study [30] was dismissed because it did not include soil culturing, but leaf litter based feedbacks.

[g] At this stage it was decided to focus only on biomass responses due to lack of survivorship, germination or growth data.

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5. Callaway RM, Thelen GC, Rodriguez A, Holben WE. 2004. Soil biota and exotic plant invasion. *Nature* 427: 731–3.

6. Casper, BB. & Castelli, JP. 2007. Evaluating plant-soil feedback together with competition in a serpentine grassland. *Ecology Letters* 10: 394–400.

7. Ehlers, BK. & Thompson, J. 2004. Do co-occurring plant species adapt to one another? The response of Bromus erectus to the presence of different Thymus vulgaris chemotypes. *Oecologia* 141: 511–8.

8. Gustafson, D. & Casper, B. 2004. Nutrient addition affects AM fungal performance and expression of plant/fungal feedback in three serpentine grasses. *Plant Soil* 259: 9–17.

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10. Puerta-Piñero, C., Gómez, J. M. & Zamora, R. 2006. Species-specific effects on topsoil development affect Quercus ilex seedling performance. *Acta Oecologica* 29: 65–71.

11. Van Grunsven, RHA. *et al.* 2007. Reduced plant?soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology* 95: 1050–1057.

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13. Dostál, P. & Palečková, M. 2010. Does relatedness of natives used for soil conditioning influence plant-soil feedback of exotics? *Biological Invasions* 13: 331–340.

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```
   #Supporting Information Notes S2
   # Relatedness is a poor predictor of plant-soil feedbacks
   # Zia Mehrabi and Sean Tuck
   #####

   ##### Load and prepare the data
   library(metahdep)
   library(ggplot2)
   library(meta)
   library(xlsx)

   setwd('/Users/sean/Dropbox/PhD/PlantSoilMeta')   # University computer
   setwd('~/Dropbox/PhD/PlantSoilMeta')             # Home mac

   ps.meta.dat      <-      read.xlsx('SupportingInformationTableS1.xlsx',
sheetIndex = 1)
   # Order the dataset by study name, to group rows for the same studies
together
   ps.meta.dat <- ps.meta.dat[order(ps.meta.dat$Ref), ]
   # Remove one row, experiment 178, because it causes p.e to divide by 0
and creates an Inf in d.samp.var
   ps.meta.dat <- ps.meta.dat[-which(ps.meta.dat$Experiment == 178), ]
   # Remove the 1 vine life form record. n=1 not much good for estimate
pars, and means fitting a model with PD*life.form won't fit.
   ps.meta.dat <- ps.meta.dat[-which(ps.meta.dat$life.form == "vine"), ]
   #  Remove  the  vine  level.  Easily  done  by  converting  to  and  from
character class
   ps.meta.dat$life.form <- as.character(ps.meta.dat$life.form)
   ps.meta.dat$life.form <- as.factor(ps.meta.dat$life.form)

   # Calculate pooled standard deviation
   ps.meta.dat$pooled.sd <-
     with(ps.meta.dat,
          sqrt((((Naway - 1) * (Sdaway^2)) + ((Nhome - 1) * (Sdhome^2)))
/ (Naway + Nhome - 2)))

   # Calculate Hedge's d
   df.e <- with(ps.meta.dat, Naway + Nhome - 2)
   J <- with(ps.meta.dat, 1 - (3 / (4 * df.e - 1)))
   ps.meta.dat$hedges.d  <-  with(ps.meta.dat,  J  *  ((Xhome  -  Xaway)  /
pooled.sd))

   # Calculate Hedge's d sampling variance
   p.e <- J^2 * (df.e / (df.e - 2))
   ps.meta.dat$d.samp.var <- with(ps.meta.dat,
```

```
                                    (p.e * ((1 / Naway) + (1 / Nhome))) +
((p.e - 1) * hedges.d^2))

   # Calculate var-cov matrix, with sampling dependence
   V    <-    matrix(0,    nrow    =    length(ps.meta.dat$d),    ncol    =
length(ps.meta.dat$d))
   # Variance on diagonal
   diag(V) <- ps.meta.dat$d.samp.var

   #  Covariance  off-diagonal  block  matrices,  representing  sampling
dependence
   samp.deps <- list(NA)
   samp.deps.ID <- with(ps.meta.dat, paste(Ref, sp., sep = ""))
   samp.dep.groups <- names(which(table(samp.deps.ID) > 1))
   for(i in 1:length(samp.dep.groups)){
     samp.deps[[i]] <- which(samp.deps.ID == samp.dep.groups[i])
   }
   for(i in 1:length(samp.deps)){                # Loop for each sampling
dependence grouping
     for(n  in  1:length(samp.deps[[i]])  -  1){         #  Loop  for  each
observation within the grouping.
       for(m  in  n  +  1:length(samp.deps[[i]])){      #  Loop  for  each
combination needed for each n.
         V[samp.deps[[i]][n],samp.deps[[i]][m]]                         <-
V[samp.deps[[i]][m],samp.deps[[i]][n]] <-
           with(ps.meta.dat, (p.e[n] - 1) * hedges.d[samp.deps[[i]][n]] *
hedges.d[samp.deps[[i]][m]])
       }
     }
   }

   # Create hierarchical dependence groups for data.
   hierarch.deps <- list(NA)
   dep.names <- names(which(table(ps.meta.dat$Ref) > 1))
   for(i in 1:length(dep.names)){
     hierarch.deps[[i]] <- which(ps.meta.dat$Ref == dep.names[i])
   }
   M <- get.M(length(ps.meta.dat$hedges.d), hierarch.deps)
   #####



   ##### Analyse the data
   # Proportion of effect sizes that show negative plant-soil feedback
   with(ps.meta.dat, (sum(hedges.d < 0) / length(hedges.d)) * 100)

   # Intercept model, with dependencies accounted for
   ps.int.X <- model.matrix(~ 1, data = ps.meta.dat)
```

```
   ps.mod.int <- metahdep.HBLM(ps.meta.dat$hedges.d, V, ps.int.X, M,
delta.split = TRUE, two.sided = TRUE)
   grandmean.se <- sqrt(diag(ps.mod.int$cov.matrix))
   grandmean.ci <- cbind(lower = (ps.mod.int$beta.hats - (qt(0.975,
nrow(ps.meta.dat) - length(grandmean.se)) * grandmean.se)),
                         upper = (ps.mod.int$beta.hats + (qt(0.975,
nrow(ps.meta.dat) - length(grandmean.se)) * grandmean.se)))

   ### Effect of PD
   ###

   ## Zanne
   ps.Zanne.X <- model.matrix(~ PD_Soltis, data = ps.meta.dat)
   ps.mod.Zanne <- metahdep.HBLM(ps.meta.dat$hedges.d, V, ps.Zanne.X, M,
delta.split = TRUE, two.sided = TRUE)
   Zanne.se <- sqrt(diag(ps.mod.Zanne$cov.matrix))
   Zanne.ci <-
     cbind(lower = (ps.mod.Zanne$beta.hats - (qt(0.975, nrow(ps.meta.dat)
- length(Zanne.se)) * Zanne.se)),
           upper = (ps.mod.Zanne$beta.hats + (qt(0.975, nrow(ps.meta.dat)
- length(Zanne.se)) * Zanne.se)))

   png(filename = 'EffectofPD_Zanne.png', width = 14, height = 14, units
= 'cm', res = 800, pointsize = 12)
   ggplot(ps.meta.dat, aes(PD_Soltis, hedges.d)) +
     geom_point(aes(alpha = 1)) +
     #geom_abline(intercept = ps.mod.Zanne$beta.hats[1], slope =
ps.mod.Zanne$beta.hats[2]) +
     geom_smooth(method = "lm", fill = "grey", colour = "blue") +
     xlab("Phylogenetic distance (myr)") + ylab("Plant-soil feedback
(Hedges' d)") +
     theme_bw() + theme(legend.position = "none", text =
element_text(size = 14),
                        panel.background = element_blank(),
panel.grid.major = element_blank(),
                        panel.grid.minor = element_blank())
   dev.off()

   # Model stats and checking: compute I^2, the proportion of total
variance that is true variance, tau^2.
   s.sq <- with(ps.meta.dat, sum((1/d.samp.var) * (length(d.samp.var) -
1)) / (sum(1/d.samp.var)^2 - sum((1/d.samp.var)^2)))
   tau.sq <- ps.mod.Zanne$tau.hat^2 + ps.mod.Zanne$tau.var
   H.sq <- (tau.sq + s.sq) / s.sq
   I.sq <- (H.sq - 1) / H.sq
   ##
```

```
   ## Bell
   ps.Bell.X <- model.matrix(~ PD_Bell, data = ps.meta.dat)
   ps.mod.Bell <- metahdep.HBLM(ps.meta.dat$hedges.d, V, ps.Bell.X, M,
delta.split = TRUE, two.sided = TRUE)
   Bell.se <- sqrt(diag(ps.mod.Bell$cov.matrix))
   Bell.ci <- cbind(lower = (ps.mod.Bell$beta.hats - (qt(0.975,
nrow(ps.meta.dat) - length(Bell.se)) * Bell.se)),
                    upper = (ps.mod.Bell$beta.hats + (qt(0.975,
nrow(ps.meta.dat) - length(Bell.se)) * Bell.se)))

   png(filename = 'EffectofPD_Bell.png', width = 14, height = 10, units =
'cm', res = 800, pointsize = 12)
   ggplot(ps.meta.dat, aes(PD_Bell, hedges.d)) +
     geom_point(aes(alpha = 1)) +
     #geom_abline(intercept = ps.mod.Bell$beta.hats[1], slope =
ps.mod.Bell$beta.hats[2]) +
     geom_smooth(method = "lm", fill = "grey", colour = "blue") +
     xlab("Phylogenetic distance (myr)") + ylab("Plant-soil feedback
(Hedges' d)") +
     theme_bw() + theme(legend.position = "none", text =
element_text(size = 14),
                        panel.background = element_blank(),
panel.grid.major = element_blank(),
                        panel.grid.minor = element_blank())
   dev.off()
   ##

   ###
   ###

   ### Taxa subset analyses
   ###

   ## grasses
   grasses <- subset(ps.meta.dat, family == "Poaceae" & family_culture ==
"Poaceae")

   g.V <- matrix(0, nrow = length(grasses$d), ncol = length(grasses$d))
   diag(g.V) <- grasses$d.samp.var

   samp.deps <- list(NA)
   samp.deps.ID <- with(grasses, paste(Ref, sp., sep = ""))
   samp.dep.groups <- names(which(table(samp.deps.ID) > 1))
   for(i in 1:length(samp.dep.groups)) samp.deps[[i]] <-
which(samp.deps.ID == samp.dep.groups[i])
   for(i in 1:length(samp.deps)){ # Loop for each sampling
dependence grouping
```

```
     for(n in 1:length(samp.deps[[i]]) - 1){         # Loop for each
observation within the grouping.
       for(m in n + 1:length(samp.deps[[i]])){      # Loop for each
combination needed for each n.
         g.V[samp.deps[[i]][n],samp.deps[[i]][m]]                    <-
g.V[samp.deps[[i]][m],samp.deps[[i]][n]] <-
           with(grasses, (p.e[n] - 1) * hedges.d[samp.deps[[i]][n]] *
hedges.d[samp.deps[[i]][m]])
       }
     }
   }

   hierarch.deps <- list(NA)
   dep.names <- names(which(table(grasses$Ref) > 1))
   for(i in 1:length(dep.names)) hierarch.deps[[i]] <- which(grasses$Ref
== dep.names[i])
   g.M <- get.M(length(grasses$hedges.d), hierarch.deps)

   grass.X <- model.matrix(~ PD_Soltis, data = grasses)
   ps.mod.grass <- metahdep.HBLM(grasses$hedges.d, g.V, grass.X, g.M,
delta.split = TRUE, two.sided = TRUE)
   grass.se <- sqrt(diag(ps.mod.grass$cov.matrix))
   grass.ci <- cbind(lower = (ps.mod.grass$beta.hats - (qt(0.975,
nrow(grasses) - length(grass.se)) * grass.se)),
                     upper = (ps.mod.grass$beta.hats + (qt(0.975,
nrow(grasses) - length(grass.se)) * grass.se)))

   png(filename = 'EffectofPD_Grass.png', width = 10, height = 10, units
= 'cm', res = 800, pointsize = 12)
   ggplot(grasses, aes(PD_Soltis, hedges.d)) +
     geom_point(aes(alpha = 1)) +
     #geom_abline(intercept = ps.mod.grass$beta.hats[1], slope =
ps.mod.grass$beta.hats[2]) +
     geom_smooth(method = "lm", fill = "grey", colour = "blue") +
     xlab("Phylogenetic distance (myr)") + ylab("Plant-soil feedback
(Hedges' d)") +
     theme_bw() + theme(legend.position = "none", text =
element_text(size = 14),
                        panel.background = element_blank(),
panel.grid.major = element_blank(),
                        panel.grid.minor = element_blank())
   dev.off()
   ##

   ## Asteraceae
   aster <- subset(ps.meta.dat, family == "Asteraceae" & family_culture
== "Asteraceae")
```

```
   a.V <- matrix(0, nrow = length(aster$d), ncol = length(aster$d))
   diag(a.V) <- aster$d.samp.var

   samp.deps <- list(NA)
   samp.deps.ID <- with(aster, paste(Ref, sp., sep = ""))
   samp.dep.groups <- names(which(table(samp.deps.ID) > 1))
   for(i     in     1:length(samp.dep.groups))     samp.deps[[i]]     <-
which(samp.deps.ID == samp.dep.groups[i])
   for(i in 1:length(samp.deps)){               # Loop for each sampling
dependence grouping
     for(n  in  1:length(samp.deps[[i]])  -  1){         #  Loop  for  each
observation within the grouping.
       for(m  in  n  +  1:length(samp.deps[[i]])){      #  Loop  for  each
combination needed for each n.
         a.V[samp.deps[[i]][n],samp.deps[[i]][m]]                      <-
a.V[samp.deps[[i]][m],samp.deps[[i]][n]] <-
           with(aster,  (p.e[n]  -  1)  *  hedges.d[samp.deps[[i]][n]]  *
hedges.d[samp.deps[[i]][m]])
       }
     }
   }

   hierarch.deps <- list(NA)
   dep.names <- names(which(table(aster$Ref) > 1))
   for(i in 1:length(dep.names)) hierarch.deps[[i]] <- which(aster$Ref ==
dep.names[i])
   a.M <- get.M(length(aster$hedges.d), hierarch.deps)

   aster.X <- model.matrix(~ PD_Soltis, data = aster)
   ps.mod.aster  <-  metahdep.HBLM(aster$hedges.d,  a.V,  aster.X,  a.M,
delta.split = TRUE, two.sided = TRUE)
   aster.se <- sqrt(diag(ps.mod.aster$cov.matrix))
   aster.ci  <-  cbind(lower  =  (ps.mod.aster$beta.hats  -  (qt(0.975,
nrow(aster) - length(aster.se)) * aster.se)),
                       upper   =   (ps.mod.aster$beta.hats   +   (qt(0.975,
nrow(aster) - length(aster.se)) * aster.se)))

   png(filename = 'EffectofPD_Aster.png', width = 10, height = 10, units
= 'cm', res = 800, pointsize = 12)
   ggplot(aster, aes(PD_Soltis, hedges.d)) +
     geom_point(aes(alpha = 1)) +
     #geom_abline(intercept   =   ps.mod.aster$beta.hats[1],   slope   =
ps.mod.aster$beta.hats[2]) +
     geom_smooth(method = "lm", fill = "grey", colour = "blue") +
     xlab("Phylogenetic  distance  (myr)")  +  ylab("Plant-soil  feedback
(Hedges' d)") +
```

```
     theme_bw()    +    theme(legend.position    =    "none",    text    =
element_text(size = 14),
                         panel.background        =        element_blank(),
panel.grid.major = element_blank(),
                         panel.grid.minor = element_blank())
   dev.off()
   ##

   ###
   ###


   ### Estimate effects of other explanatory variables on the PD
relationship
   ###

   # Estimate life form and PD interaction
   ps.PDlifegrp.X  <-  model.matrix(~  life.form  *  PD_Soltis,  data  =
ps.meta.dat)
   ps.mod.PDlifegrp     <-     metahdep.HBLM(ps.meta.dat$hedges.d,     V,
ps.PDlifegrp.X, M, delta.split = TRUE, two.sided = TRUE)
   lifegrp.betahats     <-     with(ps.mod.PDlifegrp,     c(beta.hats[1],
beta.hats[2] + beta.hats[1], beta.hats[3] + beta.hats[1],
                                                  beta.hats[4]            +
beta.hats[1], beta.hats[5], beta.hats[6] + beta.hats[5],
                                                  beta.hats[7]            +
beta.hats[5], beta.hats[8] + beta.hats[5]))
   lifegrp.se <- sqrt(diag(ps.mod.PDlifegrp$cov.matrix))
   lifegrp.ci   <-   cbind(lower   =   (lifegrp.betahats   -   (qt(0.975,
nrow(ps.meta.dat)-length(lifegrp.se)) * lifegrp.se)),
                        upper    =    (lifegrp.betahats    +    (qt(0.975,
nrow(ps.meta.dat)-length(lifegrp.se)) * lifegrp.se)))

   # Estimate life cycle and PD interaction
   ps.PDlifecycle.X  <-  model.matrix(~  life.cycle  *  PD_Soltis,  data  =
ps.meta.dat)
   ps.mod.PDlifecycle    <-    metahdep.HBLM(ps.meta.dat$hedges.d,    V,
ps.PDlifecycle.X, M, delta.split = TRUE, two.sided = TRUE)
   lifecycle.betahats    <-    with(ps.mod.PDlifecycle,    c(beta.hats[1],
beta.hats[2] + beta.hats[1], beta.hats[3] + beta.hats[1],
                                                     beta.hats[4],
beta.hats[5] + beta.hats[4], beta.hats[6] + beta.hats[4]))
   lifecycle.se <- sqrt(diag(ps.mod.PDlifecycle$cov.matrix))
   lifecycle.ci  <-  cbind(lower  =  (lifecycle.betahats  -  (qt(0.975,
nrow(ps.meta.dat)-length(lifecycle.se)) * lifecycle.se)),
                         upper   =   (lifecycle.betahats   +   (qt(0.975,
nrow(ps.meta.dat)-length(lifecycle.se)) * lifecycle.se)))
```

```
   # Estimate between PD and experimental setting
   ps.PDexp.X <- model.matrix(~ Exp.setting * PD_Soltis, data =
ps.meta.dat)
   ps.mod.PDexp <- metahdep.HBLM(ps.meta.dat$hedges.d, V, ps.PDexp.X, M,
delta.split = TRUE, two.sided = TRUE)
   PDexp.betahats <- with(ps.mod.PDexp, c(beta.hats[1], beta.hats[1] +
beta.hats[2], beta.hats[1] + beta.hats[3],
                                          beta.hats[1] + beta.hats[4],
beta.hats[5], beta.hats[5] + beta.hats[6],
                                          beta.hats[5] + beta.hats[7],
beta.hats[5] + beta.hats[8]))
   PDexp.se <- sqrt(diag(ps.mod.PDexp$cov.matrix))
   PDexp.ci <- cbind(lower = (PDexp.betahats - (qt(0.975,
nrow(ps.meta.dat)-length(PDexp.se)) * PDexp.se)),
                     upper = (PDexp.betahats + (qt(0.975,
nrow(ps.meta.dat)-length(PDexp.se)) * PDexp.se)))

   # Estimate native/exotic
   ps.PDnatexo.X <- model.matrix(~ native.exotic.interaction * PD_Soltis,
data = ps.meta.dat)
   ps.mod.PDnatexo <- metahdep.HBLM(ps.meta.dat$hedges.d, V,
ps.PDnatexo.X, M, delta.split = TRUE, two.sided = TRUE)
   PDnatexo.betahats <- with(ps.mod.PDnatexo, c(beta.hats[1],
beta.hats[2] + beta.hats[1], beta.hats[3], beta.hats[4] + beta.hats[3]))
   PDnatexo.se <- sqrt(diag(ps.mod.PDnatexo$cov.matrix))
   PDnatexo.ci <- cbind(lower = (PDnatexo.betahats - (qt(0.975,
nrow(ps.meta.dat)-length(PDnatexo.se)) * PDnatexo.se)),
                        upper = (PDnatexo.betahats + (qt(0.975,
nrow(ps.meta.dat)-length(PDnatexo.se)) * PDnatexo.se)))

   # Estimate whole vs inoculum soil
   ps.PDsoil.X <- model.matrix(~ Soil * PD_Soltis, data = ps.meta.dat)
   ps.mod.PDsoil <- metahdep.HBLM(ps.meta.dat$hedges.d, V, ps.PDsoil.X,
M, delta.split = TRUE, two.sided = TRUE)
   PDsoil.betahats <- with(ps.mod.PDsoil, c(beta.hats[1], beta.hats[1] +
beta.hats[2], beta.hats[3], beta.hats[3] + beta.hats[4]))
   PDsoil.se <- sqrt(diag(ps.mod.PDsoil$cov.matrix))
   PDsoil.ci <- cbind(lower = (PDsoil.betahats - (qt(0.975,
nrow(ps.meta.dat)-length(PDsoil.se)) * PDsoil.se)),
                      upper = (PDsoil.betahats + (qt(0.975,
nrow(ps.meta.dat)-length(PDsoil.se)) * PDsoil.se)))

   # Include life form, life cycle, PD, native.exotic
   ps.full.X <- model.matrix(~ life.form + life.cycle +
native.exotic.interaction + PD_Soltis, data = ps.meta.dat)
```

```
   ps.mod.full <- metahdep.HBLM(ps.meta.dat$hedges.d, V, ps.full.X, M,
delta.split = TRUE, two.sided = TRUE)
   # Model checking
   # Model stats and checking. Compute I^2, the proportion of total
variance that is true variance, tau^2.
   s.sq <- with(ps.meta.dat, sum((1 / d.samp.var) * (length(d.samp.var) -
1)) /
                  (sum(1 / d.samp.var)^2 - sum((1 / d.samp.var)^2)))
   tau.sq <- ps.mod.full$tau.hat^2 + ps.mod.full$tau.var
   H.sq <- (tau.sq + s.sq) / s.sq
   I.sq <- (H.sq - 1) / H.sq
   #####


   # Calculate residuals from ps.mod.full.
   # Method for calculating BLUP from:
   # Henderson, C.R. (1975) Best linear unbiased estimation and
prediction under a selection model. Biometrics, 31(2), p.423-447.
   # With help from John Stevens to modify it to the metahdep case.
   I <- id(nrow(V))
   delta <- (tau.sq * I) + (ps.mod.full$varsigma.hat * M)
   psi <- V + delta
   delta.hat <- delta %*% solve(psi) %*% (ps.meta.dat$hedges.d -
(ps.full.X %*% ps.mod.full$beta.hats))
   theta.hat <- (ps.full.X %*% ps.mod.full$beta.hats) + delta.hat
   resids <- ps.meta.dat$hedges.d - theta.hat

   # Use resids to create funnel plot, with Egger's regression test of
funnel plot asymmetry, and a trim and fill
   # assessment of asymmetry impact.
   png(filename = 'FunnelPlot.png', width = 10, height = 10, units =
'cm', res = 800, pointsize = 12)
   funnel(x = resids, y = sqrt(ps.meta.dat$d.samp.var), level = 0.95,
yaxis = 'invse',
          xlab = 'Residuals', cex = 0.4, cex.lab = 0.7, cex.axis = 0.5,
mgp = c(1,0.4,0), pch = 20)
   dev.off()
   metabias(x = resids, seTE = ps.meta.dat$d.samp.var, method.bias =
'linreg')
   trimfill.default(x = resids, seTE = sqrt(ps.meta.dat$d.samp.var))

   # Cumulative meta-analysis function
   CumMeta <- function(data, data.order){
     cum.met <- c()
     cum.met.l <- c()
     cum.met.u <- c()
     ordered.data <- data[order(data.order), ]
```

```
     for(i in 3:nrow(data)){
       print(i)
       sample.data <- ordered.data[1:i, ]
       sample.theta <- sample.data$hedges.d
       sample.X <- model.matrix(~ 1 + PD_Soltis, data = sample.data)

       sample.V <- diag(sample.data$d.samp.var)
       sample.sampdeps <- list(NA)
       sample.sampdeps.ID <- with(sample.data, paste(Ref, sp., sep = ""))
       sample.sampdep.grps <- names(which(table(sample.sampdeps.ID) > 1))
       if(length(sample.sampdep.grps) >= 1){
         for(j in 1:length(sample.sampdep.grps)){
           sample.sampdeps[[j]]     <-     which(sample.sampdeps.ID     ==
sample.sampdep.grps[j])
         }
         for(x in 1:length(sample.sampdeps)){
           for(n in 1:length(sample.sampdeps[[x]]) - 1){
             for(m in n + 1:length(sample.sampdeps[[x]])){
               sample.V[sample.sampdeps[[x]][n],sample.sampdeps[[x]][m]]
<-

sample.V[sample.sampdeps[[x]][m],sample.sampdeps[[x]][n]] <-
                 with(sample.data,       (p.e[n]       -       1)       *
hedges.d[sample.sampdeps[[x]][n]] * hedges.d[sample.sampdeps[[x]][m]])
             }
           }
         }
       }

       if(!any(table(sample.data$Ref) > 1)){
         fit <- metahdep.HBLM(theta = sample.theta, V = sample.V, X =
sample.X)
         cum.met[i] <- fit$beta.hats
       } else {
         deps <- list(NA)
         dep.names <- names(which(table(sample.data$Ref) > 1))
         for(counter in 1:length(dep.names)){
           deps[[counter]]       <-       which(sample.data$Ref       ==
dep.names[counter])
         }
         sample.M <- get.M(length(sample.theta), deps)

         fit <- metahdep.HBLM(theta = sample.theta, V = sample.V, X =
sample.X, M = sample.M, delta.split = TRUE)
         cum.met[i] <- fit$beta.hats[2]
       }
```

```
       fit.se <- sqrt(diag(fit$cov.matrix))[2]
       cum.met.l[i] <- fit$beta.hats[2] - (qt(0.975, i-length(fit.se)) *
fit.se)
       cum.met.u[i] <- fit$beta.hats[2] + (qt(0.975, i-length(fit.se)) *
fit.se)
     }
     return(cbind(beta.hats = cum.met, lower = cum.met.l, upper =
cum.met.u))
   }

   # Run cumulative meta-analysis for sampling variance
   cum.meta.var <- CumMeta(ps.meta.dat, ps.meta.dat$d.samp.var)
   cum.meta.var[1,                          ]                          <-
ps.meta.dat$hedges.d[order(ps.meta.dat$d.samp.var)][1]
   cum.meta.var[2,                          ]                          <-
ps.meta.dat$hedges.d[order(ps.meta.dat$d.samp.var)][2]
   cum.meta.var <- rbind(data.frame(theta = cum.meta.var[1:328,1], lb =
cum.meta.var[1:328,2],
                                    ub = cum.meta.var[1:328,3], Type =
"Study"),
                         data.frame(theta = cum.meta.var[329,1], lb =
cum.meta.var[329,2],
                                    ub = cum.meta.var[329,3], Type =
"Final"))
   cum.meta.var$n <- 1:329
   ### Plot sampling variance cumulative meta-analysis
   png(filename = 'CumMetaVar.png', width = 7, height = 16, units = 'cm',
res = 800, pointsize = 12)
   theme_set(theme_bw(base_size = 10))
   ggplot(data = cum.meta.var[3:nrow(cum.meta.var), ],
          aes(x = n, y = theta, ymin = 0, ymax = ub, colour =
factor(Type), size = factor(Type))) +
     geom_hline(aes(x = 0), lty = 2, size = 0.4) +
     geom_pointrange(aes(ymin = lb, ymax = ub, colour = factor(Type))) +
scale_x_reverse() +
     coord_flip() + scale_size_manual(values = c(0.175, 0.25)) +
     scale_colour_manual(values = c("grey","black")) +
     ylab("Effect of PD (slope)") + xlab("Sample size (cumulative
total)") + theme_bw() +
     theme(legend.position = "none",
           text = element_text(size = 7),
           plot.background = element_blank(),
           panel.grid.major = element_blank(),
           panel.grid.minor = element_blank())
   dev.off()
   ###
   # Get slope estimate for sampling variance.
```

```
   var.X <- model.matrix(~ d.samp.var, data = ps.meta.dat)
   ps.mod.var <- metahdep.HBLM(ps.meta.dat$hedges.d, V, var.X, M,
delta.split = TRUE, two.sided = TRUE)

   # Run cumulative meta-analysis for publication date
   cum.meta.date <- CumMeta(ps.meta.dat, ps.meta.dat$year)
   cum.meta.date[1, ] <- ps.meta.dat$hedges.d[order(ps.meta.dat$year)][1]
   cum.meta.date[2, ] <- cum.meta.date[2,1]
   cum.meta.date <- rbind(data.frame(theta = cum.meta.date[1:328,1], lb =
cum.meta.date[1:328,2],
                                     ub = cum.meta.date[1:328,3], Type =
"Study"),
                          data.frame(theta = cum.meta.date[329,1], lb =
cum.meta.date[329,2],
                                     ub = cum.meta.date[329,3], Type =
"Final"))
   cum.meta.date$n <- 1:329
   ### Plot sampling variance cumulative meta-analysis
   png(filename = 'CumMetaDate.png', width = 5.5, height = 16, units =
'cm', res = 800, pointsize = 12)
   theme_set(theme_bw(base_size = 10))
   ggplot(data = cum.meta.date,
          aes(x = n, y = theta, ymin = 0, ymax = ub, colour =
factor(Type), size = factor(Type))) +
     geom_pointrange(aes(ymin = lb, ymax = ub, colour = factor(Type))) +
scale_x_reverse() +
     coord_flip() + scale_size_manual(values = c(0.2, 0.4)) +
     scale_colour_manual(values = c("grey","black")) + geom_hline(aes(x =
0), lty = 2, size = 0.4) +
     ylab("Effect size (Hedge's d)") + xlab("Sample size (cumulative
total)") +
     theme(legend.position = "none", text = element_text(size = 7))
   dev.off()
   ###
   # Get slope estimate for publication date.
   date.X <- model.matrix(~ year, data = ps.meta.dat)
   ps.mod.date <- metahdep.HBLM(ps.meta.dat$hedges.d, V, date.X, M,
delta.split = TRUE, two.sided = TRUE)

   ### Subset analysis
   ## Subset data to remove multiple species records across studies,
creating a data set with no species replication.
   n.sp.by.studies <- apply(table(ps.meta.dat$sp., ps.meta.dat$study), 1,
function(x) sum(x > 0))
   sp.to.subset <- names(n.sp.by.studies[which(n.sp.by.studies > 1)])
   studies.to.subset <- ps.meta.dat$study[which(ps.meta.dat$sp. ==
sp.to.subset[1])]
```

```
   w.study <- table(studies.to.subset)[which(table(studies.to.subset) ==
max(table(studies.to.subset)))]
   ##

   sp.subset <- read.csv('PSF_Dataset_SpSubset2013-12-16.csv')

   # Remove row that calculates Inf sampling variance
   sp.subset <- sp.subset[-which(sp.subset$Experiment == 178), ]
   # Remove the 1 vine life form record. n=1 not much good for estimate
pars, and means fitting a model with PD*life.form won't fit.
   sp.subset <- sp.subset[-which(sp.subset$life.form == "vine"), ]
   # Remove the vine level. Easily done by converting to and from
character class
   sp.subset$life.form <- as.character(sp.subset$life.form)
   sp.subset$life.form <- as.factor(sp.subset$life.form)

   sp.subset$pooled.sd <-
     with(sp.subset, sqrt((((Naway - 1) * (Sdaway^2)) + ((Nhome - 1) *
(Sdhome^2))) / (Naway + Nhome - 2)))

   df.e <- with(sp.subset, Naway + Nhome - 2)
   J <- with(sp.subset, 1 - (3 / (4 * df.e - 1)))
   sp.subset$hedges.d <- with(sp.subset, J * ((Xhome - Xaway) /
pooled.sd))

   p.e <- J^2 * (df.e / (df.e - 2))
   sp.subset$d.samp.var <- with(sp.subset, (p.e * ((1 / Naway) + (1 /
Nhome))) + ((p.e - 1) * hedges.d^2))

   V <- matrix(0, nrow = length(sp.subset$hedges.d), ncol =
length(sp.subset$hedges.d))
   diag(V) <- sp.subset$d.samp.var

   samp.deps <- list(NA)
   samp.deps.ID <- with(sp.subset, paste(Ref, sp., sep = ""))
   samp.dep.groups <- names(which(table(samp.deps.ID) > 1))
   for(i in 1:length(samp.dep.groups)) samp.deps[[i]] <-
which(samp.deps.ID == samp.dep.groups[i])
   for(i in 1:length(samp.deps)){ # Loop for each sampling
dependence grouping
     for(n in 1:length(samp.deps[[i]]) - 1){ # Loop for each
observation within the grouping.
       for(m in n + 1:length(samp.deps[[i]])){ # Loop for each
combination needed for each n.
         V[samp.deps[[i]][n],samp.deps[[i]][m]] <-
V[samp.deps[[i]][m],samp.deps[[i]][n]] <-
           with(sp.subset,
```

```
                (p.e[n]    -    1)    *    hedges.d[samp.deps[[i]][n]]    *
hedges.d[samp.deps[[i]][m]])
       }
     }
   }

   # Create hierarchical dependence groups for data.
   hierarch.deps <- list(NA)
   dep.names <- names(which(table(sp.subset$Ref) > 1))
   for(i in 1:length(dep.names)){
     hierarch.deps[[i]] <- which(sp.subset$Ref == dep.names[i])
   }
   M <- get.M(length(sp.subset$hedges.d), hierarch.deps)

   ## Matrix checks
   library(matrixcalc)
   # Check they are symmetric
   all.equal(V, t(V))
   all.equal(M, t(M))
   # Check if they are positive definite
   is.positive.definite(V)
   is.positive.definite(M)
   #

   sp.subset$PD_Soltis <- NA
   for(i in 1:nrow(sp.subset)){
     sp.subset$PD_Soltis[i]                                                <-
ps.meta.dat$PD_Soltis[which(ps.meta.dat$Experiment                         ==
sp.subset$Experiment[i])]
   }

   sub.PD.X <- model.matrix(~ PD_Soltis, data = sp.subset)
   sub.mod.PD <- metahdep.HBLM(theta = sp.subset$hedges.d, V = V, X =
sub.PD.X, M = M,
                               delta.split = TRUE, two.sided = TRUE)
   sub.PD.se <- sqrt(diag(sub.mod.PD$cov.matrix))
   sub.PD.ci  <-  cbind(lower  =  (sub.mod.PD$beta.hats  -  (qt(0.975,
nrow(sp.subset)-length(sub.PD.se)) * sub.PD.se)),
                        upper   =   (sub.mod.PD$beta.hats   +   (qt(0.975,
nrow(sp.subset)-length(sub.PD.se)) * sub.PD.se)))

   #####

   ###   Final   subset   analysis   checking   sensitivity   against
disproportionate representation across levels
   ### of phylogenetic distance.
```

```
   # Run multiple simulations each time randomly sampling a subset from
the data that provide one data
   # point for each unique level of PD.

   num.sims <- 100
   subset.size <- length(unique(ps.meta.dat$PD_Soltis))
   PD.sub.res <- numeric(length = num.sims)

   for(k in 1:num.sims){
     sub.dat <- c()
     cat("PD subset analysis: simulation", k, "of", num.sims, "\n")

     for(j in 1:subset.size){
       PD.subset     <-     ps.meta.dat[which(ps.meta.dat$PD_Soltis     ==
unique(ps.meta.dat$PD_Soltis)[j]), ]
       sub.dat <- rbind(sub.dat, PD.subset[sample(x = 1:nrow(PD.subset),
size = 1), ])
     }

     sub.dat <- sub.dat[order(sub.dat$Ref), ]

     # Fit model using sub.dat
     pooled.stdev <-
       with(sub.dat, sqrt((((Naway - 1) * (Sdaway^2)) + ((Nhome - 1) *
(Sdhome^2))) / (Naway + Nhome - 2)))

     df.e <- with(sub.dat, Naway + Nhome - 2)
     J <- with(sub.dat, 1 - (3 / (4 * df.e - 1)))
     d.es <- with(sub.dat, J * ((Xhome - Xaway) / pooled.stdev))

     p.e <- J^2 * (df.e / (df.e - 2))
     d.samp.var <- with(sub.dat, (p.e * ((1 / Naway) + (1 / Nhome))) +
((p.e - 1) * d.es^2))

     V <- matrix(0, nrow = length(d.es), ncol = length(d.es))
     diag(V) <- d.samp.var

     samp.deps <- list(NA)
     samp.deps.ID <- with(sub.dat, paste(Ref, sp., sep = ""))
     samp.dep.groups <- names(which(table(samp.deps.ID) > 1))
     for(l     in     1:length(samp.dep.groups))     samp.deps[[l]]     <-
which(samp.deps.ID == samp.dep.groups[l])
     for(i  in  1:length(samp.deps)){                    # Loop  for  each
sampling dependence grouping
       for(n  in  1:length(samp.deps[[i]])  -  1){        # Loop  for  each
observation within the grouping.
```

```
         for(m in n + 1:length(samp.deps[[i]])){     # Loop for each
combination needed for each n.
           V[samp.deps[[i]][n],samp.deps[[i]][m]]                      <-
V[samp.deps[[i]][m],samp.deps[[i]][n]] <-
             with(sub.dat,
                  (p.e[n]    -    1)    *    d.es[samp.deps[[i]][n]]    *
d.es[samp.deps[[i]][m]])
         }
       }
     }

     hierarch.deps <- list(NA)
     dep.names <- names(which(table(sub.dat[ ,2]) > 1))
     for(i in 1:length(dep.names)){
       hierarch.deps[[i]] <- which(sub.dat[ ,2] == dep.names[i])
     }
     M <- get.M(length(d.es), hierarch.deps)

     subset.X <- model.matrix(~ 1 + PD_Soltis, data = sub.dat)
     PD.sub.mod <- metahdep.HBLM(theta = d.es, V = V, X = subset.X, M =
M, delta.split = TRUE, two.sided = TRUE)

     PD.sub.res[k] <- unname(PD.sub.mod$beta.hats[2])
   }

   ################ END OF SCRIPT
```

Chapter 4 Appendix: Conflicting conclusions between Mehrabi and Tuck (2015) *New Phytologist*, 205, 1071-1075, and Parker, I. M (2015). *Nature*, 520, 542-544.

The conclusions of Parker et al. (2015) contrast with Chapter 4 of this thesis (Mehrabi and Tuck, 2015). Parker et al claim that phylogeny can be used as a predictor of plant community structuring. I claim that this is unlikely to be true. Below I discuss why this discrepancy might arise.

In chapter 4, I tested if phylogenetic distances separating plant species can be used to predict the sign and magnitude of interspecific plant-soil feedback interactions. Whilst I found evidence to suggest that plants on average culture soil to the relative disadvantage of their-own species, there was large variation in this effect (Chapter 4, Fig 2a). I found no evidence to suggest that phylogeny could explain the variation in feedback sign and magnitude. That is, the difference in the fitness of a species growing on its own soil versus an alternative species soil was independent of how closely those two species were related. This finding held across different life forms, life cycles, provenances, and most importantly different phylogenetic scales. I concluded from this analysis that phylogenetic distance is a poor general predictor of plant-soil feedback effects.

For the evolutionary distance separating interacting species to be a good predictor of the relative strength of intraspecific versus interspecific competition, two stringent assumptions need to be met. First, closer relatives must be more likely to share similar phenotypic expressions or functional traits. Second, species with more similar functional traits must compete more strongly (cf. Mayfield & Levine, 2010). Empirical tests within plants, animals and fungal groups show that the relationship between phylogenetic distance and trait dissimilarity often saturates very quickly to a slope of zero (Kelly *et al.*, 2014). This means that phylogenetic distance is only likely to predict functional distance across a very limited number of species comparisons

that occupy to the far left of the phylogenetic distance to functional dissimilarity curve: for the majority of species comparisons its predictions may be no different from random.

However, even within the short range where a phylogenetic signal in trait similarity is likely to be seen, functional trait dissimilarity is by itself uninformative for predicting the fitness of interacting species (Mayfield & Levine, 2010; Adler *et al.*, 2013). Dissimilarity in pathogens or nutrient depletion traits might increase the relative strength of intraspecific to interspecific competition, but dissimilarity in beneficial symbionts or nutrient exploitation rates might decrease it. The net effects of trait dissimilarity will depend on which traits dominate the relative strength of interspecific to intraspecific competition, which in turn is likely to change with environmental context (HilleRisLambers *et al.*, 2011; Adler *et al.*, 2013). In retrospect, given the limited range of prediction space of the first assumption, and ambiguous nature of the second assumption implicit to using phylogenetic distance to proxy the outcome of species competition, it is not at all surprising I found the results I did in chapter 4 of this thesis.

Parker et al. tested the idea that the phylogenetic distance separating plant species could be used to predict the relative amount of disease on those plant species in the field. They hypothesised that (1) local abundances of plant species will be positively correlated to the species percent plant tissue covered in disease, and (2) that reducing the phylogenetic distance between a focal species from the rest of the local community would increase this effect. The reasoning they gave for (2) is that the likelihood of local spill over of pathogens between species will be related to the evolutionary distance separating those species. They measured the area cover in necrotic and chlorotic lesions as a measurement of disease on 43 plant species in 10 sites in the field and correlated these disease measures to both the relative abundance of those species in each sites, and the phylogenetic rarity of those species. They then took 44 non-native species and introduced them into each of the 10 sites and measured their accumulated disease, and then correlated this disease to the output of

a model that predicted the probability of disease infection for a given plant species based on its phylogenetic distance to other plant species in the local community (the 'Phylosusceptibility' model).

Parker et al. found correlations between local disease and phylogenetic rarity in the wild plants, and correlations between local disease and the predicted probability of that species share pathogens with the neighboring community from their Phylosusceptability model. They concluded that the phylogenetic distance separating plant species strongly influenced pathogen infection on plant hosts in addition to local host abundances, and that their findings have broad utility for ecology and for applied situations such as agriculture and forestry. Whilst an interesting study, which clearly took a lot of effort to conduct, I think there are some shortcomings of their methods sufficient enough to limit the strength of the conclusions they made.

The first issue relates to how well the main results in the paper link to the theoretical arguments they present. Parker et al. predicted that disease on the most 'phylogenetically common' species was 14.7%, and on the most 'phylogenetically rare' one was 4.1%, leading to an overall difference of 10.6% between the full phylogenetic range of species used in their study (95% CI's: 5.6%: 10.7%) (Note: we do not know the biological significance of a 5% - 10% shift in this disease measure from the base levels it is predicted to change between).

To reach their estimate Parker et al. aggregated their data across 10 study sites. As shown in Fig 2 of their manuscript, there can be considerable variation in a species disease response within any given site. In addition not all species were represented at all sites, with 30% of the species averages plotted in Fig 2 of their paper only having 1 or 2 site level replications. Species estimates with one site level replication are plotted with SE's of 0 (!) It is not clear why these site level differences were not formally incorporated into the analysis. A key hypothesis of their paper is that phylogenetic rarity of plants reduce the likelihood of the local "spill over" of

disease from neighboring plant species. Incorporating the differences and variation in site effects therefore seems important to understanding the predictive utility of phylogeny for local disease epidemics.

In addition, the logic of figure 1 of their paper suggests that the effect of relative abundances on disease will depend on the phylogenetic relationship of a species to other species in the community. However they did not fit the necessary interaction between phylogenetic rarity and local relative abundances to test this explicitly (I downloaded their aggregate dataset supplied with the paper and tested this but found no evidence to suggest any interaction occurred: $F_{1,39,} = 0.6$; $P = 0.42$). Further, in the methods, they report that they found no relationship between phylogenetic rarity and species relative local abundances in their plots. How they then conclude that phylogenetic rarity has any influence on community structuring is unclear. These inconsistencies between results and theory are not adequately addressed. Clearly an experimental manipulation that orthogonally treats abundances and phylogeny is needed.

The second issue, relates to the validity of the statement Parker et al. make about the utility of the Phylosusceptibility model for predicting disease of plants introduced into resident communities. Parker et al. use the model to calculate the probability that introduced plant species will share pathogens with native species. They use models with independent datasets that predict that the probability of any two species sharing a pathogen is likely to drop off quickly with phylogenetic distance (cf. the relationship between phylogeny and functional trait similarity I outlined above). They then fit exploratory linear and non-linear trends to model the relationship between the probability of shared pathogens and their disease survey data.

They state that the probabilities of shared pathogens (from their Phytosusceptibility model) predict the percentage of disease in the field well across all species comparisons in their study: that a linear relationship is reasonable, and that a non-linear positive decelerating function fits the relationship better. They also state

that: “The introduced species that escaped disease were all species with few close relatives, while those closely related to many residents always showed high levels of disease.”. I think these claims might be misleading. High levels of disease were frequently observed on introduced species that were were not predicted to share pathogens (see below Fig 1: label 1). Low levels of disease were also observed on introduced species that were predicted to share more pathogens (Fig 1: label 2). And the highest levels of disease were not observed on plants predicted to share the most pathogens- but on plants that were predicted to share only intermediate numbers of pathogens (Fig 1: label 3). The simple observation, shown in Figure 3 of their paper, albeit hidden by a logarithmic transform, that disease increases and peaks at mid relatedness, shows that highest disease is not observed in the most closely related plant species.

The third issue, is that Parker et al. measured the chlorotic and necrotic lesions on leaves as a measure of fungal disease pressure in their plots. In fact these kind of disease symptoms can be caused by a variety of different abiotic and biotic factors (Agrios, 2005). Parker et al. cite their previous work showing a link between these disease symptoms and fungal diseases as a justification for this approach, but this previous work was conducted on only 2 of the 41 genera included in the present study and so is not likely to be a good evidence base for their approach (Parker & Gilbert, 2007). Sequencing technology is readily available and cheap enough for them to have, at least on a sub-sample of their plots, confirmed that the chlorotic and necrotic lesions were correlated with fungal diseases. Following this, to prove a causal link, they would have had to infect the plants in a manipulative fashion and observe the disease symptoms. The lack of validation using Koch’s postulates means that is unclear if if the disease symptoms were caused by fungal pathogens. Without any of this type of information, it is actually very difficult to say what was driving the disease symptoms they measured.

The fourth point, (mentioned in passing above), is that Parker et al. did not measure the fitness effects of disease infection on the species in their study. This

shortcoming makes it very difficult to make any conclusions on the importance of their disease measures for structuring of the plant communities. At the very least, a subsample of biomass measures from some of their plots would have been good to show a correlation between the disease measure and plant fitness in the field. For their second survey on introduced plants, they actually retrieved all the plants at the end of the experiment, and so it is also unclear why they didn't dry and measure them. If they had done, they could have then explored how this correlation between disease and fitness might change between different species. Of course, this would still not be causal proof of the diseases effects on fitness, but it would have at least allowed them to approach the question of how the disease they measured might in theory influence community structuring. It is unclear from the current data, if the differences in disease loads across species are likely to have any effect on plant yields or population growth, how these effects might differ between the different species, and what the consequences of these different effects might be for the realized relative abundance of the species in their plots.

In summary, I have concerns about both on how well the patterns observed in their aggregate data analysis of disease can predict the dynamics of disease spread on the local scale, and how well the results presented actually support the logic of the main arguments. Whilst the Phylosusceptability model is argued to perform well, the experimental introductions showed that disease was highest on species that were not most closely related to the rest of the community. Furthermore, without any proof of the causal agents of the disease measures, Parker et al. did not actually collect the right kind of data needed to conclude if phylogenetic distance can be used to predict the degree of *pathogen spillover* between species. Finally, with no measure of how plant fitness relates to the disease they measured, and how this relationship changes between species, they also did not collect the necessary data needed to confirm if phylogeny had any role in the structuring of plant communities in nature. These shortcomings limit the stength of the conclusions that can be made from the Parker et al.'s study, and leave many fundemental questions unanswered.

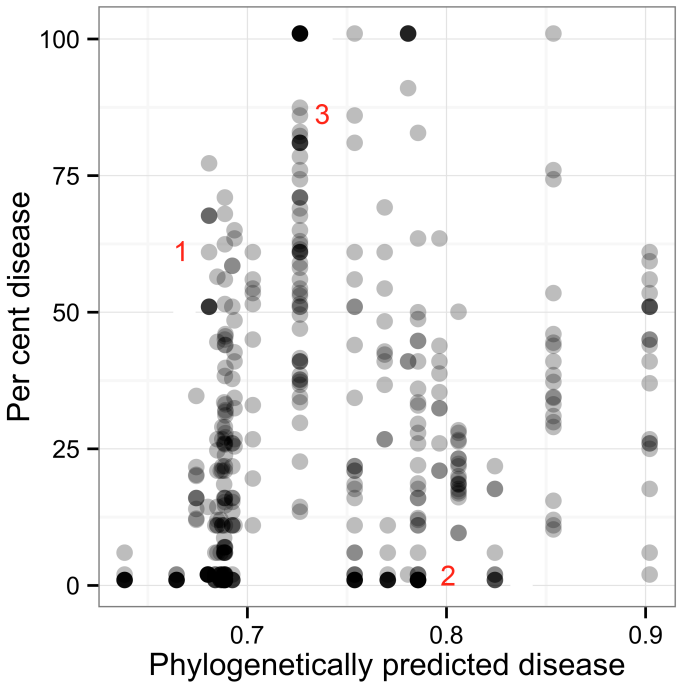


Figure 1. Scatter plot of the percent disease on introduced species and the predicted probability of the likelihood of sharing pathogens with resident species in the community (replotted from Fig 3 in Parker et al.) (1) shows that plants that are predicted to have few shared pathogens also show very high levels of disease, (2) shows that plants that are predicted to have mid-high levels of shared pathogens show low disease levels and (3) shows that the highest disease records occur in species that are predicted to have moderate sharing of pathogens

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5. Plant-soil feedbacks and the resource economics spectrum[4]

Abstract

Plants often culture soil to the relative disadvantage of their own species. In natural systems this process can maintain diversity, whilst in agricultural systems it is a principle factor driving yield loss. A key problem for plant ecologists is predicting how much better plants will perform on soil cultured by different species than their own. Despite a large volume of experiments and research to date, interspecific plant-soil feedbacks remain poorly understood. Here we show, using a hierarchical meta-analysis of 95 plant species interacting in 199 plant-soil feedback experiments, that the benefits of heterospecific soils are related to trait differences between species pairs. We found that soil cultured by resource exploitative heterospecifics (e.g. plants with higher specific leaf area, leaf [N], leaf [P], and lower leaf dry matter content) was associated with a reduction in the benefits of escaping conspecific soils, and that soil cultured by more conservative and resource efficient heterospecifics (plants with lower specific leaf area, leaf [N], leaf [P], and higher leaf dry matter content) was associated with an increase in

[4]Typescript from Mehrabi, Z., Tuck, SL., Kattge,J., Reich, P.B. Plant soil feedbacks and the resource economics spectrum. *In review*

that benefit. When isolated from whole soil chemistry effects, we found some evidence that plants experienced better growth with soil biota cultured by more resource exploitative species, although this effect was less well supported that observed for whole soils. Our work provides the first analysis attempting to quantitatively predict interspecific soil interactions from plant functional traits across multiple experimental settings. Our results suggest that resource exploitative plant species can disadvantage more conservative species, reducing the benefits of escaping fitness costs imposed by conspecifics.

5.1 Introduction

Negative plant-soil feedbacks occur when plants culture soil microbiota and chemistry to the relative disadvantage of their own species (Kulmatiski *et al.*, 2008; Mangan *et al.*, 2010; Van der Putten *et al.*, 2013). This phenomenon has widespread importance in natural systems (Petermann *et al.*, 2008; Mangan *et al.*, 2010; Bagchi *et al.*, 2010), and in agriculture where it is a principle driver of crop yield losses (Dick, 1992; Bennett *et al.*, 2012). Despite its significance, the direction and strength of plant-soil feedbacks has proved extremely difficult to predict (Van der Putten *et al.*, 2013; Mehrabi & Tuck, 2014; Kardol *et al.*, 2015). Evidence for directional effects of plant leaf traits on nutrient cycling (Cornwell *et al.*, 2008; De Deyn *et al.*, 2008; Orwin *et al.*, 2010) and soil microbial community composition (Van Der Heijden *et al.*, 2008; de Vries *et al.*, 2012; Grigulis *et al.*, 2013) suggests that plant functional strategies might be used to determine the direction of plant-soil feedback effects. This idea is hinged on the assumption that 'fast' growing exploitative species with highly decomposable root and leaf tissues will have higher fertilizing effects on soil than 'slow' growing resource conservative species (Baxendale *et al.*, 2014; Ke *et al.*, 2014) (Fig 1).

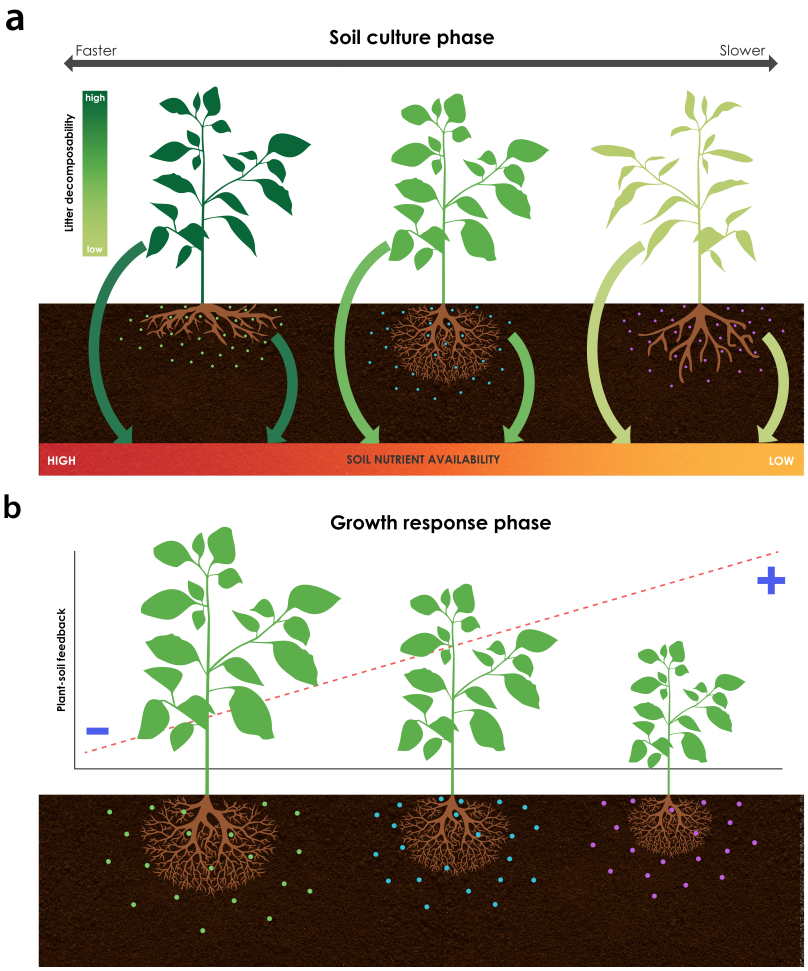


Figure 1. The classic hypothesis on the relationship between the plant resource economics spectrum and nutrient driven plant-soil driven feedbacks a) 'Faster' plants with highly decomposable litter inputs are expected to culture soils to have higher nutrient availability than 'slower' plants with lower litter decomposability b) All else being equal, plant growth is expected to increase on soil cultured by heterospecifics that are 'faster' and decrease on soils cultured by heterospecifics that are 'slower' relative to that of similar strategy conspecifics. Plant-soil feedback is defined as the average of a species biomass accumulated on conspecific soil minus its average biomass accumulated on heterospecific soil.

Although the idea of classifying plant species into strategies is a potentially powerful concept, with some evidence for co-ordination of resource acquisition and nitrogen status across above and below ground plant organs (Laughlin *et al.*, 2010; Liu *et al.*, 2010; Freschet *et al.*, 2013; Reich, 2014), the generality of directional strategy effects on plant-soil feedbacks has yet to be fully explored. Nutrient driven proliferation of pathogens (Veresoglou *et al.*, 2013) and losses of beneficial fungi (Hoeksema *et al.*, 2010; Orwin *et al.*, 2010; Grigulis *et al.*, 2013), alongside phytotoxic effects of highly decomposable tissue inputs (Bonanomi *et al.*, 2006, 2011), or disruptions and lags to recycling of plant materials (Hobbie, 2015), could all drive the effect of plant strategy in the opposite way to expected, with plants performing better on soil cultured by more conservative species. To date however the relative impact of plant strategy on plant-soil feedbacks experienced by multiple species and study systems remains unknown.

Here we present a formal meta-analysis of 95 plant species interacting in 199 experiments that compared the growth responses of plant species to heterospecific vs. conspecific soil. Assigning plant functional trait data to the species pairs in each experiment, we are able to begin to test the utility of the fast-slow strategy concept for predicting interspecific plant-soil feedback effects. Our chosen effect size is Hedges *d* standardized difference, calculated from mean plant biomass responses to heterospecific vs. conspecific cultured soil, and we use a continuous metric of functional differences in plant strategies between interacting species pairs, computed from easy to measure above-ground traits (Leaf [N], Leaf [P], Specific leaf area, Leaf dry matter content). We utilize these four leaf traits for their strong links to the plant resource economics spectrum (Wright *et al.*, 2004; Reich, 2014) and documented importance in driving soil nutrient status and food web structure (Cornwell *et al.*, 2008; De Deyn *et al.*, 2008; Orwin *et al.*, 2010).

We use a hierarchical Bayesian meta-analysis to examine the evidence for a directional effect of plant strategies on the sign and strength of feedback effects, whilst accounting for study and sampling level dependencies in the dataset. We separated studies that accentuated soil biota effects over other soil chemistry effects (hereafter 'soil biota feedbacks') from studies giving more equal weighting to both root soil biota and soil chemistry feedback pathways (hereafter 'whole soil feedbacks'). We fitted separate models for whole soil and soil biota community led feedback and estimated the coefficients for functional difference effects with 95% credible intervals for these estimates. We checked the stability of the relationships to study level reliability, and to a range of potentially confounding covariates, such as N_2-fixing status interactions between species pairs, experimental settings, the time periods of soil culture or response measurements.

5.2 Methods

5.2.1 Study selection

Full details of the study selection, including a PRISMA statement outlining the flow of information in the review process, are given in Mehrabi & Tuck (Mehrabi & Tuck, 2014). Briefly, we searched the ISI Web of Knowledge for 20 years (1993-2013) alongside additional references and unpublished works for studies that recorded interspecific plant-soil feedback biomass responses. All studies in the final selection had data on means and SD that were readily accessible from figures or through author correspondence. We subsetted the dataset of Mehrabi & Tuck to only represent experiments from which (1) dominant species in each pair were identifiable (2) which tested either soil biota feedbacks or whole soil feedbacks and (3) to ensure that a given focal species was only represented once in the dataset, avoiding the non-independences arising from using the same species in multiple studies which could not be accounted for in the modeling procedure (The studies included were: Bever, 1994; Callaway *et al.*, 2004; Ehlers & Thompson, 2004; Bonanomi & Mazzoleni, 2006; Puerta-Piñero *et al.*, 2006; Kardol *et al.*, 2007; Van Grunsven *et al.*, 2007; Petermann *et al.*, 2008; Casper *et al.*, 2008; Kueffer, 2009; Te Beest *et al.*, 2009; McCarthy-Neumann & Kobe, 2010a,b; Jiang *et al.*, 2010; Fitzsimons & Miller, 2010; Yelenik & Levine, 2011; Dostál & Palečková, 2011; Burns & Strauss, 2012; Perkins & Nowak, 2012; Pendergast *et al.*, 2013; Hendriks *et al.*, 2013). We guided the trimming process for (3) by first maximizing the total number of effect sizes and unique species interactions in the dataset and then retaining the effect sizes that carried the highest level of confidence (lowest variance). Approximately 41% of experiments used soil cultures of <1 year, 29% used soil cultures of 1-5 years, and the remaining 30% used field soil. The feedback response phases in these experiments ranged from 1.5-6 months.

5.2.2 Functional differences

We determined the "fast-slow" strategy of species in the plant-soil feedback interactions using leaf [N], [P], Specific Leaf Area and Dry Matter Content, extracted from TRY-DB (species coverage ~69%), and from other published sources (species coverage ~31%) (Supplementary Information Fig S6). The similarity in "fast-slow" strategies was formalized as the absolute distance between the species positions on the first principle co-ordinate axis from a PcOA computed using a Gower dissimilarity matrix from a minimum of two "fast-slow" strategy traits per species (with functional differences being the strategy of focal conspecifics – the strategy of heterospecifics) (Fig S1). This axis was highly correlated with [N], SLA, LDMC and moderately correlated with leaf [P] (Fig S2). Specific Root Length and fine root [N] (<2mm) were coded from TRY-DB and published literature sources (species coverage ~31% for SRL, ~24% for fine root [N]) (Fig S7). N_2-fixing interactions between species pairs ('Culture-Response' species N_2–fixing status: 'No-No', 'Yes-No', 'No-Yes', 'Yes-Yes') in the dataset were coded from the N_2-fixing status of species and genera given in *Werner et al.* (Werner *et al.*, 2014).

5.2.3 Soil biota vs. whole soil feedbacks

We determined separated studies into those that controlled for soil chemistry effects and those which allowed for both biotic and abiotic feedbacks. Studies that controlled for soil chemistry included soil innoculum experiments and soil wash extractions, which the authors of these studies made to accentuate soil biota mediated feedbacks over the conjoined effect of soil biota and soil chemistry. The majority of soil dilution experiments used ratios of ~1:6 or less (e.g. volumes of <15% fresh soil inoculum of

conspecific or heterospecific sources to sterile soil media), although other references (Te Beest *et al.*, 2009; Pendergast *et al.*, 2013) were also included that used slightly higher ratios (20-25% inoculum by volume) but minimized interspecific soil chemistry effects by homogenizing soil across species. Other experiments used whole soil cultures that were considered to give more equal weighting to both root microbial and whole soil feedback pathways (e.g. Petermann *et al.*, 2008 was included in this category and used 80% fresh soil inoculant). This differentiation between 'whole soil' and 'soil biota' feedback pathways is widely used in the literature, and the separation of these studies in our analysis was in line with the stated rationale made by the authors of these studies in their published articles and datasets. In our sensitivity analysis, we further split 'soil biota' based feedbacks into those that inoculated the complete soil biotic community versus those that used a filtrate-based inoculums (20-30`m) that attempted to remove mychorrizal fungi and microfauna.

5.2.4 Hierarchical Bayesian meta-analysis

To determine the influence of interacting functional identities on plant-soil feedbacks we fitted a hierarchical Bayesian model accounting for sampling and hierarchical dependencies in our data. This approach is particularly valid in this case, where the effect of plant strategy differences is tested independent of study or research group differences unaccounted for by the coded covariates.

The model in matrix form is:

$$d = \mathrm{Xb} + \mathrm{d} + \mathrm{e} \qquad (1)$$

where d is the vector of effect size estimates from all the experiments; X, a design matrix with our covariates, b, a vector of parameters; d, a vector of hierarchical errors; and e a vector of sampling errors. It assumes the distributions:

$$\mathrm{d} \sim N(0, \mathrm{D})$$

$$\mathrm{D} = \mathrm{t}^2\mathrm{I} + \mathrm{zM}$$

$$\mathrm{e} \sim N(0, V) \qquad (2)$$

Where V is the sampling variance-covariance matrix, with known sampling variances and covariances. D represents the variance and covariance among random deviations of Xb from the effect sizes being estimated. D is a block-diagonal matrix, with hierarchical variance t^2 on the diagonal (I) and blocks (M) of hierarchical covariance z on the off-diagonal for pairs of hierarchically dependent effect sizes. The effect of functional identity interactions was tested by constructing a design matrix for functional differences in "fast-slow" traits, along with other study or experiment specific covariates e.g. N_2-fixing status interactions, experimental settings, above vs

total (above & below) ground measurements, the time period of soil culture and response, and competition (intraspecific density at measurement). In order to code the time period of culture as a continuous covariate "field" soil was assigned with a culture period of 10 years, assuming that natural stands had observed at least that length of culture. Separate models were fitted for whole soil and soil biota feedbacks. Coefficients were estimated for functional difference effects with 95% credible intervals for these estimates, calculated by multiplying the posterior standard error of the coefficients by the 95% quantile of a *t*-distribution with *N*-*k* degrees of freedom. The meta-analysis was carried out using R 3.0.3 (R Core Development Team, 2013) with the R package *metahdep* (Stevens & Nicholas, 2009).

5.3 Results and Discussion

Our analysis shows that strategy differences between conspecific and heterospecifics are related to the net-direction and strength of plant-soil feedbacks: with the direction of the effect differing between biotic and whole soil manipulations. In whole soils, on average plant species did moderately better on soils cultured by different species to their own (Intercept only model: d= -0.42 95%CI: lower= -0.85, upper= 0.009), with plant species accumulating more biomass on soil cultured by "slower" than "faster" species (Slope: -2.02; 95% credible interval (CI): lower= -3.37, upper= -0.67) (Fig 1a). For soil biota feedbacks, plants also on average performed better on soils cultured by different species to their own (Intercept only model: d= -0.33 CI: lower= -0.63, upper= -0.03), although the strategy of the heterospecific had the opposite effect from that in whole soils, with plants accumulating more biomass on soils cultured by 'faster' species (Slope: 0.81; CI: lower= 0.02 upper= 1.61) (Fig 1b). There was however high variability in interspecific feedbacks around the prediction lines, and the explanatory power of plant strategy in soil biota feedbacks was low (Whole soil $R^2 = 0.15$; Soil biota $R^2 = 0.05$: computed from residuals conditional on the random effects in models with and without the functional difference predictor).

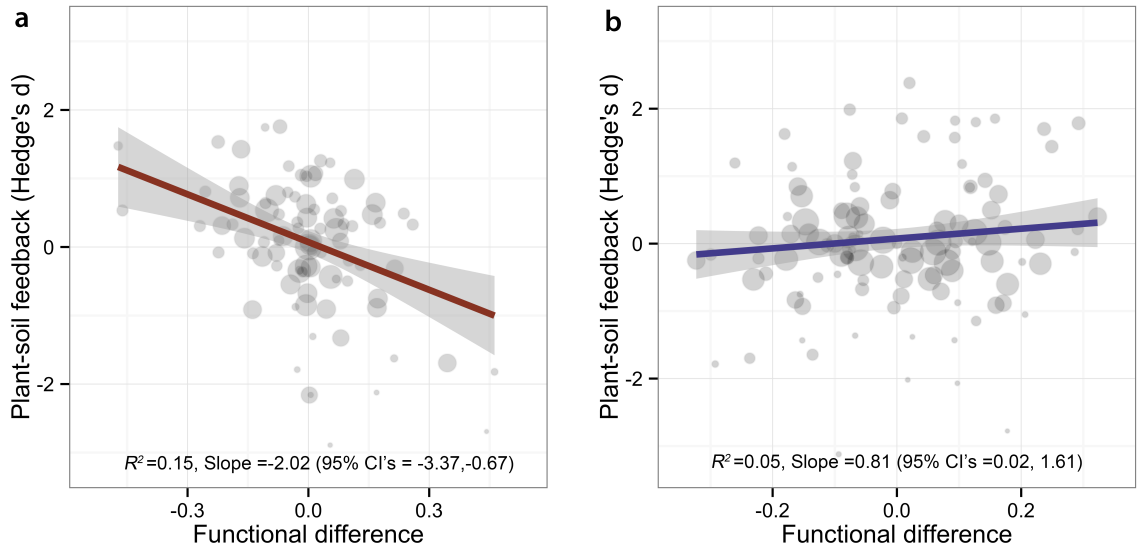


Figure 2. Plant soil feedbacks and the resource economics spectrum. Meta-regressions of experimental plant–soil feedbacks against plant resource economics differences between interacting species pairs (total species = 95, experiments= 199). (a) In whole soils plants performed better on soils from heterospecifics with conservative strategies relative to that from more exploitative species (b) Controlling for soil chemistry with soil biota inoculations removed this effect. The weighted partial regressions plots and 95% intervals shown here (whilst accounting for moderators and study identity effects) assume fixed models. Negative and positive functional differences represent a heterospecific strategy "faster" or "slower" to that of conspecifics, respectively (Fig 1).

We examined the additive influence of study and experiment level covariates, on the estimated effect of functional strategy differences. As N_2-fixers may have positive nutritional effects, negative phytotoxic influences (Bonanomi *et al.*, 2006), and positive symbiont culture effects (Lira *et al.*, 2015) average interspecific plant-soil feedback effects may depend on N_2–fixing identities of interacting species pairs. We found that controlling for the N_2-fixing status of conspecific and heterospecifics (i.e. by fitting separate intercepts for each type of N_2–fixing interaction) improved our estimate of the effect of plant strategy on plant-soil feedbacks (Figs 2a-b). The time of soil culture or growth response period are both likely to be important for feedbacks effects by influencing soil biota colonisation dynamics, tissue inputs and decomposition processes (Bardgett & Wardle, 2010; Ke *et al.*, 2014), and biomass allocation may differ between above and belowground organs during the feedback process (Kulmatiski *et al.*, 2008; Bardgett & Wardle, 2010; Baxendale *et al.*, 2014). Controlling for these and other study level covariates (e.g. provenance, conspecific density and the experimental setting) had little impact on the estimated relationship between plant strategy differences and plant-soil feedback effects (Figs 2a-b). These results support the idea that plant species realize the greatest benefit from escaping the costs of negative conspecific effects when moving to heterospecific soils cultured by more resource efficient conservative species, and that removing soil chemistry effects through soil biota inoculation removes this effect.

We checked our models for publication bias (e.g. a tendency for publication of negative or positive plant-soil feedback effects) and the influence of less reliable experiments (e.g. with high sampling variance). We found little evidence of publication bias that could cause skew in the distribution of residuals conditional on the random effects in a full model (including all 199 experiments and moderators (Funnel Plot: Fig S3). Any bias present did not have any effect on the results we present (Trim and Fill: 22 studies added, 0.003 increase in effect size, $z = 0.064$, $p = 0.95$). A cumulative meta-analysis approach was used to evaluate how the slope of the relationship between functional differences and plant-soil feedbacks was influenced by experiments with high sampling variance. Although these models do not allow the inclusion of all moderators they may give some insight into the reliability of our results gained from analysis of the whole dataset. We found that the reliability of experiments had little influence on the relationship between functional strategy differences and whole soil feedbacks, but that high within study error weighted the sign of the final effect of strategy on soil biota plant-soil feedbacks (Fig 2 c-d). This latter result suggests that we need more, well replicated, studies to support the observed positive effects fast plant strategy on soil biotic feedbacks.

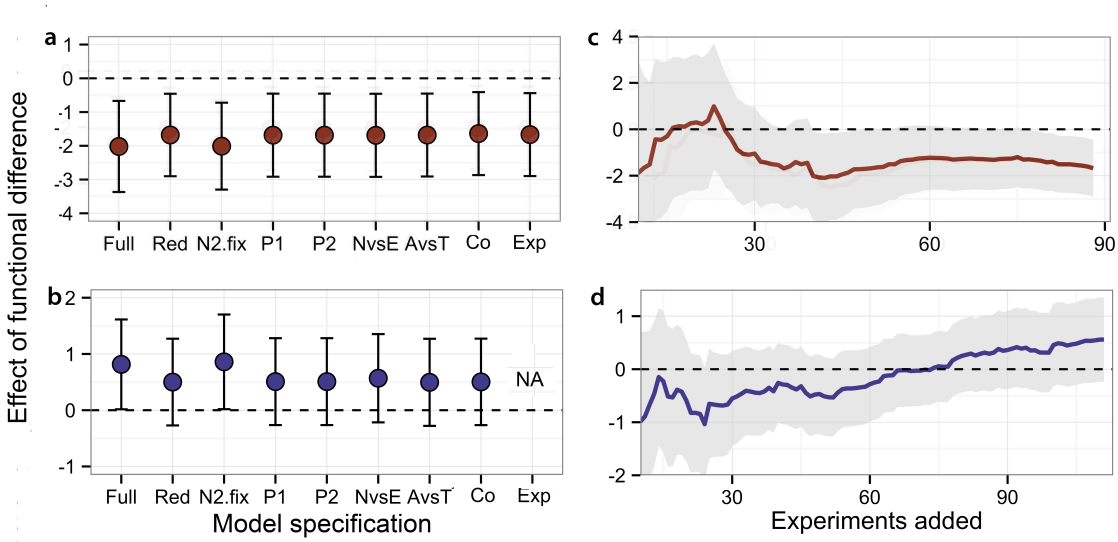


Figure 3. Stability of model estimates of functional difference effects to covariates and sampling variance. The y-axis shows posterior slope estimates and their 95% credible intervals for the effect of functional difference on experimental plant-soil feedbacks. The additive influence of different covariates on the slope estimates was checked in whole soils (a) and soil biota inoculations (b). X-axis labels represent the covariates included in meta-regressions with the functional difference predictor. N2.fix= N_2 fixing status of species pairs (e.g. Culture-Response status: No-No, Yes-No, & No-Yes), P1= period of soil culture, P2= growth response period, NvsE= provenance (native vs. exotic status of the species pairs), AvsT= measurement type (above or total biomass responses), Co= competition in response phase (alone vs. co-cultures of conspecifics), Exp= experimental setting (e.g. field vs. laft vs. pots in field vs. greenhouse), Full= all covariates, Red= no covariates. A cumulative meta-analysis was also performed to check for the effect of sampling variance (low to high) on the reduced model estimates for whole (c) and soil biota mediated (d) feedbacks.

We explored the poor prediction over soil biota based feedbacks experiments further. As filtrate based biotic inoculations can give a highly skewed representation of the soil biotic community (e.g. by eliminating fungal hyphae, microarthopods and adult nematodes), we removed these experiments (all with 20-30μm exclusions) from our dataset and re-ran our analysis. We found that removing filtrate-based studies increased the effect of functional difference on soil biota based feedbacks (R^2 = 0.30, Slope: 2.05; CI: lower= 0.64 upper= 3.46). This sensitivity analysis suggests that the positive plant strategy effects on soil biotic legacies are more predictable for the complete soil biotic community, and much more variable if the larger sized (¿30um) members of the soil community are excluded via filtration. This differences in experimental approach explains in part the poor general predictability of plant strategy on soil biota feedbacks as shown in Fig 2b.

Importantly, the net effect of plant strategy effects on interspecific plant-soil feedbacks on nutrient dynamics will result from the immobilisation (or withdrawals) and mobilisation (or inputs) mediated through both aboveground and belowground organs (Freschet *et al.*, 2013; Hobbie, 2015). We did not explicitly include root economic resource traits [e.g. specific root length, root [N], root dry matter content, root [P]) in our analysis due to the poor coverage of root traits in the literature to date. However, we did find a positive correlation between specific root length and specific leaf area (r=0.36, P<0.04), and fine root [N] and leaf [N] (r=0.77, P<0.001), for species that had these traits available, indicative of some aboveground-belowground resource economics strategy co-ordination for species in our study (Fig S4 a-b).

These results taken together clearly show that the negative effects of fast strategy plants in whole soils are unlikely to be the result of negative microbial effects (e.g. increased generalist pathogens or loss of promiscuous beneficial symbionts). The main effects observed in whole soils ran in the opposite direction to that

expected if faster strategy plants would have dominating positive nutritional effects interspecifically. In principle the contrasting positive effects of fast plants on soil biota feedbacks could be due to increased density of microbes involved in nutrient cycling (Grigulis *et al.*, 2013), although this relationship only seemed important for studies that included the whole soil biotic community in their inoculations, and was muddied by idiosyncratic filtrate based inoculants.

Phytotoxic effects of quickly decomposing leaf and root litter on seedling development might help explain the negative effects of species from the fast end of the resource economics spectrum on whole soil (Bonanomi *et al.*, 2006, 2011). However, quantification of the mass of nutrients held in standing or harvested biomass (or in losses to other sources such as herbivores in the field), alongside soil chemistry measurements of nutrients limiting for plant growth, would be needed to determine the degree to which the negative interspecific effects of fast strategy plants in whole soils result from immobilisation. Higher leaf or root [N] might actually slow down decomposition in the longer term, and negative correlations between 'fast' tissues and other nutrients limiting to decomposer growth such as Ca, could also contribute negative interspecific effects of fast strategy plants (Hobbie, 2015). The challenge is now on for biologists to include this range of variables and mechanisms in their models and experimentally test their importance in driving interspecific plant-soil feedback interactions.

Our analyses show that plant species perform worse on whole soil cultured by faster species than they do on soil cultured by more conservative heterospecifics relative to when growing on soil cultured by their own species. We detected this effect from a few easy to measure aboveground traits. However, the variation in interspecific plant-soil feedbacks around our prediction lines suggests a high degree of context dependency not accounted for in the models. There is likely to be large amount of noise introduced from species level trait measurement error (Albert *et al.*, 2010), and potentially from unobserved divergences between effects

of above or below ground economics (Comas & Eissenstat, 2004; Fort *et al.*, 2013; Cantarel *et al.*, 2014). Critically, trait metrics are influenced by the environmental settings in which they are measured, and so literature based species level estimates will always be less useful for predicting plant-soil feedbacks than if traits are directly measured in experiments by plant-soil feedback researchers. Genotype x environment interactions would exacerbate this effect, and temporally lagged trait measurements are likely to be important to assess how both root and leaf traits adaptively change throughout the plant-soil feedback cycle. Finally, we found that whilst researchers are representing longer-term cultures and field cultures in their experiments, the time of growth response periods used in feedback assays are still limited, particularly for longer-lived species. This is important because more realistic timescales of response growth periods might be needed to allow compensatory growth to occur, and to determine the overall fitness benefits species gain from escaping conspecifics. Expanding our scope on all these fronts will be essential to bringer us closer to predicting interspecific plant-soil feedbacks effects in future.

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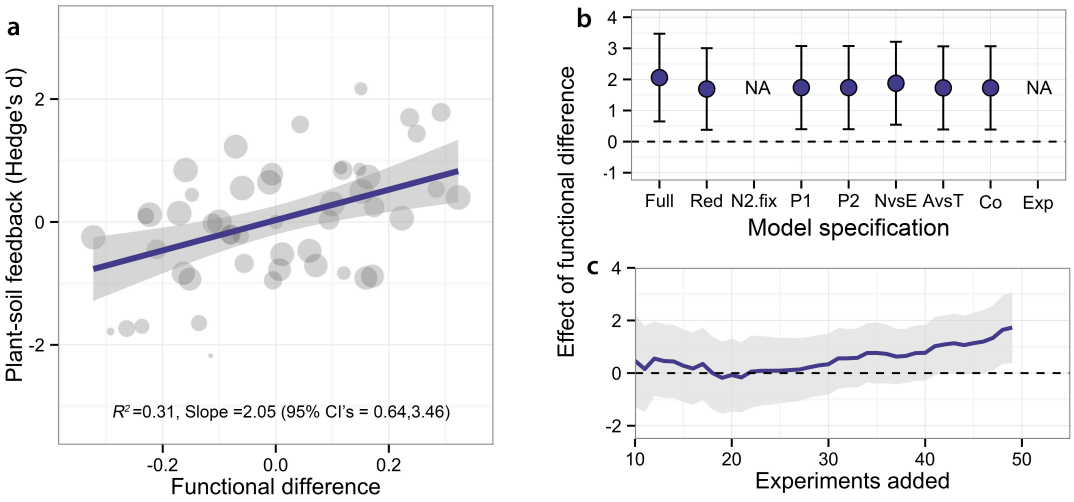


Fig S2. Re-analysis of soil biota plant-soil feedbacks and the resource economics spectrum removing filtrate inoculum (20-30 um) based studies. a) Meta-regression of experimental plant–soil feedbacks against plant resource economics differences between interacting species pairs, showing a clear positive effect of fast plants on soil biota based feedbacks b) The additive influence of different covariates on the slope estimates. X-axis labels represent the covariates included in meta-regressions with the functional difference predictor. N2.fix= N_2 fixing status of species pairs (e.g. Culture-Response status: No-No, Yes-No, & No-Yes), P1= period of soil culture, P2= growth response period, NvsE= provenance (native vs. exotic status of the species pairs), AvsT= measurement type (above or total biomass responses), Co= competition in response phase (alone vs. co-cultures of conspecifics), Exp= experimental setting (e.g. field vs. laft vs. pots in field vs. greenhouse), Full= all covariates, Red= no covariates. c) A cumulative meta-analysis was also performed to check for the effect of sampling variance (low to high) on the reduced model estimates for whole (c) and soil biota mediated (d) feedbacks. Negative and positive functional differences represent a heterospecific strategy "faster" or "slower" to that of conspecifics, respectively (Fig 1).

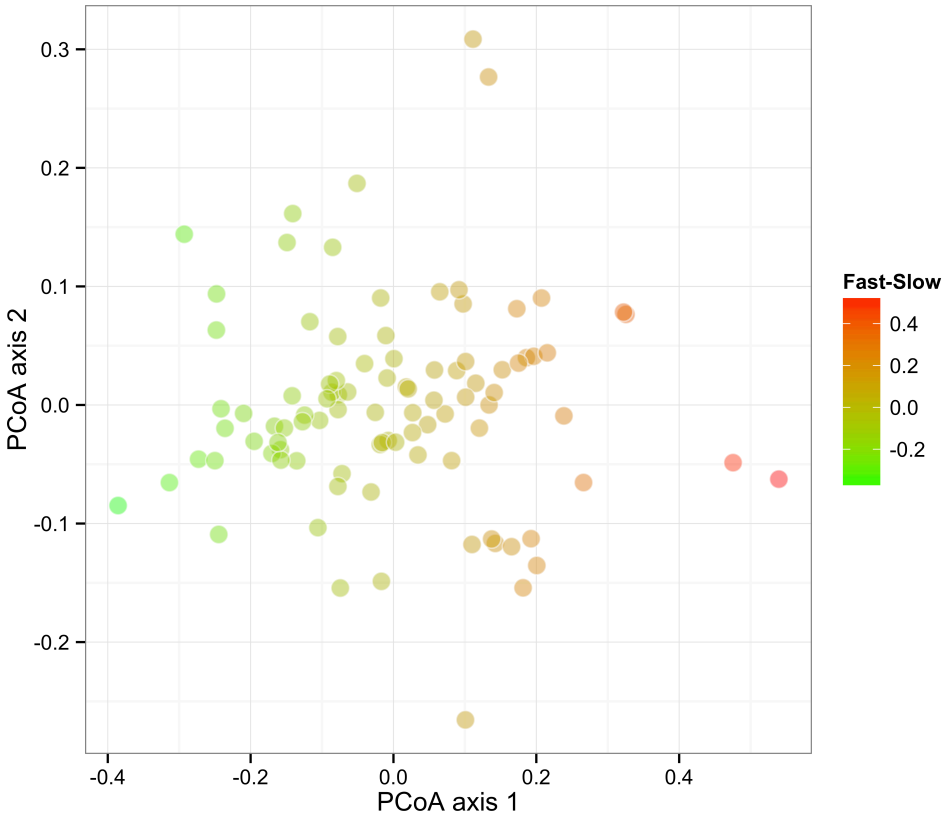


Fig S2. PcoA computed using a Gower dissimilarity matrix from a minimum of two "fast-slow" strategy leaf traits per species (Fig S5) The first PcoA axis was used to calculate functional strategy differences between plant species, and was highly positively correlated with Leaf [N], SLA, and moderately correlated with leaf [P] (Fig S2).

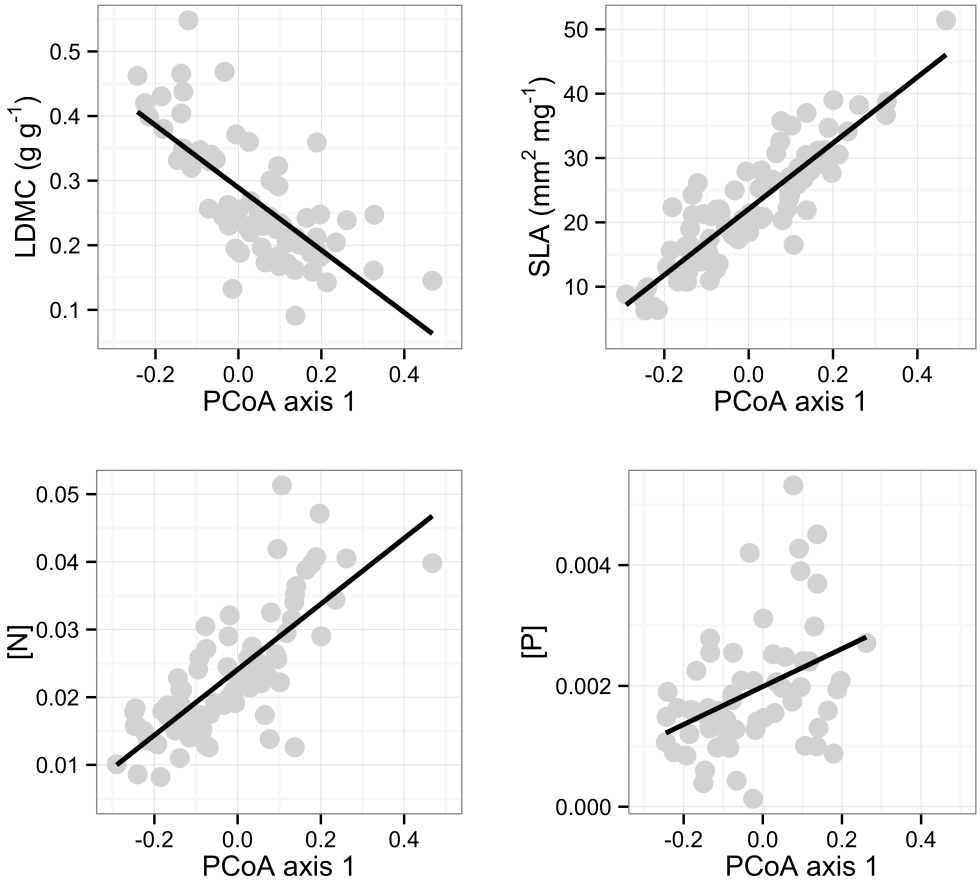


Fig S3. Correlations between PCoA axis 1 used to compute functional distances between species pairs and species level leaf trait values. LMDC: r=-0.72, P<0.0001, SLA: r= 0.90, P<0.0001; [N]: r= 0.79 P<0.0001, [P]: r= 0.45, P = 0.001.

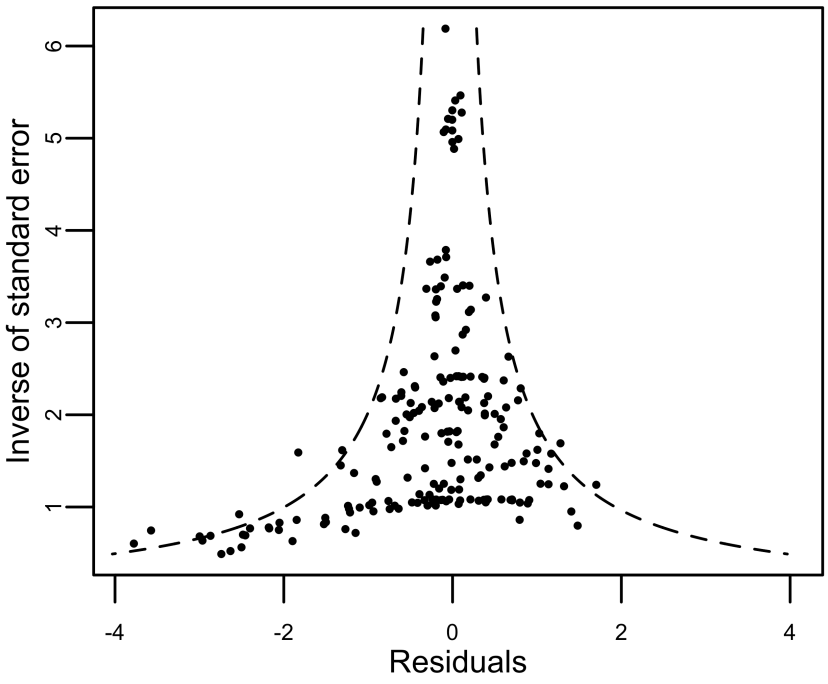


Fig S4 Funnel plot showing model residuals vs precision (inverse sampling variance). Model fitted for computation of residuals in R syntax was Hedge's d~ functional difference*feedback approach (soil biota inoculations vs. whole soil) + all other study level moderators + study & sampling level dependencies. Total experiments = 199.

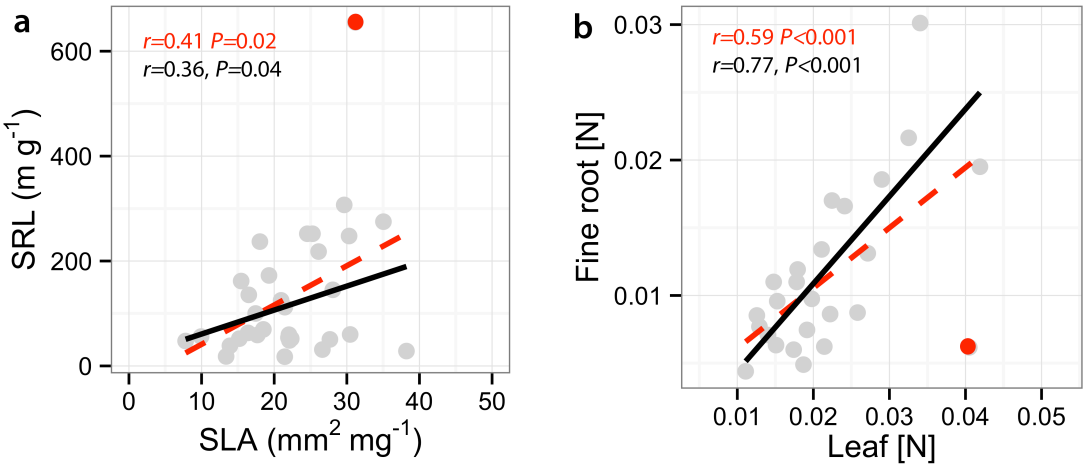


Fig S5. Correlations between leaf and root resource economic traits for a selection of species used in the study. a) correlation between specific root length and specific leaf area (n=30), b) correlation between fine root [N] and leaf [N (n=23)]. Red fits represent relationships including the potentially outlying red points.

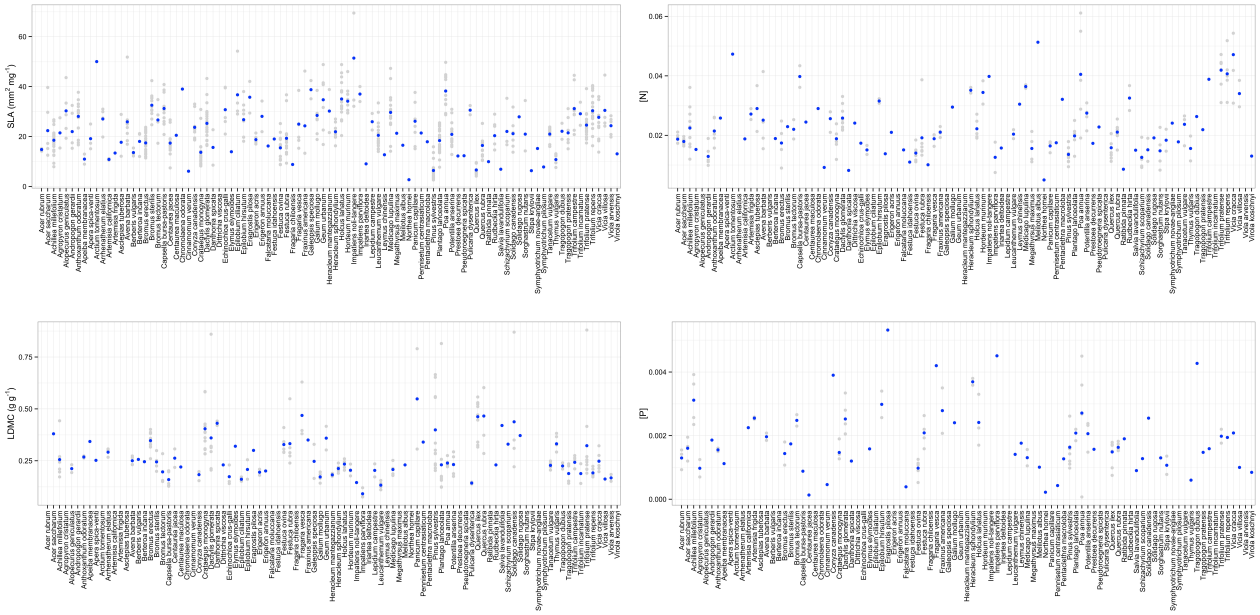


Fig S6 Continuous mean species level trait coverage for 95 species used in the study. Leaf traits were used to compute the functional distances between species pairs in 199 experiments. Blue points show means, and grey points show the underlying data used to compute the means. Data were compiled from TRY, LEDA, and the published literature (Thébault *et al.*; Hughes & Evans, 1961; Winter & Edwards, 1982; Nicola & Pickett, 1983; Oberbauer *et al.*, 1987; Alpert, 1991; Bigelow, 1993; Rich *et al.*, 1995; Cornelissen *et al.*, 2004; Zhang & Skarpe, 1996; Cornelissen, 1996; Cornelissen *et al.*, 1996, 2003; Meletiou-Christou *et al.*, 1998; Watling & Press, 1998; McKenna & Shipley, 1999; Medlyn *et al.*, 1999, 2002; Meziane & Shipley, 1999a,b; Pyankov *et al.*, 1999; Shipley & Lechowicz, 2000; Craine *et al.*, 2001, 2005, 2009; Shipley, 2002; Shipley & Vu, 2002; Quested *et al.*, 2003; Adler *et al.*, 2004; Diaz *et al.*, 2004; Harrington *et al.*, 2004; Wang *et al.*, 2004; Wirth & Buschmann, 2004; Wright *et al.*, 2004; Han *et al.*, 2005; Tjoelker *et al.*, 2005; van Staalduinen & Anten, 2005; Cornwell *et al.*, 2006; He *et al.*, 2006; Niklas & Cobb, 2006; Palacio *et al.*, 2006, 2008; Preston *et al.*, 2006; Feng *et al.*, 2007a,b; Garnier *et al.*, 2007; Kardol *et al.*, 2007; Karowe, 2007; Kazakou *et al.*, 2007; Palacio *et al.*, 2007; Cascorbi, 2007; Kleyer *et al.*, 2008; Kueffer *et al.*, 2008; Calvo-Alvarado *et al.*, 2008; Reich *et al.*, 2008, 2009; Yulin *et al.*, 2008; Cregg & Schutzki, 2009; Thorne & Frank, 2009; Laliberte *et al.*, 2010; Laughlin *et al.*, 2010; Messier *et al.*, 2010; Flory *et al.*, 2011; Kattge *et al.*, 2011; Drenovsky *et al.*, 2012; Burns & Strauss, 2012; Kraft *et al.*, 2014).

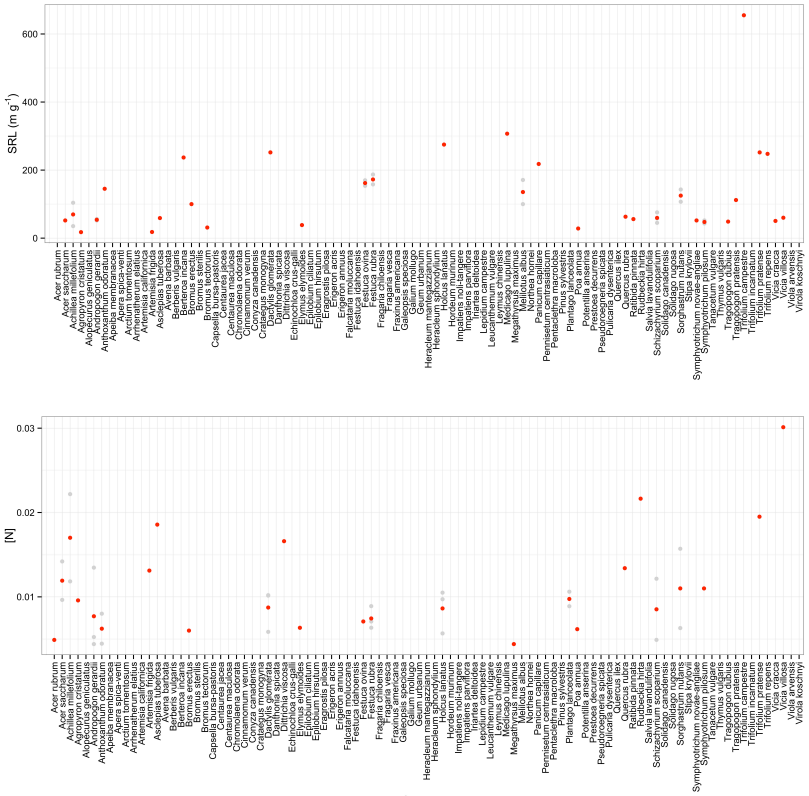


Fig S7 Continuous mean species level root trait coverage for 95 species used in the study. Red points show means, and grey points show the underlying data used to compute the means. Data were compiled from TRY, LEDA, and the published literature Meletiou-Christou *et al.*, 1998; Craine *et al.*, 2001; Comas & Eissenstat, 2004; Tjoelker *et al.*, 2005; Liu *et al.*, 2010; Kattge *et al.*, 2011; Fort *et al.*, 2013, 2015; Baxendale *et al.*, 2014; Smith *et al.*, 2014).

6. Effects of ecosystem change on coffee productivity[5]

Abstract

Loss of shade cover, agricultural expansion, and climate change have all been predicted to cause future coffee yields to decline, by increasing the frequency of, and susceptibility to, extreme weather and disease epidemics. However, field studies that simultaneously test the effects of multiple human based drivers of ecosystem change are rare, putting a limit on our ability to make realistic assessments of farmer risk and mitigation. To try and help fill this gap we assessed rain-fed coffee production across gradients of shade, elevation (a space-for-time climate warming proxy) and fragmentation within smallholder production systems in the Yayu UNESCO Man and Biosphere Reserve in Ethiopia. We found that increasing shade can offset increased understory temperature and vapour pressure deficit at lower altitudes. However, elevation and shade did not have similar effects on understory pest damage, diseases incidence, or coffee yields. Initial pinhead yields (i.e. following fruit set) showed a humpback relationship to shade, but were unrelated to elevation. Final yields (i.e. at harvest) were a function of both the humpbacked shade effect, and an additional positive effect of elevation: corresponding to patterns of lower coffee leaf rust and more stable leaf retention at higher altitudes. Fragmentation had no

[5] Typescript from Mehrabi, Z., Morel. A., Long, P., Noris, K., Mahli, Y., Davis, A., Gole, T., & Willis, K *et al.* Effects of ecosystem change on coffee productivity *In prep.*

effects on yield, but larger patches had higher tree diversity and therefore are likely to provide insurance against tree death and shade loss under future climate change linked drought events. Our results show that whilst moderate shade management can be critical for rain fed smallholder coffee production, it is unlikely to be able to mitigate coffee leaf rust epidemics and associated yield losses at low elevations. This suggests we need to concurrently promote optimal shading conditions at the local scale, and the active reduction of climate warming at international levels to prevent direct negative feedbacks onto smallholder coffee farmer income and productivity.

6.1 Introduction

Coffea arabica is a globally important non-food agricultural commodity, covering an estimated ~6 million ha of the land surface area and providing significant livelihood for its growers, traders, roasters and retailers (International Coffee Organisation, 2014; Food and Agriculture Organization of the United Nations, 2015). Emerging diseases, rising temperatures, frequent droughts and poor genetic diversity have all raised concerns over the future of the world's *C. arabica* production systems (Sylvain, 1958; Davis *et al.*, 2012; Baca *et al.*, 2014; Bunn *et al.*, 2014; Avelino *et al.*, 2015). Human driven climate change has been proposed to be a central point for concern (Jaramillo *et al.*, 2011; Davis *et al.*, 2012; Bunn *et al.*, 2014; Ovalle-Rivera *et al.*, 2015). However other factors at the local and landscape scale are also likely play important roles (Lin, 2007; Perfecto & Vandermeer, 2015). One such factor is shade cover, which can potentially buffer understory microclimate, prevent yield boom and bust cycles, control pest and disease outbreaks, maintain soil fertility and stop soil erosion (Beer *et al.*, 1998; DaMatta, 2004; Lin, 2010; Perfecto & Vandermeer, 2015). Another factor is forest

connectivity, which can maintain the movement of pest control and pollination agents across the landscape (Ngo *et al.*, 2011; Perfecto & Vandermeer, 2015), and might hold the potential to slow the spread of wind dispersed fungal diseases (Plantegenest *et al.*, 2007).

Taken together, these three human driven factors of shade, fragmentation and climate change are likely to provide a more suitable basis for prediction of risk for sustainable coffee production than any one factor considered alone. For example, systems at low elevation, with high fragmentation and low shade could be deemed to be 'high risk' and require the most immediate attention from policy or on the ground mitigation. However, the spread of fungal pathogens at high elevations (Nutman, 1970; Hindorf & Omondi, 2011) and reduced light in shaded environments (Cannell, 1985), might actually drive more of a Goldilocks like effect, with productivity peaking at an optimum elevation and shade level. Currently, however it is unclear which patterns are most relevant as there are very few field studies that actually look at the effects of this trio of human drivers together in real world smallholder production systems.

Here we report the results of a field study designed to predict the effects of shading, fragmentation and elevation (a space-for-time climate change proxy) on *C. arabica* production. We sampled coffee farms across the three gradients simultaneously, and assessed the effect of these multiple drivers on coffee system properties and productivity. Our study site is the UNESCO Yayu Man and Biosphere Reserve, an important flagship for the conservation of the world's coffee genetic resources, placed in one of the core coffee production areas in Ethiopia (Gole, 2003). The dataset we present here is based on 54 smallholder coffee farms, and is used to track coffee fruit and leaf dynamics, understory microclimate, soil chemistry, disease incidences, pest damage, and harvestable yields across the three gradients of elevation, shade and fragmentation. We

analyse these data using a two-pronged approach- first by testing for consistent effects of the three predictors on the state of yield in farmers plots, and second by performing an exploratory analysis to model the relationship between disease over the growing season and harvestable yields. We use this study to test the idea that shade reduction, fragmentation, and climate change each independently add to production risk, and to highlight the opportunities available for improving productivity into the future.

6.2 Materials and methods

6.2.1 Site selection

54 individual coffee farm plots were selected along gradients of elevation, patch size and shade intensity. To direct sampling the landscape was stratified into 9 contingencies based on 3 levels of patch size, 2 levels of elevation, and 3 levels of shade. Patch size classes were terciles of forest *ln*(area), with forest classifications based on an 0.7 thresholded Normalized Difference Vegetation Index calculated from a cloud-free Landsat image composite of the study region (NASA, 2014). Elevation classes were split above and below 1600 m based on the Shuttle Radar Topography Mission digital elevation product (USGS, 2006). A mask was built for accessible wet-dry season study areas within the boundaries of the Yayu Biosphere Reserve, and points were randomly placed at distances >250m in each of the patch and elevation classes using The Sampling Design Tool in ArcGis 10 (ESRI, 2012). Each point was geolocated by GPS (Garmin 62s) during March 2013, and farmers who owned the plot were approached and asked if they would in principle be interested in participating in the study. Canopy scope measurements (Hale & Brown, 2005) were taken at the centre and corners of a 20 m^2 grid surrounding the plot - with all plots then stratified by 3 classes of average

shade intensity. We implemented a stopping rule in our sampling of random points when 6 farms in each of the 9 classes had been located.

6.2.2 Vegetation survey

At the centre of each plot a permanent 20 m^2 study grid was established, following the Global Ecosystems Monitoring network protocols (Mathews *et al.*, 2012). Briefly, all stems > 10cm diameter at breast height (DBH) were tagged, and their DBH taken. Specimens for each tree were sampled, pressed, sorted to morphospecies and assigned scientific binomials at Addis Ababa Herbarium. Above ground biomass (AGB, kg) was estimated for each 20m^2 plot using the equation Eq.1 from Chave *et al.* (Chave *et al.*, 2005):

$$AGB = \rho e^{-1.499+2.148ln(D)+0.207(ln(D))^2-0.0281(ln(D))^3} \qquad \text{Eq. 1.}$$

Where D is the stem DBH (cm) and ρ is the mean species specific wood density retrieved from the Global Wood Density Database (Chave *et al.*, 2009). Regional or global generic means were used for species not held in the database, and species without confirmed scientific binomials at the time of analysis were assigned the species average from this dataset (0.552 g cm^{-3}).

Canopy tree α diversity was computed as the reciprocal of Simpson's diversity index (Simpson, 1949), and β diversity as the local contributions to beta-diversity (LCBD) (Legendre & De Cáceres, 2013), from relative species abundances of all stems >10cm. LCBD is calculated as the proportion of the total variation in a farm (site) x species abundance matrix accounted for by the variation in species abundances at any one farm (Legendre & De Cáceres, 2013),

6.2.3 Understory microclimate

We used a multi-data synthetic approach to estimate understory microclimate for a 365-day period from 1st Oct 2013- 31st Aug 2014 covering our growing season. To record differences in understory microclimate across farms, temporary temperature and humidity dataloggers (Lascar EL-USB 2) were installed during April-May 2014, South facing, 3 m above the ground on the trunk of a tree in each plot. To track longer term temperature and humidity, alongside precipitation and solar radiation, additional permanent meteorological stations were assembled in a subsample of plots and in unshaded conditions. All data-loggers were run and logged at 30 min intervals. We then used temporal overlap in records, to calculate the mean difference between temporary datalogger measures daily minimum and maximum temperature and humidity readings at each plot, and those measured at a single focal undercanopy meteorological station. Using these empirically estimated deviations, we were able to project undercanopy microclimate forward for each plot using the focal permanent weather station data. To project back in time we used the same calibration method, but with the Climate Forecast System Reanalysis product for 2013-2014 for a 38 km^2 grid square encompassing our study plots (Saha *et al.*, 2010) (ordered from http://globalweather.tamu.edu).

To estimate the ratio of transmitted solar radiation, hemispherical photographs were taken at the center of each plot directly above the canopy of the three coffee shrubs sampled for fruit and leaf measurements (*Coffee disease surveys*) (Sigma 4.5mm DC HSM F. 2.8 circular fisheye, a Canon 50D, Manfrotto leveled tripod, oriented North). Blue channel images were extracted and thresholded in FIJI (Schindelin *et al.*, 2012) and the ratio of undercanopy

transmitted solar radiation computed using Gap Light Analyser (Frazer *et al.*, 1999). Transmitted solar radiation (MJ m^{-2} d^{-1}) was then computed as the product of the solar radiation recorded at local permanent unshaded met stations (CFSR data for back projection) and the ratio of transmitted solar radiation for each plot. We then used the projected undercanopy air temperature and humidity estimates to compute daily potential evapotranspiration (Et_0) using the FAO Penman-Monteith equation (Allen *et al.*, 1998) implemented in the et0() function in the *sirad* package in R (Bojanowski, 2013; R Core Development Team, 2013), setting below canopy wind speed to 0.05 m s^{-1}, and clear sky solar radiation (R_{so}) to Eq. 2.

$$R_{so} = R_a\,(0.75 + 2{\times}10^{-5}{\times}z) \qquad \text{Eq. 2.}$$

Where z is the site-specific altitude in m, and R_a is the extra-terrestrial solar radiation. For back projections with CFSR data, only daily mean humidity measurements were available, and so in these cases we used the modified actual vapor pressure calculation (e_a), Eq. 3.

$$e_a = \frac{RH_{mean}}{100}\left[\frac{e^0(T_{\max})+e^0(T_{\min})}{2}\right] \qquad \text{Eq. 3.}$$

Where $e^0(T_x)$ is the saturation vapor pressure (kPa) at the specified (min or max) temperature, and RH_{mean} is the mean relative humidity (%). Throughout we assume a crop coefficient of 1(Allen *et al.*, 1998).

At the centre of each plot, equidistant from the three focal coffee shrubs sampled for fruit and leaf measurements (*Coffee disease surveys*), we non-destructively sampled soil to 1m rooting depth using a gauge augur (Eijkelkamp

Co.), and for each distinct horizon, determined the textural soil class by touch using a dichotomous texture key, and then estimated % total volume of gravel (> 2mm) by homogenizing soil and measuring its volume in a measuring cylinder with and without gravel removed. Using textural class data we estimated water held at field capacity (33 kPa tension) and permanent wilting point (1500 kPa tension) for each horizon (Saxton & Rawls, 2006), setting organic matter (OM) in topsoil (0-20cm) to 2 x empirically measured soil organic carbon (*Soil chemistry parameters)* (Pribyl, 2010), and OM at 21-40cm, 41-60cm, 61-80cm and 81-100cm to be 21%, 13%, 9% and 7% of the total (Jobbágy & Jackson, 2000). Weighted sums (by horizon depth) of water holding capacity and gravel content were computed to gain integrated estimates down to 1 m rooting depth. We then used water holding capacity estimates, and undercanopy daily Et_0 at each farm, alongside blanket precipitation records from our unshaded meteorological stations (or the CFSR product for backward projections), to run a simple plant available water simulation for the 365 day period for each plot, initiated at field capacity:

$$Paw_{i+1} = (Sw_{i+1} - WP) \times (1 - (G/100)) \qquad \text{Eq. 4.}$$

$$Sw_{i+1} = \begin{cases} FC - Et_{0i+1} & Sw_i + ppt_{i+1} > FC \\ (Sw_i + ppt_{i+n}) - Et_{0i+1} & otherwise \end{cases} \qquad \text{Eq. 5.}$$

Sw is the potential soil water content (mm), ppt is the daily precipitation (mm), Fc is the soil water held at field capacity (mm) and Et_0 is the daily potential evapotranspiration (mm). Paw is the plant available water (mm), WP is the water held at wilting point (mm) and G is the volumetric gravel content of the soil (%). Minimum Paw was used to estimate maximum potential water deficit during the year for each plot.

6.2.4 Coffee disease and yield surveys

Three productive coffee trees (>1.5 m high) were randomly selected within a 5 m radius of the hemispherical photograph taken at the center of each plot, and three plagiotrophic branches (20 cm, 40 cm and 60 cm from the apex) tagged on a single productive orthotropic main stem per tree for disease and yield survey measurements. During early May 2013, ~8 weeks after flowering, the number of leaves and pinhead fruits were measured on each marked branch. During mid July 2013, ~20 weeks after flowering, a suitable time to allow for fruit growth and the development of coffee berry disease (Mouen Bedimo *et al.*, 2007, 2010), the same branches were revisited and the number of leaves and fruits again counted. The incidence (presence-absence) of abaxial coffee leaf rust lesions (*Hemileia vastatrix*), and of leaf herbivory were recorded on each leaf, and coffee berry disease lesions (*Colletotrichum kahawae*), and berry herbivory recorded on each fruit. Leaf miner markings on leaves, and evidence of wilt on stems *(Gibberella xylarioides*) were also recorded, but were at low incidence and so were disregarded in further analysis. During November 2013, immediately prior to harvest, marked shrubs and branches within each plot were relocated and leaf and fruit surveys repeated. Fresh cherry weights for each tagged branch were measured using portable scales. In addition, we (1) measured the weight of all the remaining cherries on the tagged orthotrophic shoot on each branch, to compute a sample to total orthotropic shoot yield ratio, and (2) repeated our vegetation survey for a 10 m^2 subplot within each 20 m^2 plot, identified all stems between 1-10cm DBH within it, and multiplied *C. arabica* stem counts by a 100 to estimate the number of orthotropic stems per ha^{-1}. For each coffee shrub used in the disease and yield survey supplementary biometric measurements were taken on height, canopy width and length, and diameter at 40cm height. The stability of leaf turnover over the growing season as determined with the coefficient of variation in leaf counts between the three survey periods.

6.2.5 Coffee leaf spectra

In May 2013, we sampled coffee leaves for spectral analysis. One non-chlorotic leaf was removed from the mid-point of each of three plagiotrophic branches (each opposite to those tagged for disease and yield surveys), placed in a ziplock bag with damp tissue paper, exhaled into, and then sealed (3 leaves x 3 shrubs=9 leaves per plot x 54 plots= 486 leaves). At base camp, spectroradiometric readings were taken with an ASD Handheld 2 spectroradiometer (range 325-1075 nm +/- 1 nm accuracy; resolution of 3nm) (Analytic Spectral Devices, Boulder, USA). The device was mounted on a levelled tripod within a black out tent, and each leaf positioned in a fixed clip 5 cm away and at 90° from the bare optic (25° field of view), generating a target spot of 22 mm. Illumination was generated with a fixed position halogen lamp and reflector (2.5 W). The device was optimized and reflectance calibrated prior to acquiring each set of leaf spectra for a given shrub (every 3 leaves) (with an average of 10 white reference SPECTRALON (Spectralon, Labsphere, North Sutton, NH, USA) readings and an average of 25 dark current readings). Leaf spectra (averaged over 25 readings per leaf) were all taken on the adaxial side of the leaf, away from the midrib, and at a site unaffected by fungal growth or insect damage. Reflectance's (R) were then extracted from each leafs spectrum to calculate a mND_{705}, a chlorophyll index that performs well across a variety of plant types (Main *et al.*, 2011):

$$mND_{705} = (R750_{nm} - R705_{nm})/(R750_{nm} + R705_{nm} - 2R445_{nm}) \qquad \text{Eq. 6.}$$

6.2.6 Bait lamina assays

Bait lamina assays have been proposed as a quick and easy means to proxy soil invertebrate feeding activity in upper soil layers (Helling *et al.*, 1998; Kratz, 1998). The assay we used was comprised of a plastic strip 1mm thick, 10 mm wide, and 120 mm long, lined with 16 holes (2mm diameter, separated by 5 mm distances= 8 cm soil depth assay), each filled with a standard bait mix (cellulose, wheat bran flakes and active charcoal in ratio of 70:27:3). During April-May 2013 we placed a minimum of 2 bait lamina strips at 10 cm N and S from the stem of each of our survey coffee trees in each plot, and replaced these at three 10 day intervals (2 strips x 3 shrubs x 3 replacements= 18 strips per plot x 54 plots = 972 strips). Retrieved bait lamina sticks were carefully cleaned of soil and held against a light source to identify the total number of holes that had been eaten and degraded during exposure to the topsoil zone surrounding the coffee plants.

6.2.7. Soil chemistry parameters

In late May 2013 (following the bait lamina assays) two 10 cm deep soil cores were extracted from the root zone of each shrub in the disease surveys, in North and South directions at 10 cm distances from the coffee tree stem. The 6 soil cores were pooled for each plot, dried at 40°C, and passed through a 2-mm sieve to remove gravel. Samples were then tested for exchangeable cations (Ca, K, Mg, Na, Al, Mn, Fe) using $BaCl_2$ extraction, and for total nitrogen and carbon content (organic and inorganic fractions). Analyses were carried out by the Chemical Analysis Service at the Centre for Forestry and Climate Change, Surrey, UK.

6.2.8 Data analysis

We tested for effects of elevation (m), patch size (*ln* km^2) and shade (% openness) on all our measured responses. ABG (*ln* kg), 1/D (Simspon's reciprocal index), LCBD (local contributions to beta diversity), Daily T_{max} (°C) Daily RH_{mean} (%), Daily VPD_{max} (kPa), Daily Et_0 (mm), Soil chemistry parameters (CN, [N], CEC, Al-Mn-Fe saturation), mND_{705} and leaf turnover were all fit with linear models (LMs), disease incidences (CBD, CLR, CLH, CBH), bait lamina assays, and N_2 fixing stem incidences were fit with quasibinomial generalized linear models (GLMs) with a logit link; and mean fruit counts per shrub (pinhead and harvest) were fitted with quasipoission GLMs with a log link in R. One plot without canopy trees was excluded from analysis of vegetation survey data. Bait lamina responses and disease incidences were summed at the plot level prior to analyses. The functional form of the relationships between mean centered predictors (patch size, shade and elevation) and the responses was investigated using partial plots, and quadratic terms included to account for curvature. Diagnostics were performed graphically. For coffee berry disease incidence, legume incidences, and harvestable fruit counts valid but extreme outlying observations led to unstable parameter estimation, and so in these three cases we improved fits by extracting Huber-type weights from glmrob() objects in the *robustbase* package (Rousseeuw *et al.*, 2015), to use in classic quasilikelihood models. We checked for multicollinearity in elevation, patch size, and shade. Regressing all predictors against each other resulted in a reasonable summed R^2 value of 0.44. Variance inflation for our three predictors consistently small across models presented, with factors in the range of 1-1.6. We made inferences on each predictor using F-tests. Goodness of fit is given as R^2 and *pseudo*-R^2 (pR^2) (1- (Residual deviance/ Null deviance) (McFadden, 1974) for linear and generalized linear models respectively; and ΔR^2 values given for model comparisons with each predictor removed. It is possible that mean diameter could be a factor driving fruit counts and so we

checked this by fitting this control variable in our fruit count models but it did not change our estimates. The total number of observations varied throughout our analysis due to unplanned data losses (i.e. water ingresses in dataloggers, removed tag identifiers on shrubs, broken bait lamina assays, a spurious spectrum, and pre-harvested coffee trees prior to re-surveying) and intentional subsampling (i.e. soil chemistry). 54 observations were maximal, with reductions to 53 for bait lamina and leaf chlorophyll, 38 for microclimate, 37 for soil chemistry, and 36 for final cherry counts and leaf turnover. Despite this difference in observations, the ranges of predictor levels remained reasonably constant in all analyses.

To test for the effects of disease during the growing season on fruit drop and final yields we conducted an additional exploratory analysis. In the first exploratory model, fruit drop over the growing season (May – November) was fitted as a response and the proportion of each disease or pests fitted as predictors in a GLM with quasibinomial error and logit link. Predictors with explanatory power in the fruit drop model (coffee leaf rust and coffee berry disease) were fitted as predictors with initial pinhead fruit counts, in an second exploratory model to predict harvestable fruit counts, using a GLM with quasipoisson error and a log link. In all exploratory analysis coffee berry disease proportions were square root transformed to account for its long tailed distribution. Model checking and inferences for our exploratory analyses was conducted as above. Final yields as presented in the main text are based on a scaled harvest fruit count. Our scaling factor was generated by (1) fitting a LM to characterise the relationship between fruit counts and fresh weight cherry yields and point estimate fresh cherry weights (mean=1.033 gram per fresh cherry; $R^2 = 0.91$, $P<0.0001$) (2) by point estimating the ratio of sample to orthotropic shoot values across shrubs and plots (mean= 13.5) and (3) by point

estimating the number of *C. arabica* orthotropic shoots per ha (mean= 970 ha^{-1}, rounded to 1000 ha^{-1}).

6.3 Results

6.3.1 Agroforestry canopy

We found that above ground biomass held in woody stems increased by an additional 36.3% (95% CI's: 9.5-63%), for every 10% shade increase (Fig 1a). Canopy tree diversity was however not higher in more shaded systems, and only saw increases in larger fragments and at higher elevations (Fig 1b). Local shade tree canopy contributions to beta diversity (i.e. uniqueness) were not predicted by any of the three gradients (Fig 1c). The odds of encountering N_2-fixing stems in canopy trees showed quite variable relationships to shading and elevation, with a 33% increase (95% CI's: -5-90%) per every 10% increase in shade cover, and 17% decrease (95% CI's: 0-30%) for every 100 m ascent (Fig 1d).

6.3.2. Climate and soils

The median maximum daily farm understory temperature (measured at the height of coffee shrubs below shade trees) was 28 °C (Range: 23.7-35.7 °C). Maximum understory daily temperature in turn increased by 0.73 °C (95% CI's: 0.3-1.2 °C) for every 100 m drop in elevation, and 1.09 °C (95% CI's: 0.4-1.8 °C) every 10% drop in shade cover (Fig 1e). Maximum daily understory vapour pressure followed a similar pattern, but predictions were more uncertain for

elevation by an order of magnitude (0.07 kPa per decrease per 100m drop; 95% CI's: 0.008-0.13 kPa), than for shade cover (0.12 kPa per 10% shade drop; 95% CI's: 0.02-0.22 kPa), probably resulting from the lack of clear relationship between elevation and understory relative humidity (Fig 1f-g). We estimated that mean daily potential evapotranspiration increased by 0.6 mm per day with each 10% drop in shade cover (95% CI's: 0.56-0.71 mm) and as it was primarily dependent on solar radiation, did not change with elevation (Fig 1h). Whilst it is plausible that fragmentation could influence local climate (i.e. humidity conditions, or potentially land surface temperatures) we found that it was unable to predict microclimatic changes in the coffee understory. Soil water availability is an important aspect of coffee production (Carr, 2001), and so we ran a crude annual soil water budget simulation to estimate potential water deficit across the farms (*Methods*). We estimated minimum water budget values in a range of -271 mm to +103 mm to 1 m rooting depth. However, soil water holding capacity played an undetectable role in this (F test: $F_{1,36}$=0.6; P=0.4) , with potential soil water status simply a function of potential evapotranspiration (i.e. shade effects).

We assessed soil faunal feeding activity and soil chemical parameters at the base of coffee shrubs. We found that organic carbon in the soil surface-rooting zone of coffee decreased by 0.12% for each 1% increase in patch size (95% CI's: 0.03-0.21 %) (Fig i). The soil C:N followed a similar pattern, although in absolute terms the variability was small with an expected value of 10.3 (95% CI: 10.1-10.8). Soil cation exchange capacity (CEC) decreased with elevation (17% decrease for every 100 m drop in elevation; 95% CIs: 7-21 %) (Fig 1j). Saturation of exchangeable Mn, Al and Fe mirrored CEC and increased elevation (Fig 1k), although the maximum effect of 2.5% over the ~650 m elevation gradient in our system was small. Topsoil faunal feeding activity below coffee shrubs, was idiosyncratic and poorly predicted by shade, elevation or patch size (pR^2=0.02, P>0.05) (Fig 1l).

6.3.3 Coffee health

Coffee leaf rust (CLR: *Hemileia vastatrix*) lesions were negatively related to elevation, with the odds of a leaf being infected increasing by 43% for every 100m descent (95% CI's 21-68%). CLR was not related to shade or fragmentation factors (Fig 1 m). Leaf herbivory (CLH) increased with shade, with an odds of a leaf showing herbivore damage increasing by 58% for each 10% increase in shade (95% CI's: 16-120%) (Fig 1n). The stability of coffee leaf turnover was higher at high elevation, and to a lesser and inconclusive extent with lower shade (Fig 1o). Coffee leaf chlorophyll content (proxied through the spectral index mND_{750}) followed a very similar pattern to leaf turnover (Fig 1p). We found that whilst coffee berry disease (CBD: *Colletotrichum kahawae*) was relatively high (>30% incidence) in 4 plots at high elevation, elevation was generally a poor predictor of CBD using robustly weighted estimates (odds of infection per 100m ascent: 9%; 95% CI's: -30-75%); as was shade and patch size (Fig 1q). Signs of berry herbivory by chewing pests (CBH) could not be related conclusively to any of the three predictors (Fig 1r). We found that coffee fruit counts at the pinhead stage (e.g. immediately following fruit set) could be predicted by a quadratic relationship to shade, with fruit counts peaking at 70% shade cover and dropping by more than 40% at low shade cover and 65% at high shade cover (Fig 1s). We found that harvest fruit counts had a similar shade relationship to initial pinhead yields, accompanied by a positive effect of elevation (1.3 factor drop in fruit counts for every 100 m descent 95% CI's: 1.14-1.49 (Fig 1t).

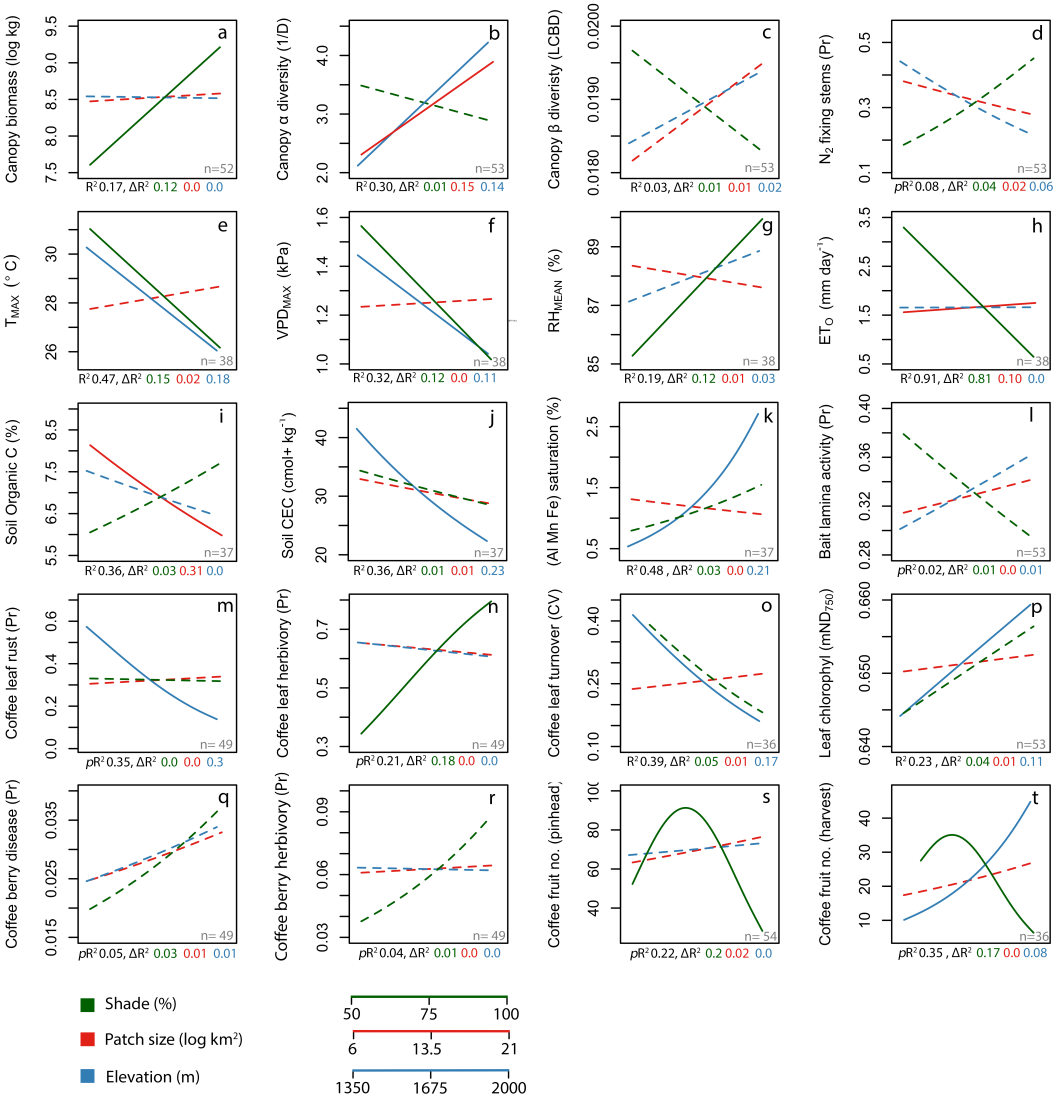


Figure 1. Effects of shade, elevation and patch size on *Coffea arabica* agroforestry. Bold lines represent significant effects, dashed lines are not significant at the 95% level. (a-d) *Agroforestry canopy* (e-h) understory daily microclimate; (i-k) topsoil chemistry; (l) topsoil faunal feeding activity; (m-i) coffee leaf pathology (j) instability in coffee leaf cover over the growing season (k) coffee leaf chlorophyll; (q-r) coffee fruit pathology; (s-t) coffee fruit counts. CV= coefficient of variation. VPD= vapour pressure deficit. Disease surveys were performed on 3 plagiotrophic branches on 3 coffee shrubs per plot, mid growing season, ~20 weeks post fruit set. Predictions are made holding other predictors at

their mean value. n= coffee farms, the unit of analyses. Differences in n are due to missing data or intentional subsampling, but the ranges of predictor levels remained reasonably constant across analyses (a-t). For full details and model specifications see *Methods*.

6.3.4 Exploratory yield modelling

To test the idea that indirect effects of diseases could be important for final yield estimation we set up an exploratory analysis. Given the results from our initial analyses, we hypothesized that (1) fruit drop over the growing season would be a function of the pests and diseases on coffee plants and (2) consequently that final yields (including the outlier CBD plots) could be best predicted based on a combination of knowledge on initial fruit sets and pest and pathogen incidences. We found that coffee berry disease and coffee leaf disease explained ~1/3 of the variation in fruit dropped by coffee shrubs over the growing season, but that coffee leaf and berry herbivores had little effect (Fig 2a). We also found that in all, coffee berry disease, coffee leaf rust and initial fruit counts, could explain nearly 50% of the final variation in harvest fruit counts. We scaled fruit counts using an empirically derived standardization factor to provide a 'potential yield' estimate (*Methods*). Using these standardized yields we estimate that a 50% incidence of either coffee berry disease on fruit, or coffee leaf rust on leaves, would result in fresh cherry yields of 131 kg ha^{-1} (95% CI's: 67-257 kg ha^{-1}) and 285 kg ha^{-1} (95% CI: 214-380 kg ha^{-1}), holding initial pinhead yields at their mean, corresponding to a 260 kg ha^{-1} and 140 kg ha^{-1} reduction in final yields relative to non-infected plants, respectively (Fig 2b).

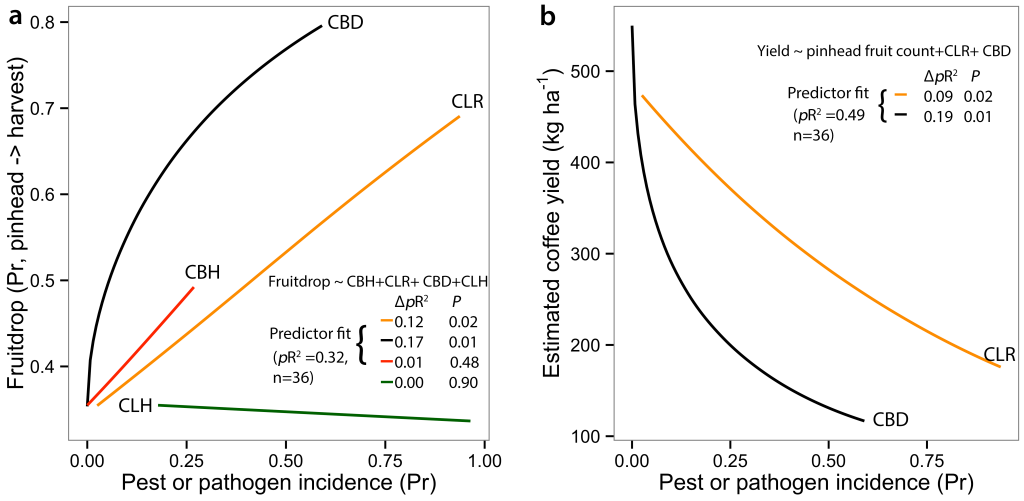


Figure 2. Exploratory modelling of *C. arabica* yields. a) predictions for probability of fruit drop over the season as a function of the proportion of different coffee pests and diseases. b) predictions for effect of fungal diseases on final harvestable yield, holding initial yields constant. Yields are potential estimates scaled directly from fruit counts, see *Main text* for full details. CBD= coffee berry disease, CLR= coffee leaf rust, CLH= coffee leaf herbivory, CBH= coffee berry herbivory. Disease surveys were performed on 3 plagiotrophic branches on 3 coffee shrubs per plot ~20 weeks post fruit set. All analyses are made on the farm level, on farms with available data at time of final harvest (n=36). Prediction surfaces in (a) are built holding other predictors at their their minimum values, and in (b) at their means.

6.4 Discussion

6.4.1. General Overview

In the introduction we asked if fragmentation, shade and elevation each add to production risk, and then asked if we could model final yields based on disease dynamics over the growing season. We found that production was optimal in moderate shading environments, was lowest at low elevations, and was not noticeably affected by fragmentation. Exploratory yield modelling showed that fruit drop over the season could in part be explained by coffee leaf rust, which was prevalent at low elevations, and accompanied by higher coffee leaf turnover and reductions in coffee leaf chlorophyll content. Additional variation in fruit drop could be explained by coffee berry disease, but this was unrelated to elevation or shading. The effects of shade and elevation were clearly non-substitutable, and despite the central importance of shade for yield management, we found little evidence that it currently offers a mechanism to mitigate fungal disease spread at low elevations.

6.4.2. Management opportunities

One key advantage of looking at multiple drivers in concert is to identify if common denominators exist for management. This is particularly relevant for low input smallholder coffee production systems, where it is critical to see if local management, such as shading, has potential to counter ecosystem changes outside farmer's control, such as climate warming. Previous studies have suggested that temperature is a key parameter for predicting *C. arabica* production: setting the optimal ranges for photosynthesis (DaMatta, 2004), rust growth (Avelino *et al.*,

2015), coffee berry disease (Nutman, 1970), and the coffee berry borer (Jaramillo *et al.*, 2011). We found that maximal understory temperatures changed with shade to the same extent as with the elevation ranges in our study. This tight correspondence might suggest shade has potential to be used as a mitigation mechanism against climate warming. However our results do not support this idea. In contrast, our results highlight some of the details of how shade and elevation may have very different effects on disease dynamics and coffee yields.

Shade regulates light transmitted into the understory, which is required for flowering (Cannell 1985). Transmitted light also controls potential evapotranspiration and thus determines the success of water filling during flower and berry development (Carr, 2001). This potential for negative effects of both high and low shade on fruit development at the pinhead stage could readily explain the hump-shaped effect of shade on coffee yields we observed. Although field studies assessing shade effects on coffee production systems are scarce, similar Goldilocks type effects of shade have been previously shown in other low input smallholder farming systems in different continents (Soto-Pinto *et al.*, 2000; Perfecto *et al.*, 2005; Perfecto & Vandermeer, 2015).

We observed an elevation effect on final harvestable yields, which was not present at the pinhead fruit stage, and thus developed during the growing season. Coffee leaf rust incidences ~20 weeks post fruit set also showed marked elevation trend. This pattern was matched by lower chlorophyll content in leaves, and higher leaf instability at low elevations indicative of higher leaf drop and shorter retention periods at low elevation where leaf rust infections were highest. Coffee leaf rust infection levels in turn explained a significant proportion of the variation in fruit drop and final harvestable yields, indicative of the commonly observed mechanistic link between rust infections and yield (Schieber, 1972; Avelino *et al.*, 2015). Unlike with elevation, coffee leaf rust was not related to shade, and did

not follow a hump-shaped relationship as would be predicted if rust infection was related to initial pinhead fruiting densities (Kushalappa, 1989).

Increases in coffee leaf rust and associated yield losses at lower elevations have previously been suggested to be the result of more optimal temperatures for rust growth (Avelino *et al.*, 2015). The question remains as to why elevation would create these optimum conditions and not shade. The likely explanation is two-fold. Firstly, reductions in shade can raise diurnal temperatures within an optimum range, but will also remove canopy buffering and expose the understory to colder nigh time and dawn temperature extremes that might limit rust growth (Kushalappa, 1989; Avelino *et al.*, 2004). Contrastingly elevation influences night and day temperature extremes in the same direction. Second, shade removal, unlike elevation decline, is accompanied by an increase in transmitted understory light, and a marked decrease in understory relative humidity, both of which are known to markedly inhibit rust growth (Kushalappa, 1989; Avelino *et al.*, 2004; López-Bravo *et al.*, 2012).

Clearly the effects of elevation (or climate warming) are distinct from those of shading. Shading is therefore unlikely to be useful as a direct mitigation strategy for warming effects on disease dynamics and yield losses. However, optimum shading is essential for pinhead fruit development, and so both shade effects and climate-warming effects need to be considered jointly for maintaining coffee productivity. Our predictions on the effects of these two factors on coffee productivity are shown graphically in Figure 3. The realised effects in this study suggest the current potential opportunities for increased production (blue arrows) largely depend on how far the distribution of shading in the population of farms is currently from the 'moderate' optimum, and how realistic reductions in a climate warming trends are based on international success in reducing green house gas emissions (Fig 3C).

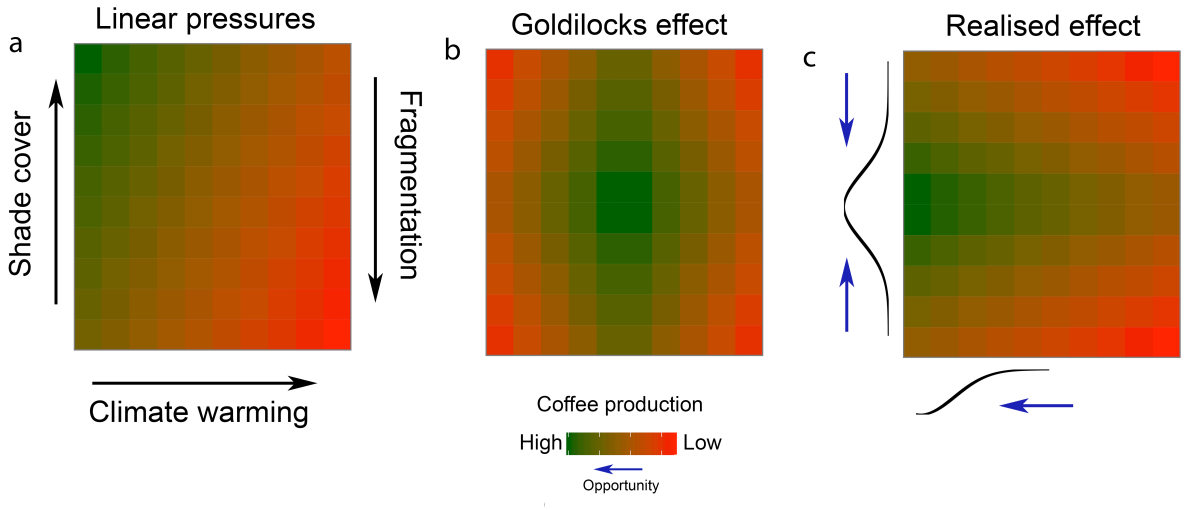


Figure 3. Management opportunities in low input smallholder coffee production systems. (a-b) the hypothesised effects of shade cover, climate warming and fragmentation on coffee productivity (c) the realised effects supported by this study. Shade shows a hump-backed relationship to yield, and elevation (our space for time climate warming proxy) showed a positive effect on yield. Fragmentation showed little effects on yield. Management opportunities are shown in blue arrows. The current scope for increasing productivity will depend on the distance of local distributions of shade cover within a given coffee production zone from the optimum, and the ability for the nations of the world to actively reduce climate warming influencing local production.

6.4.3. Fragmentation and biodiversity loss

We did not find direct effects of fragmentation on coffee productivity in this study. There are at least two ways that fragmentation might have influenced the main results we present. Firstly it might be expected that larger patches contain greater abundance and diversity of coffee pollinators, and that these in turn lead to an increased pinhead fruit set (Ricketts *et al.*, 2008; Potts *et al.*, 2010). Whilst, pollination benefits to fruit sets have previously been shown for *C. arabica* (Klein *et al.*, 2003), given its high levels of self-compatibility, it is possible that that any added pollination service benefits were simply not detectable against the shading effects on pinhead fruit counts. Second it might be expected that larger patches and higher tree diversity dilute or obstruct fungal disease spread in the landscape and within plots (Keesing *et al.*, 2006; Plantegenest *et al.*, 2007; Perfecto & Vandermeer, 2015). However, whilst dispersal obstructions within and between plots may influence the number of rust infections, if rust growth, and secondary rates of infection on a coffee tree are disproportionally increased by optimal temperatures then local warming may simply override these effects.

Despite the lack of effects of fragmentation on coffee yields, maintaining forest cover is likely to to be an important strategy in coffee agro-forestry landscapes. Not only can forest loss influence physical dynamics such as increasing surface water flows and erosion (Labrière *et al.*, 2015), it also can cause both immediate and lagged losses in biodiversity (Haddad *et al.*, 2015). In our study we found that larger patches had greater tree diversity and smaller isolated patches had were less diverse. These biodiversity losses may influence current value of the coffee agro-forestry system for honey production, the collection of tree based spices and medicines, or materials for building (Senbeta *et al.*, 2013). Retaining diversity in the longer term is also essential to ensure different tree species with similar use values but different environmental responses are kept as insurance for

productive outputs in face of drought, disease or environmental shocks (Oliver *et al.*, 2015; Trumbore *et al.*, 2015).

6.4.4. Study limitations and future directions

Looking forward, it is pressing to determine how relevant particular ecosystem changes are to coffee yield stability between years. Short-term studies such as this one may underestimate the benefits of shade management over the long term. For example, one of the commonly cited values of maintaining a shaded system in coffee is to avoid boom and bust cycles (Beer *et al.*, 1998; DaMatta, 2004). Similarly, the value of shade cover or canopy tree diversity in larger patches for safeguarding coffee productivity against climate shocks such as El Nino events, or extreme weather such as high winds and storms are important in the long term, and have largely been overlooked by this study (Perfecto & Vandermeer, 2015). Longer term monitoring of the effects of shade on yields, with respect to the more general effects of climate change is thus greatly needed.

Conceptual typologies of intensified versus extensified farm management are often used to characterise the benefits and costs of farmer decision-making (Avelino *et al.*, 2004; Perfecto & Vandermeer, 2015). The coffee farms we used in our study are all low input rain fed agroforestry systems, with no organised irrigation, fertilizer or pesticide application regimes. The value of understory microclimate regulation via shade management, or the importance of climate warming on disease dynamics, and associated impacts on coffee yields are likely to be increased in our study relative to systems where these effects may be masked by other inputs. It is therefore likely that there are limitations in applying our findings to the economics of more intensified systems.

Whilst we used elevation as a climate warming proxy in our study, and carefully sampled across our elevation gradient with respect to shade and fragmentation gradients, we were unable to completely rule out the effects of hidden factors that covaried with elevation. For example although we found little evidence that elevation effects on temperature was confounded with other microclimatic variables, farm canopy tree diversity increased, and soil cation exchange capacity beneath coffee trees decreased with elevation. Whilst we expect that coffee fruit and leaf drop resulting from leaf rust infections may have contributed to changing soil chemistry below the coffee shrubs, further experimentation would be needed to disentangle this effect from contributions of higher soil CEC to coffee leaf rust infections.

Finally, work in smallholder coffee production systems in Central America; have shown that coffee leaf rust dynamics can be controlled by a complex web of multi-trophic interactions (Perfecto & Vandermeer, 2015). The poor performance of climate change negotiations and efforts to curb green house gas emissions further suggest that 'on farm management' of climate warming linked disease outbreaks may be quickest way for farmers to manage productivity in the years ahead. Understanding the trophic control on fungal diseases in coffee is therefore high priority. The fact that wild *C. arabica* exists in Ethiopian forests adjacent to managed systems, offers a unique opportunity to understand how intensification and domestication has led to the loss of natural biocontrol services, and to develop new solutions to build natural biocontrol services back into current management practice.

6.5 Conclusion

Climate change, landscape degradation, and agricultural intensification, are all likely to present a grand challenges to the future of smallholder coffee production systems. Here we argue that central to overcoming this challenge is simultaneously identifying the functional form of each of these effects in real world settings. We show that shade management shows a hump-shaped relationship with yield. Elevation shows a positive effect on yield, and is inversely associated with increase in coffee leaf rust and leaf drop. Fragmentation had little effect on coffee yields, but larger patches maintained higher levels of tree diversity, wish may provide insurance in shade cover under unpredictable climate forcing in future. The current opportunities for increased production largely depend on how far the distribution of shading in current population of smallholder farms is from the optimum, and how realistic reductions in a climate warming trends are based on international success in reducing green house gas emissions.

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7. General Discussion

7.1. Overview

This thesis uses a wide range of different approaches, techniques and study systems. It is concerned with understanding how productive ecosystems can be designed. An important question addressed within this context is if we can predict the relationship between biological diversification and productivity. I asked why spatial diversification in plant systems might lead to higher belowground productivity, and then I asked what the effects of spatial diversification in microbial systems might have for the control of plant pathogens. Following this I asked if we could predict the effects of temporal diversification on plant productivity through the study of plant-soil feedbacks. Finally I went on to study how already existing diverse ecosystems, such complex coffee agroforestry intercrops, can be practically managed to maintain or even increase productivity. As discussed in the *Introduction,* predicting how multiple species interact in space and time is not simple. It is however essential to understand if we are to design productive ecosystems that do not negatively feedback in the long-term. The best predictions are underpinned by causal explanations. This thesis does not attempt to offer all the answers, but advances some novel ideas and fills some important research gaps. Below I summarise the main findings from each of the research chapters. I then synthesise the findings and discuss the future directions ahead.

7.2. Main findings

In chapter 2, I performed an experimental test of the effect of plant diversity on complementarity between belowground bacteria. My work laid out a new framework for investigating opposing selection pressures on population versus community level microbial productivity in the belowground rooting environment. I found that it was possible to niche differentiate soil bacteria *in situ* from a common source inoculum and that more niche-differentiated microbes are more complementary. My experiments support the idea that diverse plant communities are likely to contain more belowground niches, harbouring less productive microbial populations and potentially more complementary microbial communities. I found that the optimum community level productivity occurred at the intersection between the maximum population level productivity and minimum inter-population antagonism. Using common garden experiments to mix soil microbiota across different plant root systems is likely to offer a new way to test selection for ecological interactions in real world settings. My findings might help explain the to date often unexplained variation in aboveground diversity-belowground productivity relationships.

In Chapter 3, I performed *in vitro* BEF experiments to test the effect of increasing bacterial diversity on inhibition of the take-all fungal pathogen, and genotyped and analysed the bacterial diversity patterns on wheat root systems in take-all suppressive and conducive soils to see if field patterns matched the expectations from my simple models. I found that, when forced together, rhizosphere bacteria can undergo fierce competition, suppressing putative biocontrol isolates in mixture, and preventing the community from inhibiting fungal pathogen growth. My experiments orthogonally partitioned different

components of bacterial diversity and I found that increasing phylogenetic diversity and resource utilisation diversity does not reduce this within trophic level antagonism in spatially mixed or biofilm environments. I found that some wheat varieties can prevent take-all build up in the soil prior to a commercial wheat crop and that this benefit is negatively correlated with the richness of putative biocontrol bacteria in the rhizosphere zone. This chapter provides the preliminary insights into the belowground ecology of what might be a novel alternative to the continuous monocropping and yield losses needed for take-all decline to establish.

In chapter 4, I compiled a meta-analytic dataset of all available interspecific plant-soil feedback experiments (329 experiments, 276 unique species pair interactions) and then built a phylogeny for all species interacting in these experiments to test the hypothesis that phylogenetic distance is a useful predictor of negative interspecific plant-soil feedback effects. I found that plant species are not more productive when grown on soils cultured by species further away from them on the evolutionary tree. I also found that this pattern holds across many different species, life histories, life cycles, and exotic and native interactions and within monophyletic clades and for recently diverged species. These results suggest that using evolutionary distance as a rule of thumb in the design of temporal diversification plans is likely to be of limited use. This paper contributes to a building body of evidence that questions the general use of phylogenetic distance as a proxy for niche differences in ecology.

In chapter 5, I used the database I had built on interspecific plant-soil feedbacks, alongside plant resource economics traits (SLA, Leaf [N], Leaf [P], LDMC) gathered from the literature to test for a directional effect of plant resource economic strategy on interspecific plant-soil feedbacks. I found that plant strategy effects can be used to predict the outcome of plant soil feedback interactions. This is based on the action of dominating directional effects of

resource strategy differences that consistently influence species responses to interspecific soil cultures. Specifically, plants grown on soil cultured by species with 'faster' strategies to themselves perform more poorly than on soils cultured by species with 'slower' strategies. Controlling for soil chemistry removed these effects, suggesting a mechanistic link to soil nutrient depletion or immobilisation rather than soil biotic feedbacks. Although I found some correlations between above and below ground resource economics traits, the literature was lacking sufficient information on belowground rooting traits to verify if these effects are fully synchronised at the organismal level, and future work will need to focus better on measuring belowground traits, especially for predicting soil biota legacy effects. This chapter provides an important step forward in understanding the extent to which current data on plant resource economics traits can be used to predict temporally lagged competition mediated through the soil medium.

In Chapter 6, I conducted a carefully designed field study to assess the independent effects of shading, fragmentation, and elevation (a space-for-time climate warming proxy) on *Coffea arabica* productivity. I found that shade has a hump shaped relationship to yield, and that elevation had a negative relationship with yield. The drop in yield at low elevations was associated with a considerable increase in coffee leaf rust and leaf drop. However shading showed no relationship to coffee leaf rust questioning the ability for shade to be used as a direct mitigation strategy for its spread. I found no relationship between coffee productivity and fragmentation, but I found that larger patches contained more diverse canopy trees and might therefore be useful as insurance to maintain a persistent canopy under extreme weather events such as drought in the future. The current management options for increasing productivity seem to depend on how far the population of coffee farmers are from the optimum shading regime, and how well international efforts fare in curbing climate warming effects.

7.3. Synthesis and future directions

7.3.1 Design and the nature of antagonism

"Man selects only for his own good: Nature only for that of the being which she tends." — Charles Darwin

Competition within and between populations lays the heart of the success and failure in combining species together in synthetic ecosystems. The goal of the ecosystem engineer is to identify where antagonism is best placed in a system to maintain the desired functional outputs of a community. Experimental work in chapter 3 showed that competition amongst organisms within a given trophic levels can increase with diversity and reduce in the ability of that trophic level to control pathogen growth. This type of competition between potential biocontrol strains and the rest of the ecological community presents a long-standing issue to the application of biocontrol agents into complex ecosystems (Xu *et al.*, 2011). In complement to my model experiments I show, for the first time, that high or low build up of fungal pathogen caused disease in the field is correlated with the diversity of a biocontrol containing group of bacteria on wheat root surfaces, and that wheat variety specific soil culturing is responsible for this effect. Whilst the underling traits in wheat driving this effect are still a mystery, and more *in planta* work is required to validate my abstract experiments further, this work presents a fascinating opportunity for exploring how we might be able to engineer antagonism in the belowground environment for optimal crop productivity.

An exciting prospect for the architects of newly engineered ecosystems is to use evolution to shape the building blocks for productive communities. However, facilitation and complementarity between organisms will only persist when it is selectively advantageous for them to do so (Denison *et al.*, 2003). Furthermore,

even if species have evolved specific resource preferences, complementarity within communities in any given locality will only be possible if the abiotic environment supports it (Eisenhauer *et al.*, 2013). Gravel *et al.* (2011) seminally showed that it is possible to artificially select for complementarity between bacteria and optimise the positive slope of the BEF relationship. Recent work in plant systems has also shown that selection can drive divergence between plants and that this can lead to a self optimisation of community productivity in multispecies mixtures (Zuppinger-Dingley *et al.*, 2014). These examples give us insights into how we might begin to selectively breed multispecies ecosystems. In Chapter 2, I show that is also possible to niche differentiate populations of bacteria in different plant root systems. Whilst the relative impact of sorting, and *in situ* evolution in this system has still to be fully understood, these results show that inter-population antagonism can clearly be cultured against. My work advances our understanding by providing a conceptual framework in which we can predict optimal community level productivity. Counterintuitive as it seems, whilst antagonism between populations can negatively impact productivity, some level of antagonism might be essential to prevent frugal types evolving in natural populations, and maintain maximal productivity in mixture.

7.3.2 Design and the belowground environment

"We have to remember that what we observe is not nature itself, but nature exposed to our method of questioning"— Werner Heisenberg

In the introduction I argued that the belowground environment represents a 'final frontier' in our understanding of human designed productive ecosystems. A pertinent question is how much of this gap has been filled by this thesis. The advances in management of antagonistic belowground interactions discussed in the previous section, are a critical step forward, but the ideas on the factors controlling belowground microbial competition I have presented need to be tested and monitored across spatially structured environments and in a range of different settings. In terms of synthesis, I have compiled the world's first database on interspecific plant-soil feedback experiments. Previous works (Kulmatiski *et al.*, 2008) only considered the identity of response species, and not culture species identity. By identifying the plant species involved in both the culture and the response component of the soil feedback phenomenon, I was able to parameterise characteristics of each species and build these into predictive frameworks and to test relevant theories on plant competition mediated through the soil medium. I refuted a long-standing idea that implied that it was possible to predict interspecific feedbacks using phylogenetic distance, and then was able to begin to develop a mechanistic trait based lens to explore how dominant fitness differences between plants might operate through the soil medium. However, it is clear that many important plant traits have yet to be measured in the belowground system, and trait plasticity has been largely overlooked by my work. It is clear from both my experimental and synthetic chapters is that we still have much further to go before the we can say we have truly mapped the interactions taking place in the landscapes beneath our feet.

One of the key challenges we continue to face is that some components of the belowground community that associate with plant roots are simply intractable. Simple experiments and theory are critical but need to be validated at the level most relevant to practical application. In the case of bacteria there are billions in a small patch of soil, the majority unculturable. How they all interact amongst each other or in the context of plant root systems still remains largely unclear. Joining metagenomics, transcriptomics and metabolomics can help us get a picture of what is going on in the darkness underground (i.e. linking taxonomy to function). However, there are many challenges for inferring causality with these approaches. Consider the concept of the keystone species. Classic ecological work shows that loss of keystones can lead to the trophic collapse of ecosystems. Although we can identify putative microbial keystones belowground based on cross-correlations of residence times with the rest of the community (Fisher & Mehta, 2014; Faust *et al.*, 2015; Konopka *et al.*, 2015), there is no known way to selectively knock them out to see what the effects on ecosystem functioning would be. Bridging these gaps in future will be essential to mastering the design of the belowground environment.

7.3.4 Design applications

A central argument running through this thesis is that we need to gain better predictability over species interactions so that we can go on to design more sustainable ecosystems. Many of the ideas I have presented have been abstract. It is important to ask therefore how the findings of the chapters presented can be applied in real world settings, and if they can, to what degree they can be scaled in industry. Some applications are discussed below.

My work has shown that the idea of using relatedness to predict plant-soil feedback effects may be ill founded. Whilst I did not study agricultural crop species in my meta-analyses, the experiments I analysed covered a large range of the angiosperm phylogeny, different life cycles, and life forms. This approach is very powerful to ask from an ecological perspective if general patterns exist across a wide range of different species. My work has shown that we cannot simply expect diversification in time with more distantly related species to improve productivity or avoid negative feedbacks through the soil. Knowing which plants to grow best together in sequence within the constraints of market forces is a real world practical requirement for any consultant. As the focus on diversifying diets increases (Khoury *et al.*, 2014), and more underutilized crops come into production (Mayes *et al.*, 2012), then an even greater variety of potential crop sequences will become available. My work should be used to add as background knowledge in this decision-making process.

It is easy to see how the work I present on bacterial diversity associations with take-all suppression might be scaled. Firstly, it is important that the hypothesis I have forwarded hold after more extensive testing and *in planta* validation. If the ideas I present are indeed repeatedly proven valid, then the possibility of breeding the traits for variety based selection of belowground bacterial richness would be

massive step forward in the control of take-all disease dynamics. Currently take-all suppressive soils have only been observed to establish after large initial yield losses (Kwak & Weller, 2013). The prospect that initial densities of the disease can be modified simply by wheat variety choice would represent a huge social benefit to wheat growing regions by avoiding pesticide usage, improving yield stability, and subverting economic losses. The hypothesised mode of action (i.e. bacterial richness selection in root zone) essentially represents a stable mechanism for control of this pathogen, with little scope for direct resistance. However a question remains as to the stability of this type of control from pathogens on multiple trophic levels. Work has shown clearly that bacterial pathogens find it harder to invade more diverse bacterial communities in root systems and this can protect crops from succumbing to soil borne bacterial diseases (van Elsas *et al.*, 2012; Wei *et al.*, 2015). Engineering wheat plants to select low bacterial diversity in their root systems may allow putative biocontrol strains to combat take-all, but would make these systems vulnerable to new bacterial pathogens.

Finally, my work on the design of coffee agroforestry systems has application to the farmers I work with, and more generally for informing social actors trying to mitigate climate change through local diversification practices. Currently there has been much concern on the effect of climate change on coffee production across the world. The idea that local management of shade might counteract the effect of climate change driven physiological stress or disease dynamics in the coffee system and increase long term yield sustainability is highly relevant to the management of canopy structure in these systems. My work has shown, that whilst shading can be used to manage yields it cannot be used to combat the disease dynamics expected with warming events. This finding is crucial to our understanding of the extent to which climate change can be mitigated by current management practices, and to identify key leverage points for future work in these systems. Specific attention should be directed towards management of coffee leaf rust diseases in this system so that farmers can realise a reasonable

income. My work shows that a new integrated management planning system is greatly needed for these coffee farmers, and that investment in developing this should be prioritised by the Ethiopian government.

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8. Conclusion

Humans critically depend on the adequate design of productive ecosystems. Our current systems produce goods in abundance, but at a severe environmental cost. It is now beyond reasonable doubt that something radical needs to change in the way we design and engineer ecosystems for our needs. Diversification can offer a means to improve productivity and concurrently prevent environmentally costly losses, or negative feedbacks inherent in current monoculture designs. However, in order for this idea to be effective we have to develop better predictions of how species interact in space and time. Practically, we need to specify the productive outputs required for any given system, and then evaluate the functional forms of relationships, within and across trophic levels, needed for their delivery. More focussed trait based approaches are much needed. Critical gaps between niche theory, biodiversity research, agriculture and synthetic ecology need to be bridged. Only once this has been done will be possible to realise the full potential of mixed species designs in agriculture and industry.