

Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation

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Summary

1. Root symbionts (rhizobia and arbuscular mycorrhizae) are often assumed to increase agricultural productivity consistently. However, rhizobial and mycorrhizal strains vary in effectiveness, resulting in symbiotic associations that range from parasitic to mutualistic.

2. The extent to which different farming practices mediate evolutionary changes along this continuum of symbiont effectiveness is rarely discussed. However, evolutionary theory suggests that (i) fertilizer use will favour parasitism unless host-plants impose sanctions against less-effective mutualists; (ii) tillage will have contrasting effects because it decreases within-plant symbiont relatedness but also decreases the risk that mutualism will benefit future competitors; (iii) crop rotation can act as a selective agent against dominating symbiont genotypes; and (iv) rhizobial inoculation adds beneficial strains to the soil but may increase the frequency of mixed nodules that allow parasitic strains to escape host sanctions.

3. However, the existing empirical data are inadequate to test our predictions thoroughly. Changes in species composition have been documented as a result of management practices, but evolutionary changes in symbiont effectiveness are difficult to detect. Therefore, a major aim of this study was to stimulate research that will assesses directly changes in symbiotic effectiveness as a function of management practices.

Key-words: arbuscular mycorrhizae, crop rotations, inoculation, parasitism, rhizobia, symbiosis, tillage.

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Introduction

Studies of mutualisms in agriculture have traditionally focused on the potential of nitrogen (N_2)-fixing bacteria (rhizobia) and arbuscular mycorrhizae (AM) to improve crop yields. At least 70 million metric tons of nitrogen per year are produced by legume symbioses (Brockwell, Bottomley & Thies 1995), often contributing 100–300 kg N ha⁻¹ year⁻¹ (Burns & Hardy 1975; Paul 1988; Peoples, Herridge & Ladha 1995). Arbuscular mycorrhizae are considered valuable components in most agricultural systems due to their role in plant nutrition and soil health (Bethlenfalvay & Linderman 1992). For decades, crop scientists have attempted to maximize agricultural productivity by improving the effectiveness of these symbiotic relationships

(Roughly, Blowes & Herridge 1976; McLoughlin, Hearn & Alt 1990). However, manipulation to increase field-scale nitrogen fixation and phosphorous transfer continues to be met with limited success (Hamel 1996; van Kessel & Hartley 2000).

Symbiotic effectiveness describes the benefits to the host derived from the symbiotic association. The more effective a symbiont, the more benefits it provides to its host. Different genotypes of rhizobia and mycorrhizae vary in the benefit that they provide their host-plant (Thrall, Burdon & Woods 2000). For example: (i) in a study of the effectiveness of association between native rhizobia and *Acacia* species, Burdon *et al.* (1999) found that some rhizobial strains resulted in plants only 10% the size of those inoculated with the best strains; (ii) Denton *et al.* (2000) surveyed 61 Australian pastures and found that in 60% of these sites, the mean level of nitrogen fixation was less than half that of reference strains; (iii) of 150 mycorrhizae isolates tested on cassava *Manihot esculenta*, 40% were shown to be either ineffective or have marginal effectiveness (Howeler,

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Sieverding & Saif 1987). The effectiveness of some strains of mycorrhizae may depend on host genotype or soil conditions (Krishna *et al.* 1985; Manske 1989). However, symbiotic effectiveness should not be confused with the host-specificity of a strain. We define effectiveness as the benefit derived from the symbiont after the relationship has been formed. Specificity refers to the ability to form the relationship, not the benefits derived (Chanway, Turkington & Holl 1991).

The evolutionary dynamics underlying the relative success and abundance of strains with various mutualistic/parasitic tendencies is rarely discussed in agronomic studies. However, this is a fundamentally important topic, as different agricultural practices may favour more (or less) parasitic strains, leading to: (i) a physiological change in the effectiveness of rhizobia or mycorrhizae; or (ii) an evolutionary change in relative abundance of these strains. If we understand how agricultural practices affect the relative Darwinian fitness of different strains, then we could employ agronomic practices that would favour an increase in the relative abundance of the better strains. Practical applications of this information will depend on balancing long-term benefits derived from better mutualists with any short-term costs.

In recent years, understanding how selection pressures favour the evolution of mutualistic and parasitic tendencies has been the focus of much work by evolutionary biologists and ecologists. A large body of theory has been developed aiming to predict the factors that influence shifts in symbiotic functioning. These include very general models on the evolution of mutualists/parasites (Axelrod & Hamilton 1981; Bull & Rice 1991; Frank 1994, 1995, 1996, 1998; Ebert & Herre 1996; Doebeli & Knowlton 1998; Hochberg *et al.* 2000; van Baalen & Jansen 2001) as well as models developed for mycorrhizae and rhizobia (Jimenez & Casadesus 1989; Olivieri & Frank 1994; Simms & Bever 1998; Parker 1999; Bever & Simms 2000; West *et al.* 2002; West *et al.* 2002b). A crucial point in all these models is the recognition that symbionts face trade-offs between using or storing resources for their own reproduction vs. providing resources to their host. Symbionts that sacrifice their own fitness in favour of that of the host will be displaced by symbionts that maximize their own inclusive fitness. Therefore, mutualism will persist or increase only when some factor favours symbionts that contribute to their host-plants. Host-plants that provide less resources to or completely discontinue interactions with symbionts found to be less effective (termed host-plant sanctions; Denison 2000) could be one such factor.

Our major aim in this study was to demonstrate that the predictions of evolutionary theory can be applied to agricultural systems. We show how existing theoretical models can be used to predict how symbiotic effectiveness will be selected to increase or decrease under certain agricultural practices. Specifically, we considered the consequences of (i) fertilization regimes,

(ii) tillage management, (iii) crop rotations and (iv) symbiont soil inoculation. We argue that specific agronomic practices may increase the reproductive success (proliferation) of parasitic rhizobia/mycorrhizae strains relative to more mutualistic/beneficial strains, and so lead to a long-term deterioration in the composition and functioning of the symbiont communities.

Our second aim was to examine the extent to which existing data on rhizobia and mycorrhizae can be used to test our theoretical predictions.

Variation in resources and productivity: effects of increased fertilizer use

High productivity, within environmental and economic constraints, is the ultimate goal of modern agricultural systems. Global utilization of N fertilizer has increased sevenfold since 1960 (Peoples, Herridge & Ladha 1995); P fertilization use is forecasted to increase anywhere from 1.6-fold to 3.4-fold by 2050 (Tilman *et al.* 2001). It is well appreciated that in the short term, high fertilizer use will decrease the benefits derived from root symbionts. These effects are due to physiological rather than evolutionary change, and they do not necessarily involve decreases in the symbiont's N or P contribution per unit C invested by the host-plant, i.e. costs might decrease proportionally. For instance, addition of N fertilizer can lead to lower production of nodules in legumes (Rubio Arias *et al.* 1999; Vargas, Mendes & Hungria 2000) as well as lower N₂ fixation rates per nodule (Denison & Harter 1995). Mycorrhizal formation may be radically reduced in high P soils (Hayman 1975; Jensen & Jakobsen 1980; Hicks & Loynachan 1987).

However, we argue that there are a number of reasons why high resource availability may also favour a long-term (evolutionary) change in symbiont effectiveness. Changes in effectiveness could result from the following.

Increasing nutrient availability to plants can lead to a decrease in resource allocation to the roots (e.g. fewer nodules are initiated by legumes and less root area is colonized by mycorrhizae under high nutrient availability; see the references above). As opportunities for mycorrhizal infection decrease under high fertilization regimes, competition for these opportunities increases, favouring the proliferation of more aggressive strains (Johnson *et al.* 1992; Scullion, Eason & Scott 1998). It has been suggested that strains characterized by increased aggression towards conspecifics in high fertility soils are unlikely to be good mutualists (Johnson 1993; Graham, Drouillard & Hodge 1996; Johnson, Graham & Smith 1997). Investment in antibiotic production, for example, can come at the expense of symbiotic performance, as seen with rhizobia (Goel, Sindhu & Dadarwal 1999).

Increases in soil resource availability mean that resources available to symbionts from their host (e.g. photosynthate) are less dependent upon the symbiont's contribution. Models developed specifically

for rhizobia, which could also be applied to mycorrhizae, suggest that, in the absence of host sanctions, this decreased dependence on symbiont contribution would select for rhizobia with a lower level of N₂ fixation (West *et al.* 2002). This is because nutrients acquired via symbionts have a smaller effect on plant photosynthesis rates and therefore the overall level of circulating resources available to the symbionts.

However, at least in rhizobia, if host-plants preferentially supply more resources to nodules containing more beneficial mutualists, as has been suggested (Udvardi & Kahn 1993), this could reduce, eliminate or possibly even reverse the effects of fertilizer on the evolution of microsymbionts. Conclusive evidence for such plant sanctions is still lacking, although there is a strong theoretical argument that they are essential to the evolutionary stability of mutualism within multiply infected hosts (Denison 2000; West *et al.* 2002).

By reviewing existing studies that focus on the effects of long-term fertilizer application, we can begin to assess the validity of these predictions. Recent studies have shown that application of fertilizer can lead to long-term changes in mycorrhizal functioning, although it is difficult to determine if this is due to evolutionary changes within a species, by displacement of one species with another or by both. Johnson (1993) studied changes in mycorrhizal communities after an 8-year period in fertilized and unfertilized plots and found (i) test plants colonized by mycorrhizae from the fertilized treatment were smaller with reduced inflorescences; (ii) roots inoculated with mycorrhizae from the fertilizer treatments were dominated by vesicles (resource storage structures, suggested to be indicative of more parasitic behaviour) rather than arbuscules (nutrient transfer structures); and (iii) mycorrhizal species composition between the two treatments was significantly different. The last result (iii) is in agreement with other studies that have found shifts in mycorrhizal species composition but not necessarily evolutionary change within a species (Thomson, Robson & Abbott 1992; Gryndler & Lipavsky 1995). It is unclear if the species-composition data of Johnson (1993) also predict evolutionary trends. More details of the fungal community dynamics are needed to assess whether this species shift involved interactions between mycorrhizal effectiveness and soil fertility, or whether the less effective species happened, coincidentally, to have a competitive advantage in high P soils. Changes in the abundance of fungal structures in the fertilized and unfertilized plots suggest a shift but not necessarily an evolutionary change. Adaptation to high P fertilization within mycorrhizal populations has been documented (Cooper 1978; Jasper, Robson & Abbott 1979; but see Porter, Abbott & Robson 1978) but under much extended periods of time (48 years and 26 years).

The rate of evolution of mycorrhizae in response to P fertilizer would depend on both the degree of genetic variability and the strength of the selection pressure (i.e. effects of soil fertility and its importance relative to

other factors that influence fitness). Kahiluoto, Ketoja & Vestburg (2000) studied the impact of 23 years of cumulative P fertilization on mycorrhizal infectivity and effectiveness using inoculum (whole soil) derived from plots under different P fertilization histories to run bioassays of mycorrhizal communities. Their results were consistent with the hypothesis that cumulative fertilization decreases mycorrhizal effectiveness in flax, even under moderate amounts (45 kg P ha⁻¹ year⁻¹) of annual P fertilizer. However, as whole soil inoculum was used these results do not exclude the possibility of plant growth differences due to other soil micro-organisms. Similarly, Scullion, Eason & Scott (1998) found evidence that inocula (spores only) derived from soil under organic management regimes (with lower available P) were more effective in increasing plant growth than inocula derived from conventional farms (varying with season, time since cultivation). However, this trend disappeared when the conventional and organic farm soils had comparable levels of P fertility (Scullion, Eason & Scott 1998).

To our knowledge, no studies have directly documented the effects of long-term fertilizer use on the evolutionary stability of rhizobial mutualism. In comparing communities practising traditional agriculture (low levels of soluble N) and high input agriculture (high levels of soluble N), Souza *et al.* (1997) found that high input agricultural practices modified the genetic structure and decreased nodule activity (as measured by colour of nodules; red usually indicates active fixation) of rhizobia in the field. However, comparisons were made *in situ* and could be attributed to various environmental conditions (including short-term physiological effects of soil N), cropping patterns, herbicide use, etc., rather than evolutionary changes in rhizobia. In a general survey of Australian pastures, Gibson *et al.* (1975) found no correlation between mean effectiveness of rhizobial isolates and annual rate of fertilizer application. Similarly, symbiotic effectiveness of rhizobial populations (whole soil inoculum) derived from 18 different pastures was not related to total soil N (Quigley *et al.* 1997).

The trends from these limited data suggest that cumulative fertilizer use may have no detectable effect on the evolution of rhizobial effectiveness. Three possible justifications for this may exist: (i) the interaction between host sanctions and soil N may result in no soil N effect on the evolution of mutualism, as suggested by West *et al.* (2002); (ii) gene flow from repeated introduction of effective strains might have overpowered natural selection; and (iii) there is simply a lack of studies that have systematically compared symbiotic effectiveness of rhizobial isolates over N fertilization gradients *ex situ*.

Spatial structuring: effects of tillage regimes

Spatial structuring is an important component of natural ecosystems as it may contribute to selection

pressures mediating pathogenicity (Frank 1998). In natural ecosystems, bacterial and fungal dispersal may be limited by soil structure (Wadisirisuk *et al.* 1989; Chanway, Turkington & Holl 1991; Bever *et al.* 1996; Bever & Simms 2000). In contrast, agricultural systems, many of which are characterized by frequent tillage and soil disturbance, lack strong spatial structuring in the soil. We suggest that tillage may modify selection pressures for hosts and symbionts in two opposing ways.

First, tillage may decrease relatedness between symbionts within a plant (more distinct lineages per plant) and also decrease relatedness between symbionts in the plant and symbiont conspecifics in nearby soil. Reducing relatedness at both these scales increases the potential for competition, which would favour more parasitic strains (Frank 1994, 1996). For example, in rhizobia the benefits of increased N₂ fixation through increased plant growth (higher circulating resources and/or root exudates) would be shared with unrelated non-mutualists leading to a reduction in any 'kin selected' advantage of N₂ fixation (Bever & Simms 2000). This would select for lower levels of N₂ fixing. Similarly, tillage could lead to decreased relatedness between mycorrhizal strains both within and between plants by decreasing the spatial structuring of the soil, decreasing kin selection for mutualism.

Second, tillage may decrease competition between related strains that had infected the same host-plants by dispersing them to different areas (West *et al.* 2002). Competition between relatives reduces the kin-selected benefit of N₂ fixing as described above: put simply, helping relatives is less beneficial if their fitness increase comes at a cost to yourself or your other relatives (Taylor 1992; Queller 1994; West *et al.* 2001; West, Pen & Griffin 2002; Griffin & West 2002). Consequently, tillage may also select for higher levels of N₂ fixation in rhizobia or increased nutrient transfer in mycorrhizae.

The net effect of these two opposing forces will be difficult to predict. None the less, they clearly emphasize that soil tillage may have long-term consequences for symbiont performance.

In six out of eight relevant studies, conventional tillage led to a decrease in N₂ fixation (or in parameters plausibly linked to N₂ fixation) compared with systems with conservation tillage (Rennie *et al.* 1988; Hughes & Herridge 1989; Wheatley, Macleod & Jessop 1995; Dalal *et al.* 1997; Matus *et al.* 1997; Höflich *et al.* 1999; but see Doughton, Vallis & Saffigna 1993; Horn *et al.* 1996). For example, Höflich *et al.* (1999) showed a significant increase of leghaemoglobin and nodule number per plant under a conservation tillage treatment, relative to an otherwise identical system with conventional tillage. Rennie *et al.* (1988) found superior yield and N₂ fixation of soybeans in zero-till plots grown in paddy rice rotations. Unfortunately, these and other studies are *in situ* comparisons of N₂ fixation and the differences found are not necessarily due to evolutionary changes in the rhizobia. However, in the one

case where symbiotic performance was directly tested in the glasshouse, rhizobial isolates derived from zero-till plots of soybean/wheat and soybean/wheat/maize rotations showed the highest rates of N₂ fixation (Ferreira *et al.* 2000).

To understand the effects of tillage on rhizobial evolution, studies are also needed to assess genetic structuring of rhizobial communities as related to spatial structuring imposed by tillage regime. Studies of both naturalized and agriculturally derived rhizobial populations suggest that there is relatively low genetic differentiation among populations but high variability within plants (Souza *et al.* 1994; Hagen & Hamrick 1996; Silva, Eguiarte & Souza 1999). Tilled and zero-till systems provide ideal models for studying the consequences of how spatial structuring modifies competitive interactions. This has been the subject of much theoretical work, although there is a lack of empirical data (West *et al.* 2001).

Correlations have also been found between increased physical disturbance in soil and decreased mycorrhizal functioning (Evans & Miller 1990; Galvez *et al.* 2001). Data are still needed to confirm that these are a result of evolutionary modifications in mycorrhizal effectiveness due to various tillage schemes. Douds *et al.* (1995) present data to support suggestions that tillage, like fertilization regimes, may exert selection pressures on mycorrhizal communities, resulting in shifts in community composition, as some species may cope with disturbance better than others. The question is whether there is any consistent relationship between disturbance tolerance and greater or lesser mutualism. If so, then selection within species could also occur. Tillage was shown to decrease mycorrhizal diversity and result in a competitive dominance by only three species in cultivated Argentinean fields (Menéndez, Scervino & Godeas 2001). Relationships between soil disturbance and mycorrhizal communities characterized by more vigorous but less mutualistic species have been hypothesized (Hamel 1996) but, to our knowledge, no empirical data have been published.

Temporal structuring: crop rotations

In agricultural systems, the same plant genotype may be planted each year, usually independent of any variation in reproductive success among genotypes. A cultivar (or species) that performs poorly may be abandoned, but differences in individual fitness of crop plants will have no effect on the genetic composition of next year's crop, except when farmers save their own seed for planting.

Current theory suggests that mutualisms can be viewed as reciprocal exploitations in which some net benefit is provided to each partner (Herre *et al.* 1999). In natural systems and on farms that save their own seed, we would expect constant co-evolution between host and symbiont, with selection favouring (i) plants that are able to avoid less effective symbionts and (ii)

symbionts that obtain more from their hosts while paying a lower cost for these benefits. To some extent, evolutionary changes in the plant host may constrain evolution of decreased effectiveness in microbial symbiont populations. Agricultural systems that replace plants each year with the same genotype may lead to situations where the microbial symbionts will continue to evolve but the host-plant does not (termed one-sided co-evolution).

Continuous monocultures not utilizing crop rotation will tend to speed up the evolution of micro-symbiont adaptations (mutualistic or parasitic) to particular hosts; host genotype is replanted each year, regardless of changes in the effectiveness of the symbiont. Root symbionts under these management conditions are predicted to evolve measures to counter plant strategies that were once effective in enforcing co-operation by microbial symbionts, perhaps leading to a decrease in effectiveness over time. We predict that crop rotation may change symbiont evolution in ways that may differ between mycorrhizae and rhizobia.

Mycorrhizal fungi will encounter different host-plants in successive years, thereby increasing the diversity of plant selection pressures. For mycorrhizal fungi there may be trade-offs in the ability to overexploit different hosts, as suggested by evidence for host specificity in arbuscular mycorrhizae (Harley & Smith 1983; Sanders & Fitter 1992; Kiers *et al.* 2000).

If legumes are rotated with non-legumes, data suggest that there are selection pressures favouring rhizobia that survive for longer in the soil without a host (Triplett, Albrecht & Oplinger 1993), perhaps leading to a shift towards a saprophytic lifestyle, but not necessarily driving changes towards parasitism or mutualism. However, when the same legume species is replanted each year (soybean monocultures), changes are more likely to occur along the parasitism–mutualism continuum. Local changes in mutualistic functioning of rhizobia to annual hosts in natural ecosystems have been demonstrated (Parker 1995).

Therefore, if host-plants employ a variety of strategies for controlling microsymbionts, greater host diversity in either space (mixed cropping or greater genetic diversity within a crop species) or time (crop rotation) may reduce selection for host-specific adaptations that allow symbionts to overcome possible plant sanctions.

Studies of the effectiveness of root symbionts have suggested that continuous monocultures may select for decreased mutualistic functioning, relative to crop rotation. Johnson *et al.* (1992) evaluated the symbiotic effectiveness of the mycorrhizal communities by running reciprocal transplants in plots that had been in either continuous corn or soybean for the preceding 5 years. Spore populations of mycorrhizae derived from plots of continuous corn were negatively correlated with corn yields but positively correlated with yields of soybeans. This mainly appeared to involve a species shift, rather than evolutionary change within a species.

Reciprocal patterns were found in the soybean plots, although the statistical correlation was not as strong. These results suggest a certain degree of host specificity between crops and mycorrhizal species resulting in negative correlations between continued host presence and effectiveness on that host. Spore abundance was negatively correlated with crop yield (Johnson *et al.* 1992). This is in contrast to various studies that have suggested abundant sporulation is an indicator of a successful mutualism (Koomen, Grace & Hayman 1987; Brundrett 1991; Bever *et al.* 1996).

The association of the Glomales mycorrhizal fungi *Glomus macrocarpum* on tobacco *Nicotiana tabacum* illustrates a particularly extreme case of mutualistic modification (Johnson, Graham & Smith 1997). This mycorrhizal species causes tobacco stunt; increased root colonization is correlated with increased disease severity (Modjo & Hendrix 1986). Increased tobacco yields have been correlated with changes in mycorrhizal communities when crops are in rotation with fescue (Hendrix, Jones & Nesmith 1992). This suggests that crop rotation may act as a strong selective agent by preventing particular mycorrhizal strains (in this case *G. macrocarpum*) from dominating the soil profile. However, whether crop rotation simply prevents the build-up of detrimental symbionts or actually favours the increase of mutualistic symbionts warrants further investigation. Both of these cases could be seen as instances of the ecological control of soil-borne disease by crop rotation. However, they are also consistent with the hypothesis that consistent presence of the same host could favour the evolution of parasitism even in species that are often mutualistic.

Symbiont competition: crop inoculation

Inoculation of legumes with introduced rhizobial strains is standard agronomic practice in the majority of legume cropping systems; mycorrhizal inoculation with highly effective strains has been suggested as a nutrient management strategy (Brockwell, Bottomley & Thies 1995; Hamel 1996). In general, inoculation is considered beneficial in that it introduces symbiotic genes into the soil (see the discussion below). However, we suggest that inoculation simultaneously increases the degree of symbiont competition and this may, in turn, modify host benefits derived from the relationship (for a related discussion on competition effects on parasite virulence see Read & Taylor 2001). It is well established that roots of crop plants can be colonized simultaneously by more than one strain of mycorrhizae but, due to the lack of empirical information on host effects of mycorrhizal inoculum strain competition, we will focus on the effects of rhizobial inoculation. Specifically, we predict the following.

High rhizobial inoculum densities may result in increased incidences of nodules containing more than one strain of rhizobia (Lindemann, Schmidt & Ham 1974). If mixed nodules undercut nodule-level host

sanctions hypothesized to constrain the evolution of rhizobial parasitism, high inoculum densities could increase the absolute reproductive success of less effective rhizobia (Denison 2000; West, Pen & Griffin 2002; West *et al.* 2002). The direct effects of introducing highly effective rhizobia could swamp this evolutionary effect in the short term, increasing the average effectiveness of rhizobia in nodules in the months following inoculation. But if the introduced strain survives poorly in soil after release from nodules, the evolutionary effects of increasing the frequency of mixed nodules could lead to adverse long-term changes on rhizobial effectiveness.

Although competition between rhizobial strains in the rhizosphere, particularly between introduced and indigenous strains, has been well-documented (Ham, Cardwell & Johnson 1971; Roughly, Blowes & Herridge 1976; Ames-Gottfred & Christie 1989; Gibson *et al.* 1990; Thies, Singleton & Bohlool 1991), competition studies between strains within a nodule remain relatively unexplored. This may be the result of the general assumption that nodules only contain one strain of rhizobia. However, accumulating evidence suggests that when plants are exposed to high densities of inoculum, nodules can be infected by more than one strain of rhizobia (Lindemann, Schmidt & Ham 1974; Rolfe & Gresshoff 1980; Trinick, Rhodes & Galbraith 1983; Demezas & Bottomley 1986; Nambiar, Anjaiah & Srinivasa Rao 1987; Stuurman *et al.* 2000).

Few studies have determined the frequency of mixed nodules in the field, but the mere existence of mixed nodules raises important questions. Do the high inoculum densities promoted by agronomic inoculation practices contribute to an increase of mixed infections in the field? How do mixed infections affect symbiotic functioning in the short and long term? Is this effect greater than the gene flow effect? Under field conditions, 5–59% of nodules tested contained more than one strain when inoculation rates ranged from 10^6 cells/seed to 10^8 cells m^{-1} (Diatloff & Brockwell 1976; May & Bohlool 1983; Moawad & Schmidt 1987; McLoughlin, Hearn & Alt 1990). It has been suggested that occupancy is largely based on inoculum population sizes and various environmental controls (Renwick & Jones 1986). However, whether it is inoculum population sizes or total soil population sizes that determine double occupancy rates is unknown. Inoculation could increase rhizobium populations around some parts of the roots even if there is little effect of inoculation on total rhizobial numbers in fields where the same legume has been grown previously. Legumes may employ mechanisms to limit the degree of doubly infected nodules, analogous to mechanisms that limit the number of wasps *Agaoninae* sp. per fig fruit in *Ficus* sp. (Herre 1989), but these could perhaps be overwhelmed by high inoculation rates.

Even if doubly infected nodules prove to be commonplace in both natural ecosystems and inoculated

fields, can we infer that interstrain competition leads to a decrease in effectiveness? Interstrain competition has been documented in a commercial multistrain inoculum in which low N_2 fixation rates were found, even though the mixture contained a superior N_2 fixing strain (Bromfield & Jones 1980; Rennie & Dubetz 1984; Danso & Owiredu 1988). Caldwell (1969) found increased nodule weight when plants were inoculated with two strain combinations in comparison with three-strain or single-strain inoculation, but the largest plant fresh weight was found under single-strain inoculation, suggesting a negative correlation between net host benefit and nodule fresh weight.

An assessment of the short-term advantages to inoculation vs. the longer-term evolutionary consequences of inoculum-induced symbiont competition is needed. Because inoculum strains are generally more effective than indigenous strains, mixed nodules may have higher N_2 fixation rates than singly infected nodules containing only indigenous strains. It has also been suggested that rhizobial inoculants may lead to the lateral transfer of chromosomal symbiotic genes to non-symbiotic (saprophytic) rhizobia in the soil (Sullivan *et al.* 1995), as well as lateral transfer of *sym* plasmids (Laguerre, Bardin & Amarger 1993; Sprent 1994). Segovia *et al.* (1991) found that for every one symbiotic *Rhizobium leguminosarum* isolate found, there was an average of 40 non-symbiotic strains present in a Mexican bean field. Transfer of either chromosomal or plasmid genes may lead to an increased frequency of symbiotic isolates with enhanced adaptive traits to local biotic and abiotic conditions (Sullivan *et al.* 1995). Wernegreen, Harding & Riley (1997) suggest that this type of transfer may be a result of selection pressures influenced by (i) introduction of plants in a monoculture, (ii) high inoculation densities, and/or (iii) lack of selection acting on host-plants for symbiotic compatibility with indigenous strains. However, plasmids may also be lost or undergo rearrangement leading to deterioration of acquired symbiotic capacity (Soberón-Chávez *et al.* 1986). Such instability of symbiotic functioning was noted in a *R. leguminosarum* *bv. trifolii* population introduced into five field sites in Australia; significant differences in effectiveness were found in 11 of the 19 strain \times site combinations (Gibson *et al.* 1990). Similarly, 12 years after introduction, Diatloff (1976) found a decrease in symbiotic effectiveness of 68 of 80 cultures from the field compared with their stock cultures; six isolates were more effective. Ultimately, crop plants that preferentially enhance reproduction and release of more mutualistic strains (from among the various strains that infect each plant) into the soil may be more useful than inoculation with elite strains.

Conclusion

If agronomic practices are driving symbioses towards increased parasitism, modifying the practices is a

daunting task. In examining the above-mentioned agricultural practices (fertilization regimes, tillage management, crop rotations and symbiont soil inoculation), it is clear that the short-term costs of altering certain parameters (e.g. reducing fertilizer use) to promote long-term increased efficiency of agronomic symbioses could lead to well-founded resistance. However, steps such as increasing the breadth of breeding programmes to include selection for effective symbioses under both high- and low-fertilization regimes could prove an effective tool for symbiont management. For instance, Manske (1989) compared yields of 22 land races of wheat to 22 high-yielding varieties of wheat when inoculated with mycorrhizae in low P soil and found a higher increase in average yields of the land races compared with the yield increases for high yielding varieties. In high P soils, mycorrhizal inoculation caused a greater yield depression in the high-yielding varieties than in the land races, resulting in slightly greater total yield of the land race varieties (Johnson & Pflieger 1992b). It has been demonstrated that modern wheat breeding practices tend to reduce mycorrhizal dependence, as wheat cultivars released before 1950 consistently show increased dependence on mycorrhizae compared with those released subsequently (Hetrick, Wilson & Cox 1993). Data suggest that the host-plant's ability to form effective symbioses is a heritable trait that may be selected for or against in plant breeding programmes (Kesava Rao, Tilak & Arunachalam 1990).

To conclude, there is much interest in the increased utilization of agronomic symbioses in agricultural systems. We suggest that these attempts will be met with greater success through a consideration of how natural selection shapes mutualistic interactions. Specifically, to what extent (and why?) do different agricultural practices select for more beneficial or parasitic symbionts? Once this is understood it may be practical to modify some agricultural practices to reduce selection for more parasitic symbionts (as we suggest may currently be widespread), and even specifically to farm for more effective symbionts. Increasing our ability to exploit microbial mutualisms may be an integral step in moving towards more sustainable systems of agricultural production. Our major conclusion is that there is a lack of data directly testing our predictions, especially from experimental studies. We hope that this paper will stimulate such research.

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References

- Ames-Gottfred, N.P. & Christie, B.R. (1989) Competition among strains of *Rhizobium leguminosarum* biovar *trifolii* and use of a diallel analysis in assessing competition. *Applied and Environmental Microbiology*, **55**, 1599–1604.
- Axelrod, R. & Hamilton, W.D. (1981) The evolution of cooperation. *Science*, **211**, 1390–1396.
- van Baalen, M. & Jansen, V.A.A. (2001) Dangerous liaisons: the ecology of private interest and common good. *Okios*, **95**, 211–224.
- Bethlenfalvay, G.J. & Linderman, R.G. (1992) *Mycorrhizae in Sustainable Agriculture*. Special Publication No. 54. ASA, Madison, WI.
- Bever, J.D. & Simms, E.L. (2000) Evolution of nitrogen fixation in spatially structured populations of *Rhizobium*. *Heredity*, **85**, 366–372.
- Bever, J.D., Morton, J.B., Antonovics, J. & Schultz, P.A. (1996) Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology*, **84**, 71–82.
- Brockwell, J., Bottomley, P.J. & Thies, J.E. (1995) Manipulation of rhizobia microflora for improving legume productivity and soil fertility. *Plant and Soil*, **174**, 143–180.
- Bromfield, E.S.P. & Jones, D.G. (1980) Studies on double strain occupancy of nodules and the competitive ability of *Rhizobium trifolii* on red and white clover grown in soil and agar. *Annals of Applied Biology*, **94**, 51–59.
- Brundrett, M. (1991) Mycorrhizas in natural ecosystems. *Advances in Ecological Research*, **21**, 171–313.
- Bull, J.J. & Rice, W.R. (1991) Distinguishing mechanisms for the evolution of cooperation. *Journal of Theoretical Biology*, **149**, 63–74.
- Burdon, J.J., Gibson, A.H., Searle, S.D., Woods, M.J. & Brockwell, J. (1999) Variation in the effectiveness of symbiotic associations between native rhizobia and temperate Australian *Acacia*: within-species interactions. *Journal of Applied Ecology*, **36**, 398–408.
- Burns, R.C. & Hardy, R.W.F. (1975) *Nitrogen Fixation in Bacteria and Higher Plants*. Springer-Verlag, Berlin, Germany.
- Caldwell, B.E. (1969) Initial competition of root-nodule bacteria on soybeans in a field environment. *Agronomy Journal*, **61**, 813–815.
- Chanway, B.E., Turkington, R. & Holl, F.B. (1991) Ecological implications of specificity between plants and rhizosphere organisms. *Advances in Ecological Research*, **21**, 121–169.
- Cooper, K.M. (1978) Adaptation of mycorrhizal fungi to phosphate fertilizers. *Plant Nutrition 1978* (eds A.R. Ferguson, R.L. Bielecki & I.B. Ferguson), p. 107. New Zealand DSIR Information Series No. 134. Government Printer, Wellington, New Zealand.
- Dalal, R.C., Strong, W.M., Doughton, J.A., Weston, E.J., McNamara, G.T. & Cooper, J.E. (1997) Sustaining productivity of a vertisol at Warra, Queensland, with fertilisers, no-tillage or legumes. IV. Nitrogen fixation, water use and yield of chickpea. *Australian Journal of Experimental Agriculture*, **37**, 667–676.
- Danso, S.K.A. & Owiredo, J.D. (1988) Competitiveness of introduced and indigenous cowpea bradyrhizobia strains for nodule formation on cowpeas [*Vigna unguiculata* (L.) Walp.] in three soils. *Soil Biology and Biochemistry*, **20**, 305–310.
- Demezas, D.H. & Bottomley, P.J. (1986) Interstrain competition between representatives of indigenous serotypes of *Rhizobium trifolii*. *Applied and Environmental Microbiology*, **52**, 1020–1025.
- Denison, R.F. (2000) Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *American Naturalist*, **156**, 567–576.
- Denison, R.F. & Harter, B.L. (1995) Nitrate effects on nodule oxygen permeability and leghemoglobin. *Plant Physiology*, **107**, 1355–1364.

- Denton, M.D., Coventry, D.R., Bellotti, W.D. & Howieson, J.G. (2000) Distribution, abundance and symbiotic effectiveness of *Rhizobium leguminosarum* bv. *trifolii* from alkaline pasture soils in South Australia. *Australian Journal of Experimental Agriculture*, **40**, 25–35.
- Diatloff, A. (1976) Ecological studies of root-nodule bacteria introduced into field environments. VI. Antigenic and symbiotic stability in *Lotononis* rhizobia over a 12-year period. *Soil Biology and Biochemistry*, **9**, 85–88.
- Diatloff, A. & Brockwell, J. (1976) Ecological studies of root-nodule bacteria introduced into field environments. IV. Symbiotic properties of *Rhizobium japonicum* and competitive success in nodulation of two *Glycine max* cultivars by effective and ineffective strains. *Australian Journal of Experimental Agriculture and Animal Husbandry*, **16**, 514–521.
- Doebeli, M. & Knowlton, N. (1998) The evolution of interspecific mutualisms. *Proceedings of the National Academy of Sciences, USA*, **95**, 8676–8680.
- Douds, D.D., Galvez, L., Janke, R. & Wagoner, P. (1995) Effect of tillage and farming system upon populations and distribution of vesicular–arbuscular mycorrhizal fungi. *Agriculture, Ecosystems and Environment*, **52**, 111–118.
- Doughton, J.A., Vallis, I. & Saffigna, P.G. (1993) Nitrogen fixation in chickpea. I. Influence of prior cropping or fallow, nitrogen fertilizer and tillage. *Australian Journal of Agricultural Research*, **44**, 1403–1413.
- Ebert, D. & Herre, E.A. (1996) The evolution of parasitic diseases. *Parasitology Today*, **12**, 96–101.
- Evans, D.G. & Miller, M.H. (1990) The role of external mycelial network in the effect of soil disturbance upon vesicular–arbuscular mycorrhizal colonization of maize. *New Phytologist*, **114**, 65–71.
- Ferreira, M.C., Andrade, D.D.S., Chueire, L.M.D., Takemura, S.M. & Hungria, M. (2000) Tillage method and crop rotation effects on the population sizes and diversity of bradyrhizobia nodulating soybean. *Soil Biology and Biochemistry*, **32**, 627–637.
- Frank, S.A. (1994) Genetics of mutualism: the evolution of altruism between species. *Journal of Theoretical Biology*, **170**, 393–400.
- Frank, S.A. (1995) The origin of synergistic symbiosis. *Journal of Theoretical Biology*, **176**, 403–410.
- Frank, S.A. (1996) Models of parasite virulence. *Quarterly Review of Biology*, **71**, 37–78.
- Frank, S.A. (1998) *Foundations of Social Evolution*. Princeton University Press, Princeton, NJ.
- Galvez, L., Douds, D.D., Drinkwater, L.E. & Wagoner, P. (2001) Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant and Soil*, **228**, 299–308.
- Gibson, A.H., Curnow, B.C., Bergersen, F.J., Brockwell, J. & Robinson, A.C. (1975) Studies of field populations of *Rhizobium*: effectiveness of strains of *Rhizobium trifolii* associated with *Trifolium subterraneum* L. Pastures of south-eastern Australia. *Soil Biology and Biochemistry*, **7**, 95–102.
- Gibson, A.H., Demezas, D.H., Gault, R.R., Bhuvaneshwari, T.V. & Brockwell, J. (1990) Genetic stability of rhizobia in the field. *Plant and Soil*, **129**, 37–44.
- Goel, A.K., Sindhu, S.S. & Dadarwal, K.R. (1999) Bacteriocin-producing native rhizobia of green gram (*Vigna radiata*) having competitive advantage in nodule occupancy. *Microbiological Research*, **154**, 43–48.
- Graham, J.H., Drouillard, D.L. & Hodge, N.C. (1996) Carbon economy of sour orange in response to different *Glomus* spp. *Tree Physiology*, **16**, 1023–1029.
- Griffin, A.S. & West, S.A. (2002) Kin selection: fact and fiction. *Trends in Ecology and Evolution*, **17**, 15–21.
- Gryndler, A. & Lipavsky, J. (1995) Effect of phosphate fertilization on the populations of arbuscular mycorrhizal fungi. *Rostlinná Výroba*, **41**, 533–540.
- Hagen, M.J. & Hamrick, J.L. (1996) A hierarchical analysis of population genetic structure in *Rhizobium leguminosarum* bv. *trifolii*. *Molecular Ecology*, **5**, 177–186.
- Ham, G.E., Cardwell, V.B. & Johnson, H.W. (1971) Evaluation of *Rhizobium japonicum* inoculants in soils containing naturalized populations of rhizobia. *Agronomy Journal*, **63**, 301–303.
- Hamel, C. (1996) Prospects and problems pertaining to the management of arbuscular mycorrhizae in agriculture. *Agriculture, Ecosystems and Environment*, **60**, 197–210.
- Harley, J.L. & Smith, S.E. (1983) *Mycorrhizal Symbiosis*. Academic Press, London, UK.
- Hayman, D.S. (1975) The occurrence of mycorrhiza in crops as affected by soil fertility. *Endomycorrhizas* (eds E.F. Sanders, B. Mosse & P.B. Tinker), pp. 495–509. Academic Press, London, UK.
- Hendrix, J.W., Jones, K.J. & Nesmith, W.C. (1992) Control of pathogenic mycorrhizal fungi in maintenance of soil productivity by crop rotation. *Journal of Production Agriculture*, **5**, 383–386.
- Herre, E.A. (1989) Coevolution of reproductive characteristics in 12 species of New World figs and their pollinator wasps. *Experientia*, **45**, 637–647.
- Herre, E.A., Knowlton, N., Mueller, U.G. & Rehner, S.A. (1999) The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution*, **14**, 49–53.
- Hetrick, B.A., Wilson, G.W.T. & Cox, T.S. (1993) Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. *Canadian Journal of Botany*, **71**, 512–518.
- Hicks, P.M. & Loynachan, T.E. (1987) Phosphorus fertilization reduces vesicular–arbuscular mycorrhizal infection and changes nodule occupancy of field-grown soybeans. *Agronomy Journal*, **79**, 841–844.
- Hochberg, M.E., Gomulkiewicz, R., Holt, R.D. & Thompson, J.N. (2000) Weak sinks could cradle mutualistic symbioses – strong sources should harbor parasitic symbioses. *Journal of Evolutionary Biology*, **13**, 213–222.
- Höflich, G., Tauschke, M., Kühn, G., Werner, K., Frielinghaus, M. & Höhn, W. (1999) Influence of long-term conservation tillage on soil and rhizosphere microorganisms. *Biology and Fertility of Soils*, **29**, 81–86.
- Horn, C.P., Dalal, R.C., Birch, C.J. & Doughton, J.A. (1996) Sowing time and tillage practice affect chickpea yield and nitrogen fixation. II. Nitrogen accumulation, nitrogen fixation and soil nitrogen balance. *Australian Journal of Experimental Agriculture*, **36**, 701–706.
- Howeler, R.H., Sieverding, E. & Saif, S. (1987) Practical aspects of mycorrhizal technology in some tropical crops and pastures. *Plant and Soil*, **100**, 249–283.
- Hughes, R.M. & Herridge, D.F. (1989) Effect of tillage on yield, nodulation and nitrogen fixation of soybean in far north-coastal New South Wales. *Australian Journal of Experimental Agriculture*, **29**, 671–677.
- Jasper, D.A., Robson, A.D. & Abbott, L.K. (1979) Phosphorous and the formation of vesicular–arbuscular mycorrhizas. *Soil Biology and Biochemistry*, **11**, 501–505.
- Jensen, A. & Jakobsen, I. (1980) The occurrence of vesicular–arbuscular mycorrhiza in barley and wheat grown in some Danish soils with different fertilizer treatments. *Plant and Soil*, **55**, 403–414.
- Jimenez, J. & Casadesus, J. (1989) An altruistic model of the *Rhizobium*–legume association. *Journal of Heredity*, **80**, 335–337.
- Johnson, N.C. (1993) Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications*, **3**, 749–757.
- Johnson, N.C. & Pflieger, F.L. (1992) Vesicular–arbuscular mycorrhizae and cultural stresses. *Mycorrhizae in Sustainable Agriculture* (eds G.J. Bethlenfalvay & R.G. Linderman), pp. 71–100. Special Publication No. 54. ASA, Madison, WI.

- Johnson, N.C., Copeland, P.J., Crookston, P.K. & Pflieger, F.L. (1992) Mycorrhizae: possible explanation for yield decline with continuous corn and soybean. *Agronomy Journal*, **84**, 387–390.
- Johnson, N.C., Graham, J.H. & Smith, F.A. (1997) Functioning and mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist*, **135**, 575–586.
- Kahiluoto, H., Ketoja, E. & Vestburg, M. (2000) Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization. I. Bioassays in a growth chamber. *Plant and Soil*, **227**, 191–206.
- Kesava Rao, P.S., Tilak, K.V.B.R. & Arunachalam, V. (1990) Genetic variation for VA mycorrhiza-dependent phosphate mobilization in groundnut (*Arachis hypogaea* L.). *Plant and Soil*, **122**, 137–142.
- van Kessel, C. & Hartley, C. (2000) Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? *Field Crops Research*, **65**, 165–181.
- Kiers, E.T., Lovelock, C.E., Krueger, E.L. & Herre, E.A. (2000) Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecology Letters*, **3**, 106–113.
- Koomen, I., Grace, C. & Hayman, D.S. (1987) Effectiveness of single and multiple mycorrhizal inocula on growth of clover and strawberry plants at two pHs. *Soil Biology and Biochemistry*, **19**, 539–544.
- Krishna, K.R., Shetty, K.G., Dart, P.J. & Andrews, D.J. (1985) Genotype dependent variation in mycorrhizal colonization and response to inoculation of pearl millet. *Plant and Soil*, **86**, 113–125.
- Laguerre, G., Bardin, M. & Amarger, N. (1993) Isolation from soil of symbiotic and nonsymbiotic *Rhizobium leguminosarum* by DNA hybridization. *Canadian Journal of Microbiology*, **39**, 1142–1149.
- Lindemann, W.C., Schmidt, E.L. & Ham, G.E. (1974) Evidence for double infection within soybean nodules. *Soil Science*, **118**, 274–279.
- McLoughlin, T.J., Hearn, S. & Alt, S.G. (1990) Competition for nodule occupancy of introduced *Bradyrhizobium japonicum* strains in a Wisconsin soil with a low indigenous bradyrhizobia population. *Canadian Journal of Microbiology*, **36**, 839–845.
- Manske, G.G.B. (1989) Genetical analysis of the efficiency of VA mycorrhiza with spring wheat. *Agriculture, Ecosystems and the Environment*, **29**, 273–280.
- Matus, A., Derksen, D.A., Walley, F.L., Loepky, H.A. & van Kessel, C. (1997) The influence of tillage and crop rotation on nitrogen fixation in lentil and pea. *Canadian Journal of Plant Science*, **76**, 197–200.
- May, S.N. & Bohlool, B.B. (1983) Competition among *Rhizobium leguminosarum* strains for nodulation of lentils (*Lens esculenta*). *Applied Environmental Microbiology*, **45**, 960–965.
- Menéndez, A.B., Scervino, J.M. & Godeas, A.M. (2001) Arbuscular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina. *Biology and Fertility of Soils*, **33**, 373–381.
- Moawad, S. & Schmidt, E.L. (1987) Occurrence and nature of mixed infections in nodules of field-grown soybeans (*Glycine max*). *Biology and Fertility of Soils*, **5**, 112–114.
- Modjo, H.S. & Hendrix, J.W. (1986) The mycorrhizal fungus *Glomus macrocarpum* as a cause for tobacco stunt disease. *Phytopathology*, **76**, 688–691.
- Nambiar, P.T.C., Anjaiah, V. & Srinivasa Rao, B. (1987) Factors affecting competition of three strains of rhizobia nodulating groundnut, *Arachis hypogaea*. *Annals of Applied Biology*, **110**, 527–533.
- Olivieri, I. & Frank, S.A. (1994) The evolution of nodulation in rhizobium: altruism in the rhizosphere. *Journal of Heredity*, **85**, 46–47.
- Parker, M.A. (1995) Plant fitness variation caused by different mutualist genotypes. *Ecology*, **76**, 1525–1535.
- Parker, M.A. (1999) Mutualism in metapopulations of legumes and rhizobia. *American Naturalist*, **153**, S48–S60.
- Paul, E.A. (1988) Towards the year 2000: directions for future nitrogen research. *Advances in Nitrogen Cycling in Agricultural Ecosystems* (ed. J.R. Wilson), pp. 417–425. CAB International, Wallingford, UK.
- Peoples, M.B., Herridge, D.F. & Ladha, J.K. (1995) Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant and Soil*, **174**, 3–28.
- Porter, W.M., Abbott, L.K. & Robson, A.D. (1978) Effect of rate of application of superphosphate on populations of vesicular–arbuscular endophytes. *Australian Journal of Experimental Agriculture and Animal Husbandry*, **18**, 573–578.
- Queller, D.C. (1994) Genetic relatedness in viscous populations. *Evolutionary Ecology*, **8**, 70–73.
- Quigley, P.E., Cunningham, P.J., Hannah, M., Ward, G.N. & Morgan, T. (1997) Symbiotic effectiveness of *Rhizobium leguminosarum* bv. *trifolii* collected from pastures in south-western Victoria. *Australian Journal of Experimental Agriculture*, **37**, 623–630.
- Read, A.F. & Taylor, L.H. (2001) The ecology of genetically diverse infections. *Science*, **292**, 1099–1102.
- Rennie, R.J. & Dubetz, S. (1984) Multistrain vs. single strain *Rhizobium japonicum* inoculants for early maturing (00 and 000) soybean cultivars: N₂ fixation quantified by ¹⁵N isotope dilution. *Agronomy Journal*, **76**, 498–502.
- Rennie, R.J., Rennie, D.A., Siripaibool, C., Chaiwanakupt, P., Boonkerd, N. & Snitwongse, P. (1988) N₂ fixation in Thai soybeans: effect of tillage and inoculation on ¹⁵N-determined N₂ fixation in recommended cultivars and advanced breeding lines. *Plant and Soil*, **112**, 183–193.
- Renwick, A. & Jones, D.G. (1986) The manipulation of white clover ‘host preference’ for strains of *Rhizobium trifolii* in an upland soil. *Annals of Applied Biology*, **108**, 291–302.
- Rolfé, B.G. & Gresshoff, P.M. (1980) *Rhizobium trifolii* mutant interactions during establishment of nodulation in white clover. *Australian Journal of Biological Sciences*, **33**, 491–504.
- Roughly, R.J., Blowes, W.M. & Herridge, D.F. (1976) Nodulation of *Trifolium subterraneum* by introduced rhizobia in competition with naturalized strains. *Soil Biology and Biochemistry*, **8**, 403–407.
- Rubio Arias, H.O., de la Vega, L., Ruiz, O. & Wood, K. (1999) Differential nodulation response and biomass yield of Alexandria clover as affected by levels of inorganic nitrogen fertilizer. *Journal of Plant Nutrition*, **22**, 1233–1139.
- Sanders, I.R. & Fitter, A.H. (1992) Evidence for differential responses between host–fungus combinations of vesicular–arbuscular mycorrhizas from a grassland. *Mycological Research*, **96**, 415–419.
- Scullion, J., Eason, W.R. & Scott, E.P. (1998) The effectivity of arbuscular mycorrhizal fungi from high input conventional and organic grassland and grass–arable rotations. *Plant and Soil*, **204**, 243–254.
- Segovia, L., Piñero, D., Palacios, R. & Martínez-Romero, E. (1991) Genetic structure of a soil population of nonsymbiotic *Rhizobium leguminosarum*. *Applied and Environmental Microbiology*, **57**, 426–433.
- Silva, C., Eguiarte, L.E. & Souza, V. (1999) Reticulated and epidemic population genetic structure of *Rhizobium etli* biovar *phaseoli* in a traditionally managed locality in Mexico. *Molecular Ecology*, **8**, 277–287.
- Simms, E.L. & Bever, J.D. (1998) Evolutionary dynamics of rhizopine within spatially structured rhizobium populations. *Proceedings of the Royal Society, London Biological Sciences*, **265**, 1713–1719.
- Soberón-Chávez, G., Nájera, R., Olivera, H. & Segovia, L. (1986) Genetic rearrangements of a *Rhizobium phaseoli* symbiotic plasmid. *Journal of Bacteriology*, **167**, 487–491.

- Souza, V., Bain, J., Silvia, C., Bouchett, V., Valera, A., Marquez, E. & Eguiarte, L.E. (1997) Ethnomicrobiology: do agricultural practices modify the population structure of the nitrogen fixing bacteria *Rhizobium etli* biovar phaseoli? *Journal of Ethnobiology*, **17**, 249–266.
- Souza, V., Eguiarte, L., Avila, G., Cappello, R., Gallardo, C., Montoya, J. & Piñero, D. (1994) Genetic structure of *Rhizobium etli* biovar phaseoli associated with wild and cultivated bean plants (*Phaseolus vulgaris* and *Phaseolus coccineus*) in Morelos, Mexico. *Applied and Environmental Microbiology*, **60**, 1260–1268.
- Sprent, J.I. (1994) Evolution and diversity in the legume–rhizobium symbiosis: chaos theory? *Plant and Soil*, **161**, 1–10.
- Stuurman, N., Bras, C.P., Schlaman, H.R.M., Wijfjes, A.H.M., Bloemberg, G. & Spaik, H.P. (2000) Use of green fluorescent protein color variants expressed on stable broad-host-range vectors to visualize rhizobia interacting with plants. *Molecular Plant–Microbe Interactions*, **13**, 1163–1169.
- Sullivan, J.T., Patrick, H.N., Lowther, W.L., Scott, D.B. & Ronson, C.W. (1995) Nodulating genes of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. *Proceedings of the National Academy of Science, USA*, **92**, 8985–8989.
- Taylor, P.D. (1992) Altruism in viscous populations – an inclusive fitness model. *Evolutionary Ecology*, **6**, 352–356.
- Thies, J.E., Singleton, P.W. & Bohlool, B.B. (1991) Influence of the size of indigenous populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Applied and Environmental Microbiology*, **57**, 19–28.
- Thomson, B.D., Robson, A.D. & Abbott, L.K. (1992) The effect of long-term applications of phosphorus fertilizer on populations of vesicular–arbuscular mycorrhizal fungi in pastures. *Australian Journal of Agricultural Research*, **42**, 1131–1142.
- Thrall, P.H., Burdon, J.J. & Woods, M.J. (2000) Variation in the effectiveness of symbiotic associations between native rhizobia and temperate Australian legumes: interactions within and between genera. *Journal of Applied Ecology*, **37**, 52–65.
- Tilman, D., Fargione, J., Wolff, B., D’Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D. & Swackhamer, D. (2001) Forecasting agriculturally driven global environmental change. *Science*, **292**, 281–284.
- Trinick, M.J., Rhodes, R.L. & Galbraith, J.H. (1983) Competition between fast- and slow-growing tropical legume rhizobia for nodulation of *Vigna uguiculata*. *Plant and Soil*, **73**, 105–115.
- Triplett, E.W., Albrecht, K.A. & Oplinger, E.S. (1993) Crop rotation effects on populations of *Bradyrhizobium japonicum* and *Rhizobium meliloti*. *Soil Biology and Biochemistry*, **25**, 781–784.
- Udvardi, M.K. & Kahn, M.L. (1993) Evolution of the (*Brady*) *rhizobium* symbiosis: why do bacterioids fix nitrogen? *Symbiosis*, **14**, 87–101.
- Vargas, M.T.A., Mendes, I.C. & Hungria, M. (2000) Response of field-grown bean (*Phaseolus vulgaris* L.) to *Rhizobium* inoculation and nitrogen fertilizer in two Cerrados soils. *Biology and Fertility of Soils*, **32**, 228–233.
- Wadisirisuk, P., Danso, S.K.A., Hardarson, G. & Bowen, G.A. (1989) Influence of *Bradyrhizobium japonicum* location and movement on nodulation and nitrogen fixation in soybeans. *Applied and Environmental Microbiology*, **55**, 1711–1716.
- Wernegreen, J.J., Harding, E.E. & Riley, M.A. (1997) *Rhizobium gone native: unexpected plasmid stability of indigenous Rhizobium leguminosarum*. *Proceedings of the National Academy of Science, USA*, **94**, 5483–5488.
- West, S.A., Kiers, E.T., Pen, I. & Denison, R.F. (2002b) Sanctions and mutualism stability: when should less beneficial mutualists be tolerated? *Journal of Evolutionary Biology*, **15**, 830–837.
- West, S.A., Kiers, E.T., Simms, E.L. & Denison, R.F. (2002) Sanctions and mutualism stability: why do rhizobia fix nitrogen? *Proceedings of the Royal Society, London Biological Sciences*, **269**, 685–694.
- West, S.A., Murray, M.G., Machado, C.A., Griffin, A.S. & Herre, E.A. (2001) Testing Hamilton’s rule with competition between relatives. *Nature*, **409**, 510–513.
- West, S.A., Pen, I. & Griffin, A.S. (2002) Cooperation and competition between relatives. *Science*, **296**, 72–75.
- Wheatley, D.M., Macleod, D.A. & Jessop, R.S. (1995) Influence of tillage treatments on N₂ fixation of soybean. *Soil Biology and Biochemistry*, **27**, 571–574.

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