

Review

The evolutionary ecology of rhizobia: multiple facets of competition before, during, and after symbiosis with legumes[☆]

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Rhizobial bacteria have complex lifestyles that involve growth and survival in bulk soil, plant rhizospheres and rhizoplanes, legume infection threads, and mature and senescing legume nodules. In nature, rhizobia coexist and compete with many other rhizobial strains and species to form host associations. We review recent work defining competitive interactions across these environments. We highlight the use of sophisticated measurement tools and sequencing technologies to examine competition mechanisms *in planta*, and highlight environments (e.g. soil and senescing nodules) where we still know exceedingly little. We argue that moving toward an explicitly ecological framework (types of competition, resources, and genetic differentiation) will clarify the evolutionary ecology of these foundational organisms and open doors for engineering sustainable, beneficial associations with hosts.

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Introduction

Bacterial competition for resources and habitat can occur both within and between species [1]. Competition can be

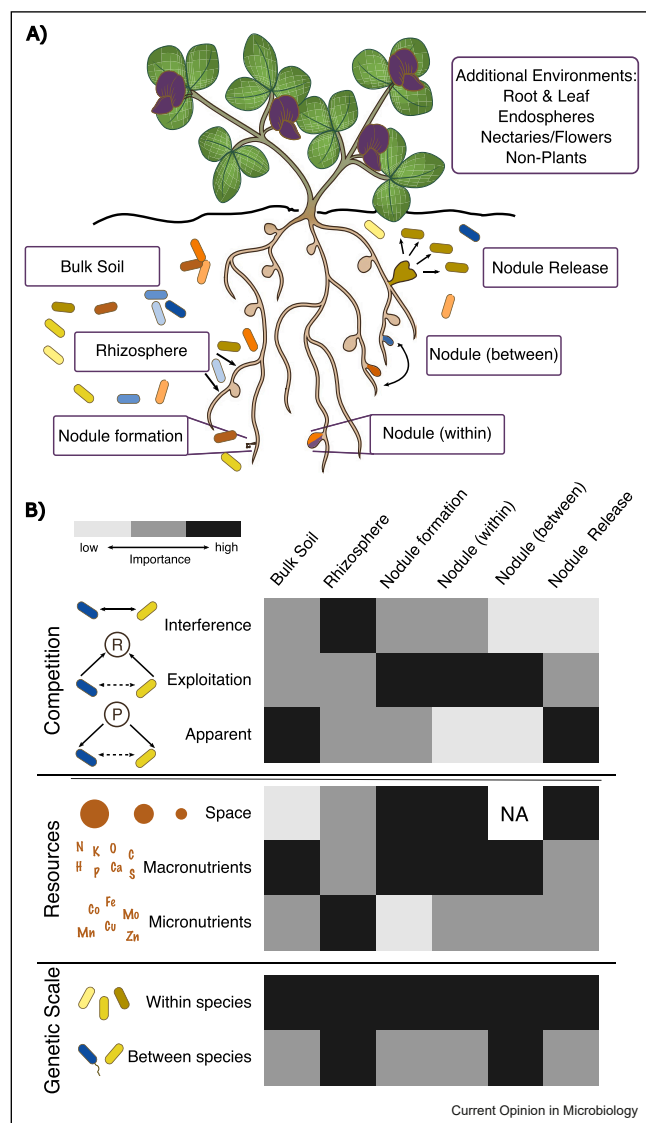
loosely divided into the nonmutually exclusive categories of exploitative competition (e.g. enzymes [2]) and interference competition (e.g. weapons [3]). Here, we refer to competition as any interaction between organisms that positively alters the fitness (i.e. reproductive success) of one organism at the expense of the other. These interactions may occur directly between competing organisms or be indirectly mediated by interactions with other organisms via cooperation or predation [4]. As bacteria reproduce via asexual reproduction, whole-organism fitness is a reflection of the ability of a strain to undergo cell division, which in turn depends on its ability to compete for access to resources, endure periods of nutrient scarcity, and survive attacks by other members of the microbial and macrobial community.

The rhizobia are a polyphyletic group of soil bacteria able to establish facultative, N₂-fixing endosymbiotic relationships with legumes. Rhizobia have complex lifestyles that involve growth and survival in bulk soil, plant rhizospheres and rhizoplanes, legume infection threads, and mature and senescing legume nodules (Figure 1a) [5]. Competition mechanisms will shift across these environments as competitor density, metabolic overlap, resource availability, degree of spatial structure, and abiotic condition changes (Figure 1b). Similarly, differences between legume hosts (e.g. determinant versus indeterminant nodule growth) may also shift multiple aspects of competitive interactions (Box 1).

Here, we review recent advances in understanding the competition mechanisms that influence the evolutionary ecology of rhizobia, focusing on competition 1) *within* members of a single rhizobial species irrespective of whether they are capable of legume symbiosis, and 2) *between* different rhizobial species that share habitat niches in host root rhizospheres and nodules. While many gaps remain, we mention our perspective on whether competition mechanisms in each environment are more likely to be based on exploitation versus interference versus apparent competition, what resources (space, macronutrients, and micronutrients) underlie competition, and if we might expect competition to be stronger

[☆] *Issue Focus:* Plant–microbe and plant–microbiome interactions with a focus on how microbes compete in the soil and colonize their plant hosts.

Figure 1



Rhizobial environments and competition mechanisms. Rhizobia compete in many different environmental contexts before, during, and after host association. Below, we summarize these major environmental niches (a) and offer hypotheses about the strength of different categories of competition, resources over which rhizobia compete, and whether competition is stronger between strains of the same species or between different species (b). Darker shades indicate categories hypothesized by the authors to be of little (light gray), intermediate (gray), and critical (dark gray) importance. For most categories, systematic experimental work will be necessary to confirm these hypotheses. Abbreviations: resources (R) and predators (P)

between versus within rhizobial species (Figure 1b). Ecological theory suggests the baseline expectation that resource competition will be stronger *within* species because they have more similar genomes and tend to exist in similar niches. However, in specific contexts, this expectation may not hold.

Competition in the soil and rhizosphere

Bulk and rhizosphere soils provide dramatically different arenas for bacterial competition. Bulk soils are often resource- (e.g. carbon) limited, with high diversity and high dormancy, whereas rhizosphere communities represent a less diverse subset of the bulk soil community with strong but more transient connections among community members [6]. Genera that contain N-fixing rhizobia are enriched in many plant rhizospheres (e.g. *Rhizobium* and *Mesorhizobium*). In croplands planted with members of the family Leguminosae, the wider rhizosphere microbial communities were enriched with more fast-growing bacteria with higher N-fixation potential, whereas nitrification was more prominent in the bulk soil [6]. These divergent environmental characteristics suggest that competitive interactions among rhizobia species and *between* strains in bulk soil and rhizosphere environments may also differ, although such interactions are rarely studied. In bulk soil, competitive outcomes may depend more on long-term survival under stress and opportunistic growth on diverse substrates. In contrast, in the rhizosphere, high resource-driven rapid growth and direct competition (e.g. bacteriocins [7]) may be more important (Figure 1b).

In bulk soils, bacterial age distributions in the soil vary widely across spatial scales, and many environments have very slow net generation times (e.g. weeks) [8]. Because these methods can only examine mean generation time, and some rhizobia can asymmetrically partition resources during division, rhizobial age distributions are likely to have long tails, which means that cells of different ages (e.g. parents and offspring) may compete against each other [9,10]. The slow net generation times suggest that long-term survival, rather than cell division, is likely to be a key determinant of competition, as well as the ability to respond to stochastic nutrient influxes rapidly. Although little research has been performed to understand traits influencing rhizobium fitness in bulk soil, we expect that the ability of rhizobia to survive in soils would depend heavily on various abiotic (e.g. pH, resource availability, temperature, and moisture) and biotic modifiers (e.g. phages, predators, and antibiotics). In particular, phages are likely to play an important role in mediating within-species apparent competition (Figure 1b) given that the phage host range is strain-specific, and there is evidence of the coevolution of rhizobia and phage populations in natural environments [11]. Similarly, many rhizobia encode bacteriocins (narrow-range antimicrobial protein toxins) that can directly alter competition between strains in, for instance, peat media [12]. Ultimately, we need new spatially explicit methods to disentangle and clarify the diverse mechanisms, frequency, and outcomes of competition occurring between rhizobia in bulk soils, including nonsymbiotic rhizobia.

Compared with bulk soil, rapid growth is likely to be a more important contributor to within- and between-species competition in the rhizosphere. High-throughput mutagenesis studies suggest that 2–2.5% of rhizobial genes contribute to growth in the rhizosphere [13,14]. Many of these are metabolic genes; organic acids are major carbon sources available in the rhizosphere [15], and the ability of rhizobia to catabolize these substrates is associated with increased competitive success [16]. In addition to organic acids, rhizobia encode a diverse array of transporters and catabolic genes that would facilitate the uptake and metabolism of the many macromolecules available in the rhizosphere (see Ref. [17] for a nice review of carbon transport and metabolism in the model rhizobium *Sinorhizobium meliloti*). Appropriate protein and RNA-based regulation of metabolism allowing for the hierarchical use of preferred carbon sources are also expected to contribute to successful competition in the rhizosphere or rhizoplane [18]. In addition to macromolecules, competition for micronutrients, such as iron, influences the success of microbes in the rhizosphere environment [19], and indeed, at least some of these siderophores can contribute to intra- or interspecies competitive success of rhizobia by inhibiting the growth of strains unable to use the siderophore [20]. While much is known about the metabolism of model rhizobium species [15,21], little is known about direct interference competition between rhizobia in the rhizosphere. Nevertheless, rhizobia have the potential to directly compete (Figure 1b) via bacteriocins or other inhibitory substances in the rhizosphere [22]; however, the impact of these molecules on rhizosphere competition remains largely unexplored.

Competition for nodule occupancy

Depending on the species, legumes will form several dozen (e.g. bean) to more than a thousand (e.g. peanut) nodules per plant. Most, but not all, legume nodules are infected by a single founder rhizobium, and thus in the majority of cases, the rhizobial population within a single nodule is isogenic [23]. Consequently, only a tiny proportion of rhizobia in a plant's rhizosphere will successfully compete for nodule occupancy. This has two

important implications. First, stochasticity, or chance, in which rhizobial strains enter nodules, has the potential to alter the evolutionary dynamics of a rhizobial population significantly. Second, the large bottleneck at this stage will create strong selective pressure for traits improving competitive fitness [24,25]. Many traits associated with intra- or interspecies competition for nodule occupancy have been described in rhizobia. These include the ability to communicate with the host plant effectively [26], chemotaxis and movement toward seed and root exudates [27,28], the production of bacteriocins or other toxins targeting other rhizobia [22], the ability to catabolize various carbon sources [29], and stress tolerance [30]. In particular, factors influencing host specificity, such as host-encoded incompatibility factors [31], can be important mediators of within- versus between-species competitive outcomes during nodule formation and beyond. The relative importance of within- versus between-species competition will vary with host promiscuity (e.g. whether the host forms nodules with several genera or a single subspecies or rhizobia), but in general, we expect greater within-species competition due to shared host compatibility factors (Box 1). Last, competition for nodule occupancy is influenced by rhizobial genotype $\times$ legume genotype $\times$ environment (G $\times$ G $\times$ E) interactions [32,33], which can influence the global ecology and distribution of rhizobial species [34,35].

Other rhizobia and nonrhizobial organisms in the rhizosphere also influence competitiveness for nodule occupancy. The effect of additional organisms can act directly or be mediated through the host legume. A recent study nicely demonstrated that *Bacillus* spp. could alter the between-species competitive dynamics of rhizobia-nodulating soybean potentially as a result of apparent competition, with the *Bacillus* improving the growth of one species but impairing the growth of the other [36]. Likewise, apparent competition mediated by phages can alter within-species competitive success, favoring strains with resistance to the phage [37]. In contrast, a recent study found that within-species competition outcomes are not altered by the presence of

Box 1 Hypothesized effects of variation in legume symbiosis types [64] on competitive interactions

- **Infection (crack entry versus root hair formation):** The method of entry could shift a) the likelihood of multistrain/species nodules, b) resource availability, and c) the likelihood of interference competition between strains.
- **Sequestration (fixation threads versus symbiosomes):** The extent of compartmentalization or rhizobia within fixation threads, located outside of plant cells, is likely less than that for rhizobia within symbiosomes, located inside host-derived membranes within plant cells. The extent of compartmentalization is likely to influence opportunities for interference competition, and competition for macronutrients and micronutrients.
- **Nodule growth (determinant versus indeterminate):** In nodules with determinant development, the space rhizobia inhabit is limited, and growth is finite. In indeterminate nodules, nodule growth and/or branching could increase space allocated to rhizobia. Influences timing and patterning of nodule senescence, release, and habitat availability.
- **Differentiation (with or without terminal differentiation):** Determines whether the nitrogen-fixing rhizobial cells can revert to a free-living state and return to the soil during nodule senescence. Could influence the primary location and type of competition in the nodule, as well as macronutrient accumulation dynamics.
- **Rhizobium partner range of plant host (broad versus narrow):** influences identity of rhizobial competitors and degree of niche overlap.

additional isolates of the same rhizobial species; instead, a competitive hierarchy emerges where the success of an isolate in multi-isolate populations can be predicted from pairwise results [32]. Last, nonrhizobial members of the rhizosphere can also positively [38] or negatively [39] influence the number of nodules formed by the host legume, thereby altering the nodule habitat size available to rhizobia and potentially altering competitive dynamics (Figure 1b).

Despite identifying numerous traits and genes influencing competition for nodule occupancy [40], a mechanistic understanding of how and when these traits influence competition is lacking. For example, it is not clear why the loss of the ability to catabolize a single carbon substrate should have a large impact on competition for nodule occupancy. Nodule occupancy reflects the cumulative fitness across multiple stages of the symbioses, including rhizosphere colonization, growth in the infection thread, and survival in the nodule, among others. However, common methods for studying competition for nodule occupancy [41,42] cannot disentangle during which stage of nodule formation is important. For instance, an often-overlooked environment with respect to competition for nodule occupancy is the infection thread. The number of infection threads formed by a legume generally far outnumbers the number of nodules that are formed, and existing infection thread growth can be stopped once nodules form [43]. It is, therefore, reasonable to expect that rhizobia capable of rapidly progressing through infection threads have a competitive advantage for nodule occupancy. Considering the agronomic importance of competitiveness for nodule occupancy [41], this is an important future research direction.

Competition in nodules (direct and indirect)

Once inside nodules, the potential types of competition depend on 1) whether multiple rhizobium strains or species coinhabit the same nodules or if each rhizobium inhabits different nodules on the same plant, and 2) the physiological state of the rhizobia in the nodules, including whether the bacteria remain as free-living bacteria outside of plant cells, or are present as N-fixing bacteroids inside plant cells (Box 1). Progress on understanding the relative importance of competition within versus between nodules in nature is hampered by a lack of understanding of 1) the relative frequency of mixed nodules in natural and agricultural contexts, 2) the difficulty of studying competition mechanisms *in vivo* inside hosts, and 3) the challenge of quantifying rhizobial fitness.

Competition between nodules is hypothesized to be primarily driven by indirect competition for limited host resources. One long-studied mechanism that could drive competitive outcomes between rhizobia is host-

mediated selective partitioning of resources [44]. If hosts can differentially allocate resources (e.g. photosynthate) to nodules based on benefits received (e.g. fixed nitrogen), this leads to adaptive sanctions/rewards whereby rhizobia fixing the most nitrogen get the most resources and fitness benefits [44]. Recent studies show that these allocation mechanisms are less effective when dealing with quantitative variation [45,46] in strain quality compared with binary effective/noneffective comparisons [47]. In other words, there is a limit to 1) plants' ability to discriminate between strains that are helpful and somewhat helpful [24], and 2) in nature where many rhizobial species and strains coexist, the benefit of a strain and thus competitive outcomes are relative to which other strains are there [32]. Nuanced new papers also show that the effectiveness of sanctions depends on external resources [45]. Moving forward, careful attempts are needed to quantify the effect of differential resource allocation during nodule habitation on the number of rhizobia released from nodules during senescence and resource accumulation during symbiosis [48].

On the other hand, the relative importance of competition *within* nodules for rhizobial evolution *in nature* is still very much up for debate [41,42] and may depend on host specificity and specific entry methods (Box 1). Carefully designed surveys of individual nodule inhabitants sampled from nature will allow these parameters to be measured explicitly. Nevertheless, in the past several years, multiple papers highlighted that in lab conditions, mixed nodules are not rare, even if they are not the majority [41], and may consist of a mixture of effective symbionts and ineffective 'cheaters' [49]. Yet, even in mixed nodules, different symbionts may be compartmentalized in different plant cells [50], limiting the opportunity for direct rhizobium competition to the infection thread. How rhizobia compete within a nodule remains poorly understood, but potentially could include interference competition, direct competition for host resources, or apparent competition via susceptibility to host-produced compounds such as nodule-specific cysteine-rich peptides [51]. If within-nodule competition is rare compared with between-nodule competition, then we expect it to be a minor contributor to the overall evolution of rhizobia. We also note that while not about competition *per se*, recent work showing increased rates of conjugation and plasmid transfer inside nodules [52,53] could allow horizontal transfer and sharing of competition mechanisms that could benefit coinfecting rhizobial strains or species in other shared environments.

Competition during nodule senescence

Nodulation can only positively impact the fitness of rhizobia in nodules if the rhizobia can return to the soil; yet, the factors affecting the success of rhizobia during

Box 2 Future directions and key questions

- What are the relative contributions of competition in different environments to rhizobial evolution?
- How vital are additional, less-studied habitats (e.g. leaves, nonlegume roots)?
- Do multiple mechanisms underlie competitive success within and across stages?
- Do the same mechanisms of competition operate within and between rhizobial species?
- What scales of genetic differentiation are the most critical drivers of rhizobial competition (strains, species, and genera)?
- How sensitive are pairwise competitive outcomes in each environment to additional rhizobia, microbes, or macrobes?
- What are the key abiotic modulators of competitive outcomes?
- How can we counteract biases resulting from rhizobial collections consisting almost entirely of strains isolated from nodules?
- How do population density and the initial ratio of competitors impact the outcomes of competition?

senescence represent one of the biggest knowledge gaps in the rhizobial lifestyle. One way that nodule-competitive outcomes could influence soil emergence is via shifts in the timing of nodule senescence and the size of rhizobial populations within nodules capable of surviving the transition to the soil [50]. Another key parameter during nodule senescence is the ability of rhizobia to generate internal nutrient and energy stores. The best-studied example is the accumulation of the carbon and energy storage compound poly-3-hydroxybutyrate (PHB) by nitrogen-fixing bacteroids of determinate, but not indeterminate, nodules [54] (Box 1). Recent work demonstrated a heritable component to the amount of PHB accumulated by rhizobia in nodules [48], and experimental and modeling studies have shown that PHB stores have the potential to support rapid rhizobial growth and long-term survival following senescence in part through the use of a bet-hedging strategy [48,55,56]. Aside from PHB accumulation, many other factors may influence rhizobial fitness during senescence. Indeed, a recent large-scale functional genomics study identified several genes potentially relevant to the fitness of rhizobia during senescence in lab conditions [13]. Senescing nodules also create a resource-rich environment with many bacteria that could attract predators and decomposers. This suggests that efficient resource use (exploitation competition) and predation and/or phages (apparent competition) may play important roles in relative success (Figure 1b). In addition, a recent study used synthetic communities to demonstrate how competitive interactions and antibiotic production of non-rhizobial nodule endophytes change as nodules age [57]. We believe that going forward, more research should be undertaken to examine the processes and ecological interactions occurring during nodule senescence, facilitated by recently developed methods such as insertion-sequencing [13], sequential passaging [24], and spatially resolved matrix-assisted laser desorption ionization coupled to time-of-flight (MALDI-TOF) mass spectrometry [57].

Rhizobial competitiveness and the effectiveness of nitrogen-fixing symbiosis

Fitness trade-offs whereby traits that make rhizobia more competitive also make them less beneficial to

plants are often assumed in mutualism models and in literature discussions. Such trade-offs set up an evolutionary scenario that destabilizes the mutualism and leads to the proliferation of ‘cheaters’. Interestingly, recent empirical results in both *Sinorhizobium* [28,46] and *Rhizobium* [23] enabled by high-throughput, cumulative competitive fitness assays and single-strain assessments of plant benefits suggest an overall lack of fundamental trade-offs between rhizobial competitive fitness and symbiotic efficiency at the phenotypic and genomic level. However, these results may be rhizobial system-specific. Gano-Cohen et al. came to the opposite conclusion in *Bradyrhizobium* using genospecies frequencies in nodules of natural *Acmispon* populations and plant benefit assessments of genospecies representatives [58]. Overall, the results offer the hope that agroecological contexts can be constructed where rhizobial and host fitness interests are aligned [41,42].

Conclusions

Rhizobia inhabit ecologically diverse niches, and their overall fitness is a product of their success in each niche (e.g. bulk soil, rhizosphere, and nodules) and their ability to move between them. Recent research suggests that the ability to compete to occupy these distinct niches, coupled with the potential for environmental trade-offs, is likely to promote the maintenance of high genetic variation within rhizobial species, as well as to contribute to the maintenance of rhizobia genotypes with poor N-fixing capabilities. While many traits impacting the ability of rhizobia to inhabit the rhizosphere, compete for nodule occupancy, and fix nitrogen have been identified, we know little about the competitive mechanisms underlying these traits. We suspect that the diversity of symbiosis formation types (infection, sequestration, growth type, differentiation, and host selectivity) plays important, mostly unexplored, roles in mediating competitive interactions between rhizobia (Box 1). Further, competition mechanisms in some niches remain largely unexplored (e.g. bulk soil and during release from senescing nodules). While outside the scope of this article, we also note that rhizobia live and compete in many more environments, including the phyllosphere and root, flower, and leaf endospheres of legumes [59], and many nonlegume niches [60]. We posit that framing

future research with careful consideration of competition mechanisms, the genetic scale of competitors, and the resources being competed for [61], will provide new clues about how competitive interactions have and will continue to evolve (see Box 2 for a list of future research directions and unresolved questions). Ecological knowledge will aid in predictions of how rhizobium inoculants will behave in agricultural settings [62]. Even more broadly, genes and mechanisms identified in rhizobia competition are also likely to be important in other host-associated bacterial interactions [63].

Author contributions

Liana T. Burghardt: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. **George C. DiCenzo:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data were used for the research described in the article.

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