Nodule branching, size, and symbiosis outcomes are shaped by natural genetic variation in rhizobial bacteria and alfalfa hosts.

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ABSTRACT

In symbiosis between hosts and microbes, hosts often create organs that house microbial symbionts. These organs are called nodules in the nitrogen-fixing symbiosis between legume plants and rhizobial bacteria. While many studies have focused on host traits, such as plant growth, nodule count, and their structural changes, these morphological changes could also be associated with rhizobial benefits (e.g., bacterial population size). Here, we investigated how the different morphologies of alfalfa root nodules influence the fitness benefits rhizobia and hosts receive from the symbiosis. We measured how host and strain genetic variation influenced 1) nodule branch number, area, and rhizobial fitness at the individual nodule level and 2) the proportion of branched nodules, nodule number, and plant benefits at the whole plant level. From the rhizobial perspective, we found that host genetics influenced rhizobial fitness more than rhizobial genetics, and that more branched and bigger nodules tend to release more rhizobia. From the host plant perspective, we found that the proportion of branched nodules was strongly negatively correlated with the total nodule number and positively correlated with host benefit across all three alfalfa genotypes. However, the height of the relationship differed by host genotype. Together, these results provide insight into the host and rhizobial genetic contributions to nodule morphology and symbiotic outcomes, suggesting a novel direction for further research in legume-rhizobium mutualism.

INTRODUCTION

In host-microbe symbiosis, microbes often live in host tissues within chimeric organs (Moran, 2007; Oldroyd et al., 2011; Visick et al., 2021). These organs, thus, create the environment that shapes microbial fitness outcomes. For example, in the rhizobia-legume symbiosis, these chimeric organs are known as nodules, which form on the roots of legumes (Oldroyd, 2013). Nodules can exhibit diverse shapes; yet we know little about the factors contributing to the various morphologies or the consequences these morphologies have on the benefits that hosts and microbes receive during symbiosis(Dupont et al., 2012; Sachs et al., 2018). More specifically, at present, little is known about how nodule branching influences rhizobial fitness and host fitness, and whether nodule branching is driven by host or rhizobial identity (Lindström and Mousavi, 2019). Here, we address these questions by examining multiple hosts and rhizobial genotypes to disentangle their contributions to nodule branching and how it affects rhizobial and host fitness.

Legume root nodules follow two developmental patterns broadly categorized as determinate or indeterminate based on the meristematic behavior of the host plant. Determinate nodules, found in soybean (Glycine max) species and common bean (*Phaseolus vulgaris),* lack a persistent meristem taking on a spherical shape. In contrast, indeterminate nodules, the focus of this investigation, found in species such as barrel clover (*Medicago truncatula)*, its close relative alfalfa *(Medicago sativa),* and pea (*Pisum sativum*), can take a more elongated shape due to having a persistent nodule meristem (Sprent et al., 2017). This persistent meristem sustains prolonged nodule growth, generating an elongated shape, and if the meristem splits, it can branch to form nodules with multiple branches, each with its own meristem (Hirsch, 1992; Łotocka et al., 2012; Gola, 2014). To our knowledge, the effect of host nodule branching on the fitness of rhizobia and the benefits provided to hosts within indeterminate nodules has never been investigated.

A common assumption in legume-rhizobium literature is that rhizobial population size increases as nodule size increases. This assumption is likely to hold in determinant nodules, where most rhizobia that fix nitrogen retain the ability to grow and divide, and the meristem only exists temporarily (Denison, 2000). However, many hosts that form indeterminate nodules house two spatially separated rhizobial populations and could be differentially impacted by nodule morphological variation (Thies et al., 1991; Sachs et al., 2018). Terminally differentiated bacteria live inside plant cells within bacteroids and are massively endoreduplicated. This is where nitrogen fixation occurs, a process that requires

an oxygen-limited environment. Bacteroids are concentrated in the nitrogen fixation zone closer to the root (Batut and Boistard, 1994). In contrast, undifferentiated bacteria live in infection threads close to the nodule meristem and serve as the source that differentiates into bacteroids as the nodules continue to elongate and grow (Sprent et al., 2017). These undifferentiated cells retain the ability to live in the soil and are released back into the soil when nodules senesce; thus, the size of the undifferentiated rhizobia population in nodules is of particular interest when considering rhizobial benefits from symbiosis (Denison and Kiers, 2004). Nodule branching could have a substantial effect on the size of this undifferentiated population because each branching event increases the meristematic area of the nodule that can be filled with infection threads and these undifferentiated rhizobia (de Carvalho-Niebel et al., 2024). Here, we test the hypothesis that nodule branching increases rhizobial population sizes, beyond what is expected due to increasing nodule size alone (Fig. 1). If branching of indeterminate nodules amplifies rhizobia population sizes, this could suggest new mechanisms by which rhizobia and hosts could alter each other's fitness (Porter et al., 2024).

Very little is known about how host or rhizobial genetics contribute to nodule branching and size. However, we do know that nodule branching and size are likely to be influenced by the genes of both host and rhizobial partner (Lindström and Mousavi, 2019). Complex host genetic pathways regulate nodule development through conserved transcription factors (Roy et al., 2020). Specifically, in *M. truncatula,* the nodule factor hydrolase, MtNFH1, promotes root hair infection and alters the form of mature nodules. The loss of MtNFH1 function results in nodules that are frequently and increasingly branched, rather than the typical elongated nodule (Tian et al., 2013; Cai et al., 2018). When there is an overexpression of MtNFH1, highly branched nodules are still produced. However, when MtNFH1 is underexpressed, fewer branched nodules are formed, indicating that either too little or too much expression disrupts normal patterning. MtFNH1 accumulates in the infection zone, where it likely establishes and maintains a nod-factor gradient concentration from the infection zone to the meristem, providing more context into the host's possible role in balancing nodule branching (Cai et. al., 2018). Confirming with this result, inoculation with nod-factor overproducing strains (1021[pEK327) lead to the increase in the number of pink, active nodules while shifting to an elongated or branched morphology; supporting the 'goldilocks' view in which the host nod-factor hydrolase activity and the rhizobial nod factor supply are involved in steering nodule multi-meristematic development (Cai et al., 2018). Rhizobia are also speculated to affect nodule morphology through phytohormones. For instance, *Rhizobium* can produce gibberellin (GA) through the *cyp* operon, specifically the GA3 oxidase enzyme that catalyzes GA2 into being bioactive, which has been identified as a nodule size regulator (Nett et al., 2022). Other

host and rhizobial genes may well influence nodule branching, but unfortunately, it is not a commonly reported phenotype. Clearly, there is much more left to uncover about how the complex crosstalk between the host and rhizobia can affect nodule morphology.

Here, we explore the effects of host and strain identity on nodule branching and rhizobial populations. We used a collection of 117 *Sinorhizobium* strains recently isolated from an alfalfa variety trial to perform a single-isolate inoculation experiment on three alfalfa varieties in a controlled greenhouse to ask questions from both rhizobia and host perspectives. To ask questions about the effect of branching on rhizobial fitness, we examined individual nodules from three host varieties inoculated with a subset of 8 strains. We pursued the following questions: a) Is nodule size and the number of rhizobia released from nodules correlated with nodule branching, and b) do these traits depend on the host variety or the rhizobium isolate identity? To ask questions about the effect of nodule branching on plant benefits, we measured per-plant traits for all three varieties inoculated with each of the 117 strains. We pursued the following questions: a) Does the host variety or rhizobial isolate influence nodule branching propensity, and b) Is the tendency for *Sinorhizobium* isolates to cause branching linked to a benefit for the host plant?

MATERIALS & METHODS

Source of *Sinorhizobium meliloti* isolates

We developed a collection of 117 *Sinorhizobium meliloti* strains from Alfalfa (*Medicago sativa*, varieties: Oneida, Vernal, and SW402) planted in 2016 at Penn-State's Russell E Larson Agricultural Station in Rocksprings, PA (40.7215,-77.9275). Full details of this collection will be published separately, along with genome sequencing information (Guha et al. in prep). Briefly, in the fall of 2020, we sampled four-year-old alfalfa plants in 0.914 by 6.019-meter strips using a randomized block design. We dug up 80 nodules from each of the three varieties growing within 5 meters of each other. After surface-sterilizing the nodules and homogenizing them in a 0.85% NaCl solution, the Isolation Bio Prospector system was used to culture individual rhizobial cells. To further purify the isolates, we streaked individual cultures from each well on a Yeast Mannitol Agar (10g Mannitol, 0.5g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.1g NaCl, 0.5g yeast extract in 1L of deionized water adjusted to a pH 6.8) plate, picked a single colony, re-streaked it, and incubated the plates at 28 °C (Vincent, 1970).

Plant preparation for the single-strain inoculation experiment

To examine the host-rhizobial interactions, we grew each of the 117 strains with four replicates of the three alfalfa varieties from the field (Oneida, Vernal, and SW0402) in nitrogen-limited conditions. We sterilized 1,200 *M. sativa* seeds of each variety in 10% commercial bleach for 90 seconds, rinsed them with de-ionized (DI) water, and transferred them onto filter paper in glass Petri dishes. We wrapped the petri dishes in aluminum foil and placed them at 4 °C for three days to stratify. In the greenhouse, we washed and bleached Heavyweight Deepot™ Cells (D40H – 2.5" diameter, 10" depth; item number: D40H), (N=1440; 4 replicates x 3 alfalfa varieties x 120 isolates & controls), filled them with 50:50 sand calcined clay. The pots were held in Deepot™ Support Tray (D20T for 2.5" & 2.7"; item number: D20T), arranged to have an empty cell between each pot, and kept in four blocks. We planted two seeds in each pot, covered them with ½ to ¼ inch of sterile vermiculite, kept pots moist with twice daily misting, and maintained humidity during germination by covering them with a clear plastic tarp. After one week, pots were moved to their randomized locations, and the plastic tarp was removed.

Plant inoculation and care

S. meliloti isolates were streaked onto Yeast Mannitol agar plates from glycerol stocks stored at –80 °C, placed in an incubator, and grown for four days at 28 °C. Then, the bacteria were transferred to Tryptone Mannitol (TY) liquid media and grown overnight in a shaking incubator at 150 RPM, 28 °C. We measured and adjusted each culture's optical density (OD600) to reach 0.1, then diluted it to 50ml with DI water. Later, we inoculated each pot eight days post-sowing with ~ 10^6 CFU. We based the OD600-to-colony-forming unit correlations on correlations made with eight strains from our collection. Greenhouse conditions were maintained at 18 °C to 23 °C during the daytime and a minimum temperature of 12°C throughout the experiment. Three weeks post-inoculation, we began fertilizing alfalfa weekly with 10 mL of 2x Fahräeus medium from the *Medicago truncatula handbook Appendix 2* (Barker et al., 2006). We doubled the fertilizer concentration every two weeks to track plant growth. We thinned to one plant per pot four weeks after sowing using sterile scissors and tweezers. Each pot was watered individually as needed to prevent cross-contamination of inoculated isolates.

Measurement of plant-level benefit traits

Eight weeks post-inoculation, we harvested plants and assessed above- and below-ground plant traits. First, we collected the aboveground biomass and dried it at 75°C for at least 72 hours. Second, we gently removed the roots from the growing media and briefly suspended them in water to remove any sand and calcined clay. We wrapped cleaned roots in a wet paper towel and stored them in a Ziplock bag at 4 °C for up to five days to allow us to manually count the number of branched and unbranched nodules using a clicker. After

recording the nodule information, we dried the roots, just as we did the shoots. We assessed biomass on a VWR® P2 balance (VWR-203P2/C).

Measurement of rhizobial fitness in individual nodules on a subset of host x strain combinations

During the above harvest, we collected six nodules from seven rhizobial isolates by three host variety combinations. The nodules were chosen, not randomly, but to span the nodule size variation on each plant. We photographed nodules on a green background to enable nodule area measurement and branch counting using the ImageJ "analyze particle tool" (Schneider et al., 2012). We then sterilized individual nodules with 20% commercial bleach and 5µl Polysorbate 20 for two to four minutes inside 1.7 mL Eppendorf tubes, lightly tapping the tube to ensure contact with all the nodule surface area. Each nodule was rinsed thrice with sterile water and stored in 100µL of 0.85% NaCl at 4 °C overnight. Using a sterile pestle, we crushed the nodules, added 900 µL 0.85% NaCl to reach 1mL, and performed a dilution series to determine the number of undifferentiated bacteria in each nodule. For smaller nodules, we plated and counted five 20 µL spots for both the 10^3 and 10^4 dilutions, while for larger nodules, we spot-plated the 10^5 and 10^6 dilutions. Spots were counted ~48 hours after treatment and then double-checked at 72 hours. We determined the dilution level at which the number of colonies mostly ranged between 10 and 100 and calculated the average number of colonies across all five spots. We multiplied the average by the dilution factor and divided by the volume plated to estimate the population size of rhizobia released from each nodule.

Statistical Analyses

All statistical analyses were performed using the R statistical environment (version 4.4.3; R Core Team, 2016). To test the relationships between single nodule area, rhizobial population size, and the number of branches, we fit linear models and tested for significant terms using an analysis of variance (ANOVA). We used the following models: To test if nodule branching increased nodule surface area across host variety *(log_{10} Nodule Surface area ~ number of nodule branches * Alfalfa host variety * rhizobial isolate + replicate);* To assess if the nodule surface area and number of nodule branches influenced the undifferentiated *Sinorhizobial* population (*log_{10} Viable rhizobia released~ nodule surface area * number of nodule branches + Alfalfa host variety + rhizobial isolate + replicate)*.

To assess the relationship between our measured predictors of plant benefit, we used the proportion of branched nodules (number of branched nodules / (number of unbranched nodules + number of branched nodules)) and host biomass (aboveground + belowground biomass). We fit linear models and tested for significance using an analysis of variance (ANOVA). We used the following models: To assess if plants with a higher

proportion of branched nodules tended to form more nodules while accounting for rhizobial strain variation *(Mean nodule count ~ mean proportion of branched nodules + rhizobial isolate);* To assess if strains that produce a higher proportion of branched nodules also support more host growth and if the effect depend on host variety we ran the following model mean (*host biomass~ mean proportion of branched nodules * Alfalfa host variety + rhizobial isolate)*.

RESULTS

Bigger and more branched nodules have larger populations of reproductively viable rhizobia.

First, we tested if nodule branching and size covary and determined if the relationship is influenced by host and strain genetics (Table S1 for full model details, $R^2=0.667$). We found, perhaps unsurprisingly, that nodules with more branches tended to have a larger surface area ($p=4.14E^{-48}$). While host genotype did not significantly alter the relationship between these traits (p=0.607), *Sinorhizobium* isolate identity did (p=0.001, Fig. 2a). This implies that some strains tend to create smaller or larger nodules independent of nodule branching. In summary, nodule branching strongly correlates with nodule surface area, and this relationship is predominantly influenced by the isolate's identity, rather than the host.

Next, we examined the relationship between rhizobial population size (inferred by the number of culturable *Sinorhizobium* in each nodule) and the number of nodule branches (Figure 2b, Table S2, full model $R^2=0.2116$). We found that the population of *Sinorhizobium* increases as more branches are present ($p=4.651E^{-11}$), and that this relationship is somewhat dependent on host variety (p=0.002) but not strain identity (p=0.409). While some unbranched nodules also house large culturable rhizobial populations, more branched nodules consistently contain large populations of undifferentiated *Sinorhizobium*. This suggests that forming branched nodules potentially increases the nodule region that undifferentiated rhizobia could inhabit (Fig. 2b).

Unsurprisingly, given that branch number and nodule area are correlated, we observed that rhizobial population sizes increased with nodule size (Fig. 2c). However, unlike nodule branch number, there is an apparent plateau, such that increasing nodule surface area does not lead to more culturable *Sinorhizobium* from nodules. We hypothesized that the decoupling of nodule surface area and population size of culturable rhizobia could be due to the spatial restriction of infection threads near the meristematic region on the nodule branches. To explore this hypothesis, we ran a third model to test whether an increase in nodule branches increases the amount of undifferentiated

Sinorhizobium released beyond what is expected based on nodule surface area alone (Table S1 for full model details, $R^2=0.453$). We found that the relationship between nodule surface area and rhizobia released significantly interacted with the number of branches a nodule had ($p=1.50E^{-21}$). Nodule area and rhizobia released are tightly coupled when nodules have only one or two branches (steep slope). Still, the relationship erodes, and rhizobia populations plateau when nodules are highly branched. Moreover, host varieties differed from each other (p=0.001). Overall, we find that nodules that have fewer than two branches and were relatively large released a similar amount of rhizobia as nodules with three or more branches (Fig. 2C). Taken together, this suggests that size isn't the sole driver of the amount of viable *Sinorhizobium* inhabiting a nodule, the architecture, branching, and host genetics play a role in applying a physical constraint to this relationship.

Rhizobial isolates that produce fewer, more branched nodules with a host tend to provide more benefits.

We extended our analysis to include the effect that nodule branching has on host benefit traits, such as nodule quantity and total host biomass. We found evidence that a tradeoff exists between forming more nodules and producing a higher fraction of branched nodules ($p=7.89E^{-17}$), and that both host variety (p=3.107E^{-11}) and specific *Sinorhizobium* isolate (p=2.275E^{-9}) significantly altered this relationship (full model $R^2=0.458$). One possibility is that branched nodules could be a resource management strategy for the host, where increased investment in branching complexity reduces the total number of nodules produced. This adds complexity to resource allocation, as a conflict arises between nodule quantity and nodule development.

Building on the previous results, we wanted to know if the nodule branching tendency of a *Sinorhizobium* isolate was associated with higher host benefits. We found that rhizobial isolates that produced a greater proportion of branched nodules with a host variety tended to be more beneficial ($p=1.13E^{-22}$)($R^2=0.530$). This relationship depends on the host variety ($p=1.75E^{-20}$) and the *Sinorhizobium* isolate identity (p=1.265E^{-9}) separately (Table S3 for full model details). This suggests that *Sinorhizobium* isolates that produced more branched nodules tended to support larger host plants. Because the plants were grown in a nitrogen-free environment, this increased growth can be attributed to increased nitrogen fixation (Fig.3b).

DISCUSSION

In this investigation, we examined nodule branching from both the rhizobial bacteria and plant host perspectives, focusing specifically on the effect of genetic variation in both the rhizobia and alfalfa. We found that larger, branched nodules release more rhizobial

bacteria regardless of the host or isolate identity. From the plant host perspective, we found that the proportion of branched nodules can be used as a low-cost and low-effort proxy for the nitrogen-fixing benefits each strain provides to the host. More broadly, these results suggest a positive correlation between rhizobial fitness benefits (rhizobia released) and plant benefits (nitrogen fixed). From an ecological perspective, our results support the hypothesis that host plant resource allocation (e.g., sanctioning or rewards) may contribute to nodule branching. Below, we discuss the implications of these findings for 1) understanding rhizobial populations, 2) use as a proxy of host benefit, and 3) maintaining mutualisms.

Nodule branching alters the relationship between nodule size and rhizobial population size.

While nodule development and rhizobial differentiation strategies have been well described in uni-branched indeterminate nodules (Rodríguez-Haas et al., 2013; Pan and Wang, 2017), the functionality of nodule branching in legume-rhizobia symbiosis remains unresolved. Infection threads within nodules are concentrated nearer the growing tip of the branches. This region is called the "infection zone" with undifferentiated rhizobia that keep reproductive viability. Near the root end of the nodules, there is a senescence zone, where older bacteroids are degraded. Transcriptomic and metabolomics studies clearly show that the environment experienced by bacteria differs between infection threads and bacteroids (Roux et al., 2014; Pereira et al., 2024). Our results suggest that when nodules branch, the growing tip space increases and thus allows for increased space for the infection threads that house undifferentiated bacteria.

The proportion of branched nodules can be used as a proxy for plant benefit.

The typical use of root nodule count as a metric of symbiotic effectiveness does not account for the size of the nodules. We observed that rhizobia isolates altered plant-level traits, such as nodule number and the proportion of branched nodules. In fact, these two metrics are negatively correlated. Further, we found that nodule branching propensity was strongly correlated with plant benefits, whereas nodule number was not. Thus, our results suggest that the proportion of branched nodules could be used as a proxy for symbiotic investment/benefit that does not require nodule drying, and allows sample processing for measuring microbial communities via sequencing of whole genomes or amplicons (Oono et al., 2011; Brockhurst and Koskella, 2013; Allito et al., 2021).

Nodule branching could play an adaptive role in mutualism evolution

Using single inoculation experiments, we found that both host and rhizobial genetic variation contribute to nodule branching and that nodule branching is linked to both

rhizobial fitness and plant fitness. Despite our observations being limited to host investment because we worked with single strains (Wendlandt et al., 2019), these results suggest that nodule branching could influence mutualism evolution and stability through the reward/sanction mechanism (Porter et al., 2024). For instance, nodules hosting more beneficial rhizobia could be receiving more rewards from hosts and therefore may be more likely to form branches to enable both additional area for nitrogen fixation in bacteroids and an increase in rhizobial population size. This would align with previous studies that have used other nodule traits such as nodule size or nodule fresh weight (Kiers et al., 2006; Westhoek et al., 2021). Previous literature, however, has found that nodule branching might not be relevant for sanctioning, as it does not positively correlate with plant benefit when inoculating a mix of *S. meliloti* in *Medicago truncatula* (Heath and Tiffin, 2009), but this could be due the little difference in nitrogen fixation of the strains they used (Oono et al., 2011). Further experiments using multi-strain inoculations are needed to assess if the number of branches is relevant for sanctioning. Overall, we suggest that nodule branching provides an additional axis of trait variation in the legume-rhizobium symbiosis that could alter the dynamics of legume rhizobia evolution (Sachs et al., 2018).

Broader Implications for evaluating nodule shape metrics for managing beneficial microbes

Overall, our results suggest that the proportion of branched nodules should be included in future studies focusing on the influence of rhizobial and host genes on symbiosis. For instance, measuring nodule traits in larger-scale experiments could reveal allelic variation that explains observed phenotypes, or assessing how different host and rhizobial benefit traits differ between leguminous species across various nodule development strategies (Burghardt et al., 2025). Alongside other metrics, using the proportion of branched nodules will add to our efforts to improve nitrogen fixation and thus decrease our reliance on synthetic nitrogen fertilizers (Herridge et al., 2008; Gopalakrishnan et al., 2015; Mendoza-Suárez et al., 2021). Similar investigations will be required in other leguminous species to determine if the positive relationship between nitrogen fixation efficiency and nodule branching is a general pattern or specific to Medicago. More broadly, our results suggest that applying evolutionary ecology perspectives to the development of symbiotic organs can provide novel insights and new traits to study even in well-studied model systems like legumes and rhizobia.

Authorship Credits: LTB devised the research idea and developed the experimental design, contributed to analysis, and revised the manuscript. SVG and MAGP also contributed to the experimental design and execution, including measuring rhizobial population sizes. AAS assisted with plant care and led the nodule image analyses. ELP managed and

executed the greenhouse experiment, analyzed the data, drafted and led the revision of the manuscript. All authors edited, reviewed, and approved the manuscript.

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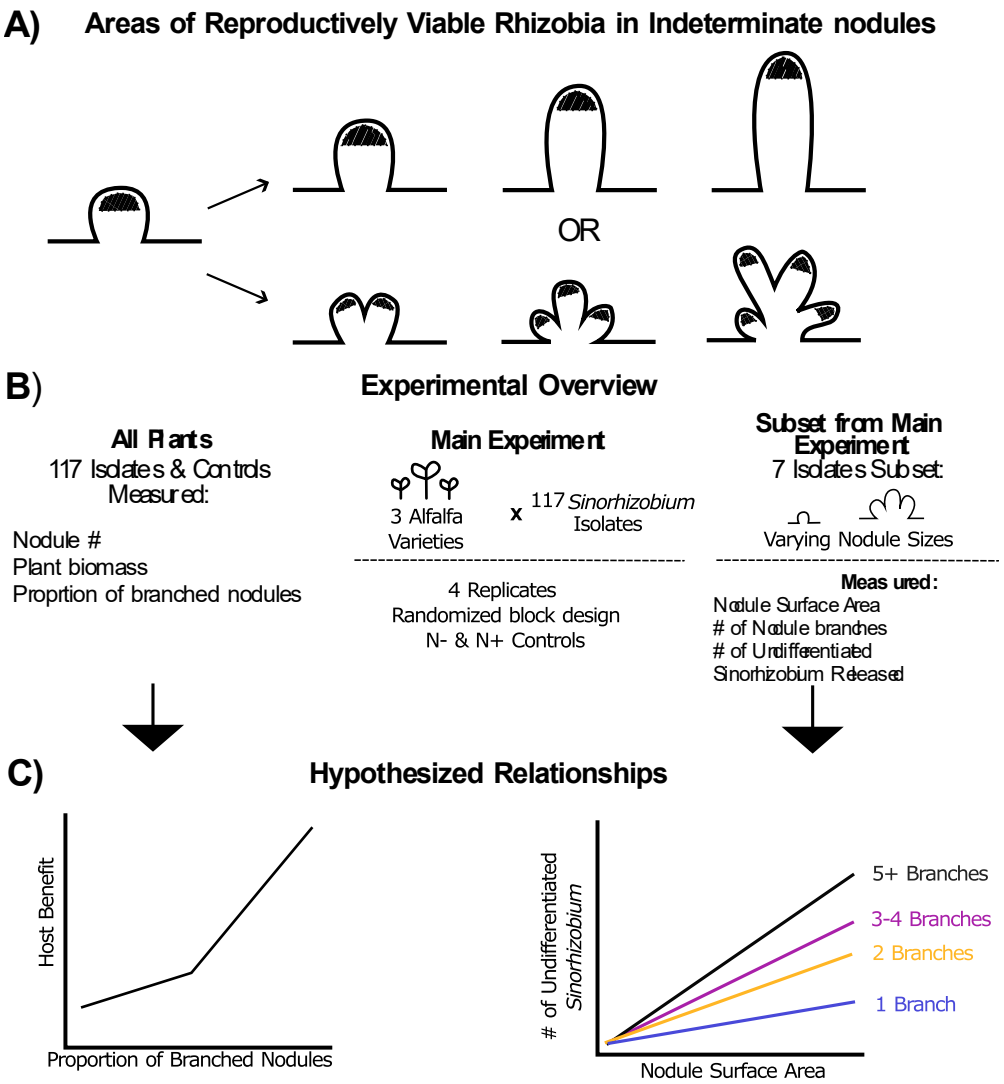

Figure 1. A) Areas within indeterminate nodules, where reproductively viable bacteroids, capable of being released back into the soil for reinfection, inhabit unbranched and branched nodule structures. **B)** Overview of our main experiment with 117 *Sinorhizobium* isolates inoculated in 3 genotypes of *Medicago sativa,* alfalfa, and an additional experiment with a subset of 7 isolates. **C)** Our hypothesis of the tested relationships between the presence of undifferentiated bacteria in nodules with more branches and possibly a higher branch size, and the relationship between host benefit and a trait like the proportion of branched nodules.

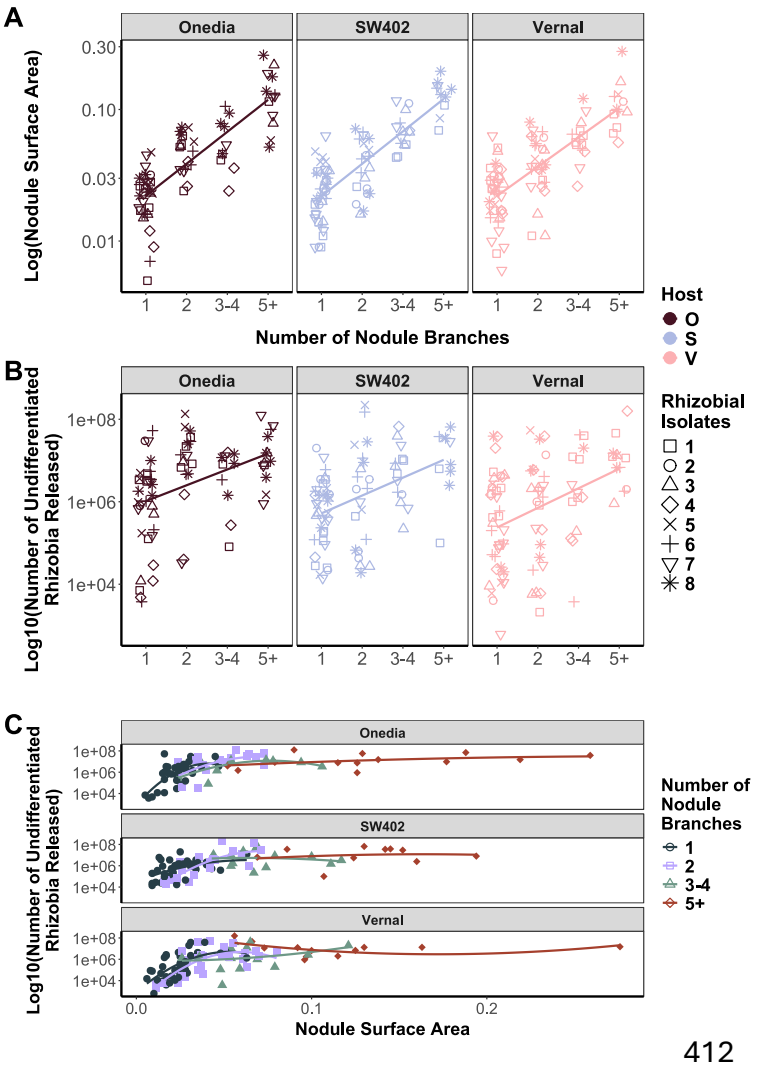

Figure 2. Examination of rhizobial benefit traits tested (number of released rhizobia and nodule surface area) compared across alfalfa host varieties *Oneida, SW402, and Vernal*. (A) Scatterplot of $\log^{10}$ of the nodule surface area across nodule branch number *($p= 4.14E^{-48}$, $R^2=0.6678$)*. **(B)** Scatter plot of $\log^{10}$ of the undifferentiated *Sinorhizobium* released from crushing nodules across nodules with 1-5 branches present *($p=4.65E^{-11}$, $R^2= 0.2116$)*. **(C)** Scatter plot of $\log^{10}$ of the undifferentiated *Sinorhizobium* about the nodule surface area based on the number of nodule branches *($p=1.50E^{-21}$, $R^2=0.453$)*. The more branches a nodule has, the higher the total nodule surface area, and the number of *Sinorhizobium released* when crushed is increased. However, there is a limit to this effect. *Additional details: Anova and model details are in (Supplement Table 1)*

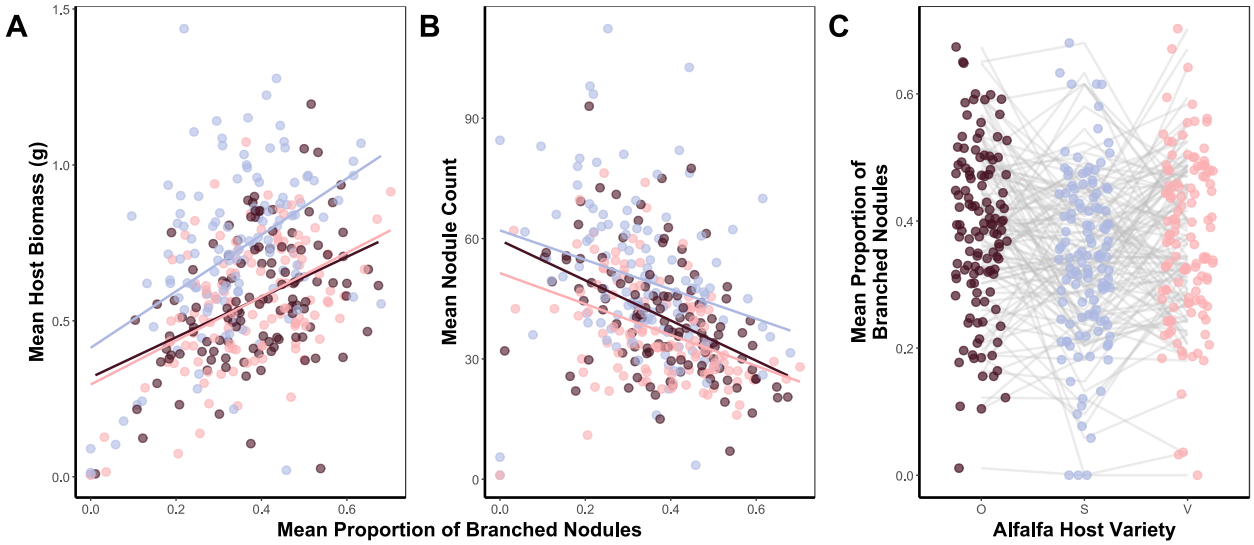

Figure 3. Relationship between nodule traits and plant benefits across alfalfa hosts. **(A)** Scatterplot of the mean proportion of branched nodules and its correlation to the mean host biomass across host plant-Sinorhizobium isolate combination replicates (*$p=1.13E^{-22}$, $R^2=0.458$)*. **(B)** Mean proportion of branched nodules and its correlation to the mean nodule count across host plant-Sinorhizobium isolate combination replicates *($p=7.89E^{-17}$, $R^2=0.530$)*. Increasing the number of branched nodules can increase the host plant's dried biomass while contributing to a lower total count of nodules overall. **(C)** Sinorhizobium isolate performance depends on the host and isolate genotype when forming branched nodules. *[Additional details: Host Alfalfa genotypes: Oneida, SW402, and Vernal. ANOVA and model details are in Supplement Table 2.]*

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