

## ***New Phytologist* Supporting information**

### **Microbiome profiling reveals that *Pseudomonas* antagonise parasitic nodule colonisation of cheater rhizobia in *Lotus***

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**Dataset S1.** Metadata of all samples.

**Table S1. Strains and plasmids used.**

Strains used	Derivation and relevant genotype	Reference
<i>Mesorhizobium</i> sp.		
Qb1E3-1	Wild type strain isolated from a healthy <i>Lotus burttii</i> nodule, Fm <sup>R</sup>	This work
Qb1E3-1-cerulean	<i>Mesorhizobium</i> Qb1E3-1 containing the pABC plasmid, Sp <sup>R</sup>	This work
DC-1.5	Wild type strain isolated from a healthy <i>Lotus burttii</i> nodule	This work
<i>Pseudomonas</i> sp.		
Lb2C2	Wild type strain isolated from a healthy <i>Lotus burttii</i> nodule, Rif <sup>R</sup>	This work
Lb2C2-GFP	<i>Pseudomonas</i> sp. Lb2C2 containing the pFAJ-GFP plasmid, Tc <sup>R</sup>	This work
PLb11B	Wild type strain isolated from a healthy <i>Lotus burttii</i> nodule, Rif <sup>R</sup>	This work
PLb11B-GFP	<i>Pseudomonas</i> sp. PLb11B containing the pFAJ-GFP plasmid, Tc <sup>R</sup>	This work
<i>Rhizobium</i> sp.		
BW8-2	Wild type type strain isolated from a starved <i>Lotus burttii</i> nodule	This work
BW8-2-DsRed	<i>Rhizobium</i> sp. BW8-2 containing the pFAJ-DsRed plasmid, Tc <sup>R</sup>	This work
<i>Escherichia coli</i>		
ST18	S17 $\lambda$ pir $\Delta$ hemA, Tp <sup>R</sup> , Sm <sup>R</sup>	(Thoma & Schobert, 2009)
Plasmids		
pFAJ-GFP	pFAJ1708 carries the GFP encoding gene, Tc <sup>R</sup>	(Kelly <i>et al.</i> , 2013)
pFAJ-DsRed	pFAJ1708 carries the DsRed encoding gene, Tc <sup>R</sup>	(Kelly <i>et al.</i> , 2013)
pABC-cerulean	pABC-cerulean plasmid, Tc <sup>R</sup>	Prof. Dr. Anke Becker

Fm, fosfomycin; Sp, spectinomycin; Tc, tetracyclin; Sm, streptomycin; Rf, rifampycin; R, resistance.

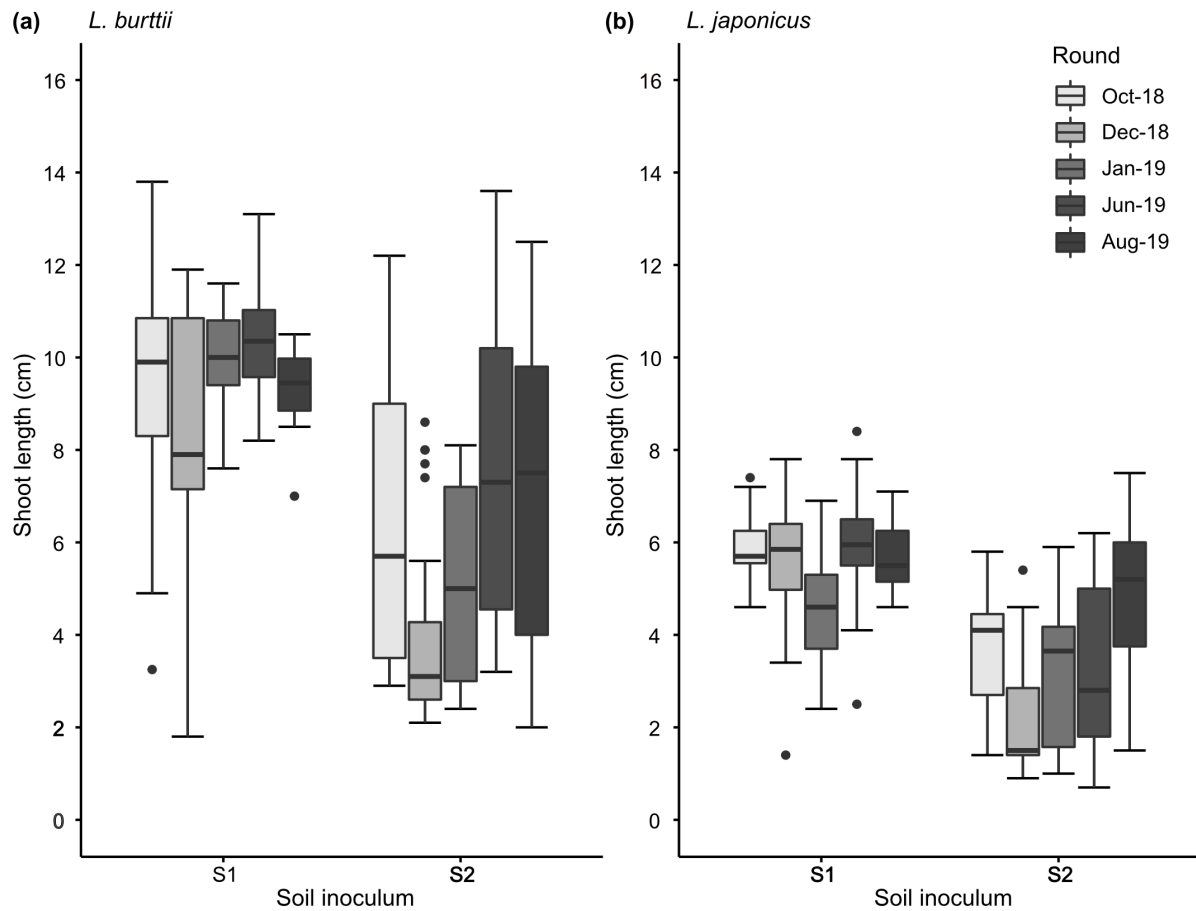
**Table S2. Physicochemical analysis of soil samples.**

	Site 1	Site 2
Soil type	huL	uL
pH-value	7.1	7.2
P <sub>2</sub> O <sub>5</sub> (mg/100g)	23	14
K <sub>2</sub> O (mg/100g)	33	19
Mg (mg/100g)	27.8	15.3
Mn (mg/Kg)	258	136
Cu (mg/Kg)	4.1	4.1
Zn (mg/Kg)	4.3	4.3
Na (mg/Kg)	3	3
B (mg/Kg)	0.62	0.12
Fe (CAT) (mg/100g)	13.1	9.7
S (mg/Kg)	5.6	5.1
K <sub>fix</sub> (mg/100g)	2	3
Org. matter %	4.7	3.5
N <sub>tot</sub> %	0.27	0.2
C/N	10	10
Ca (mg/100g)	199	231
% ton	26	24
% silt	23	45
% sand	52	31

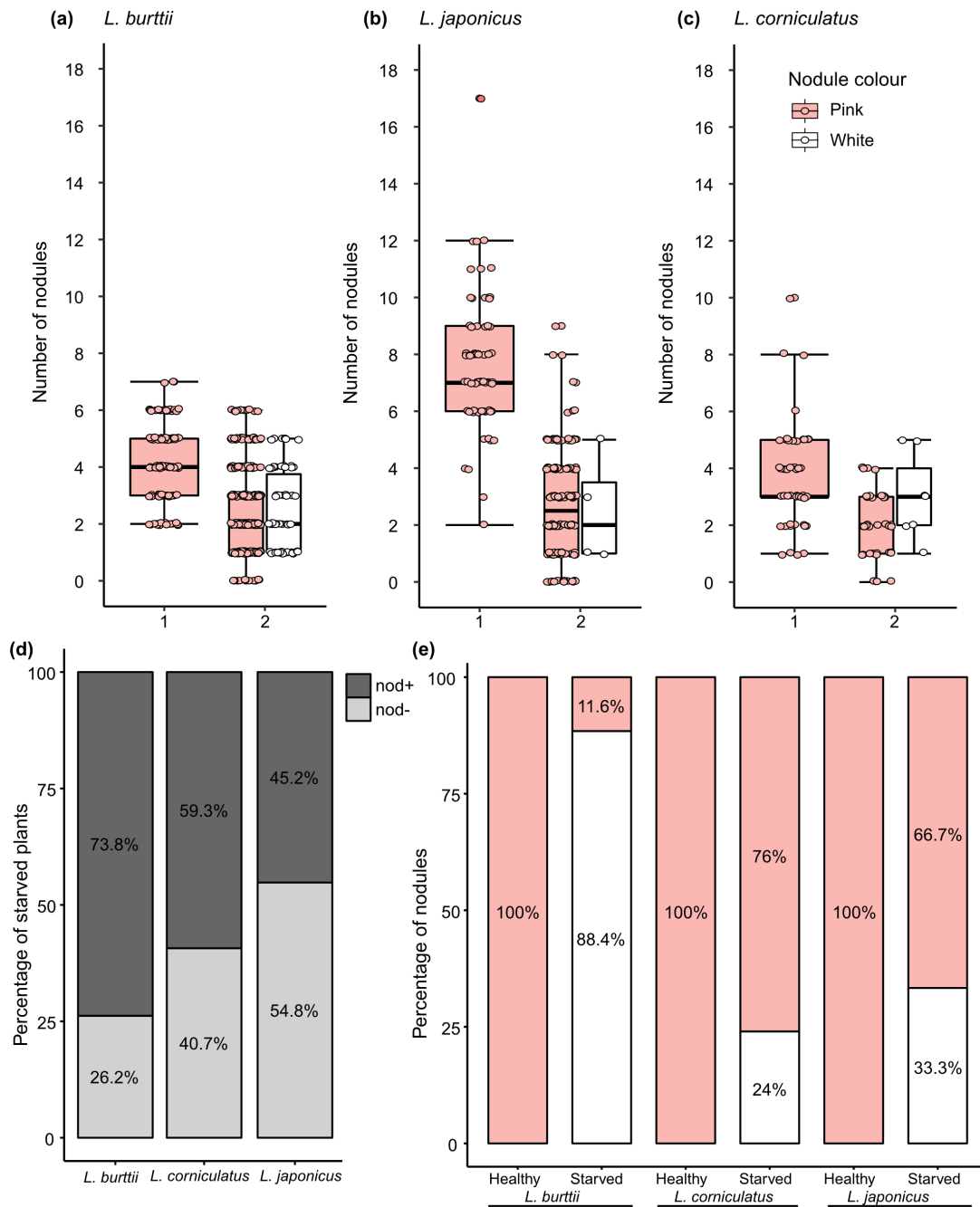
\*The analysis was conducted by AGROLAB Agrarzentrum GmbH. h = humus soil; uL = silty clay.

**Table S3. PERMANOVA analysis of beta diversity in all nodule microbiome sample types.** The Bray-Curtis dissimilarity index (Bray & Curtis, 1957) was used to provide dissimilarity measures between the samples. PERMANOVAs were then performed with 999 permutations using the vegan V 2.5.2 package in R (Oksanen *et al.*, 2018). \*\*\* indicate p-values < 0.001. \* indicate p-values < 0.05. Soil S, soil suspension.

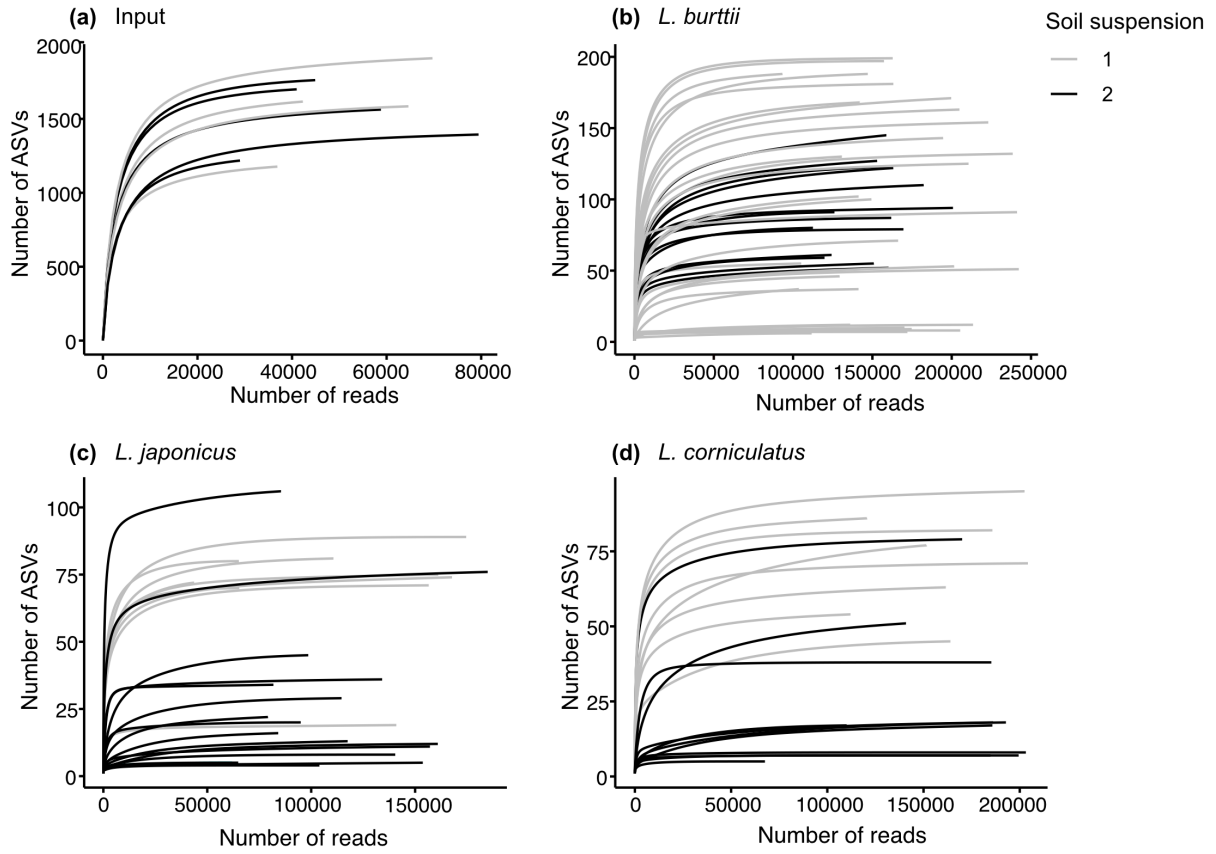
Compared sampled types	Pr(>F)	R2	F.model	Mean Sqs	Df	Sum OfSqs
Soil S1 v Soil S2 (input suspension)	0.072	0.215	1.919	0.431	1	0.4317
Soil S1 v Soil S2 (all plant nodules)	0.001***	0.213	23.843	3.535	1	3.535
Soil S1 healthy plants - <i>Lb</i> v <i>Lj</i>	0.273	0.056	1.252	0.151	1	0.151
Soil S1 healthy plants - <i>Lc</i> v <i>Lj</i>	0.042*	0.219	4.206	0.418	1	0.418
Soil S1 healthy plants - <i>Lc</i> v <i>Lb</i>	0.1	0.116	2.635	0.286	1	0.286
Soil S2 healthy plants - <i>Lb</i> v <i>Lj</i>	0.001***	0.160	5.552	0.306	1	0.306
Soil S2 healthy plants - <i>Lc</i> v <i>Lj</i>	0.093	0.088	1.753	0.120	1	0.120
Soil S2 healthy plants - <i>Lc</i> v <i>Lb</i>	0.249	0.052	1.162	0.067	1	0.067
<i>Lb</i> healthy plants - soil S1 v soil S2	0.001***	0.429	21.82	1.808	1	1.808
<i>Lj</i> healthy plants - soil S1 v soil S2	0.002***	0.461	17.98	1.489	1	1.489
<i>Lc</i> healthy plants - soil S1 v soil S2	0.109	0.175	2.562	0.210	1	0.210
<i>Lb</i> soil S2 - healthy v starved plants	0.001***	0.525	30.95	3.055	1	3.055
<i>Lj</i> soil S2 - healthy v starved plants	0.097	0.051	0.869	0.061	1	0.061
<i>Lc</i> soil S2 - healthy v starved plants	0.742	0.050	0.428	0.025	1	0.025
<i>Lc</i> soil S1 - lab grown v wild plants	0.342	0.112	0.891	0.080	1	0.080
<i>Meso</i> S1 v <i>Meso</i> Soil 2 (input suspension)	0.41	0.128	1.028	0.162	1	0.162



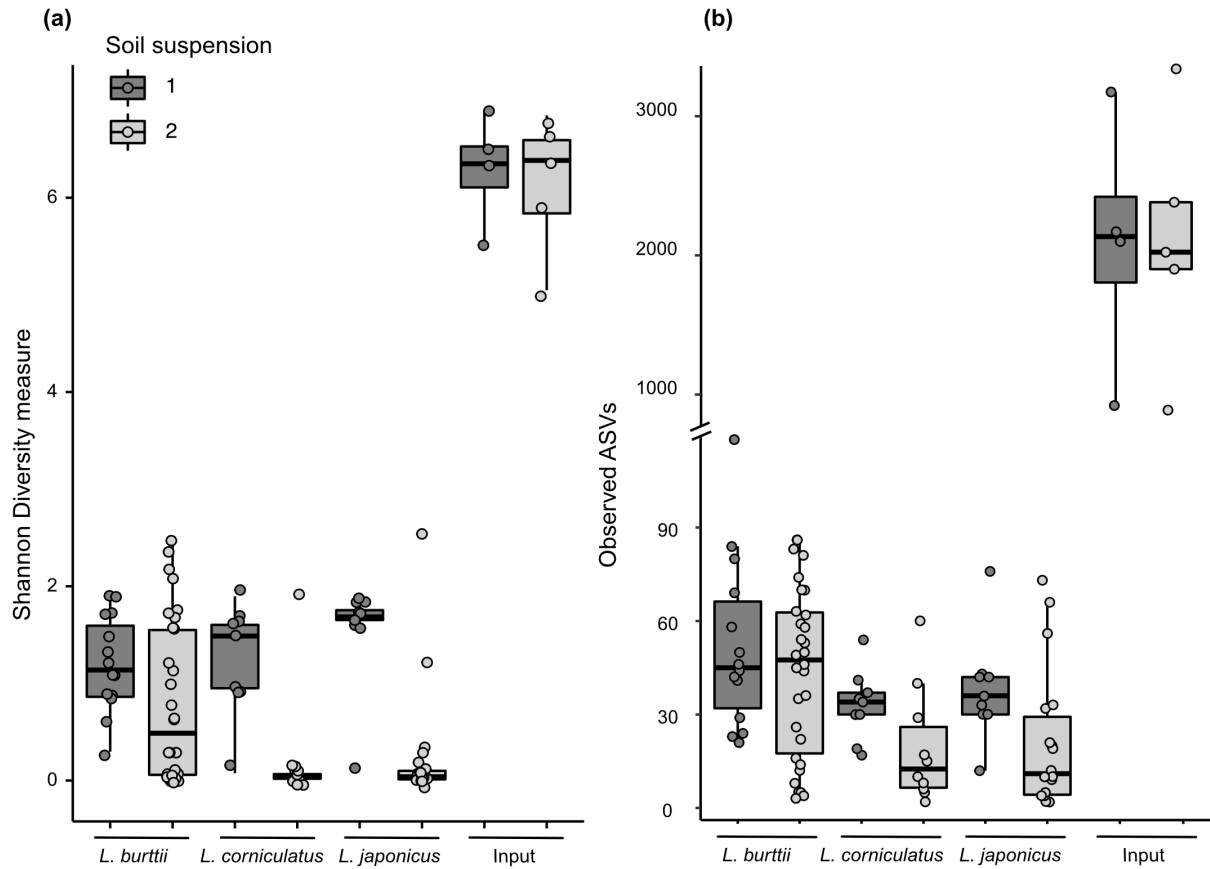
**Fig S1. Reproducibility of plant growth experiments.** Shoot phenotype of *L. burttii* (a) and *L. japonicus* (b) inoculated with either soil suspension 1 (S1) or soil suspension 2 (S2) from independent inoculations. Experiments were carried out in the months indicated with independent soil samples collected in October 2018 and May 2019. Between 50-100 plants per condition were grown in closed jars and harvested at 5 weeks post inoculation for each independent experiment. The bold black line and the box depict the median and the interquartile range, respectively. Black circles represent outliers.



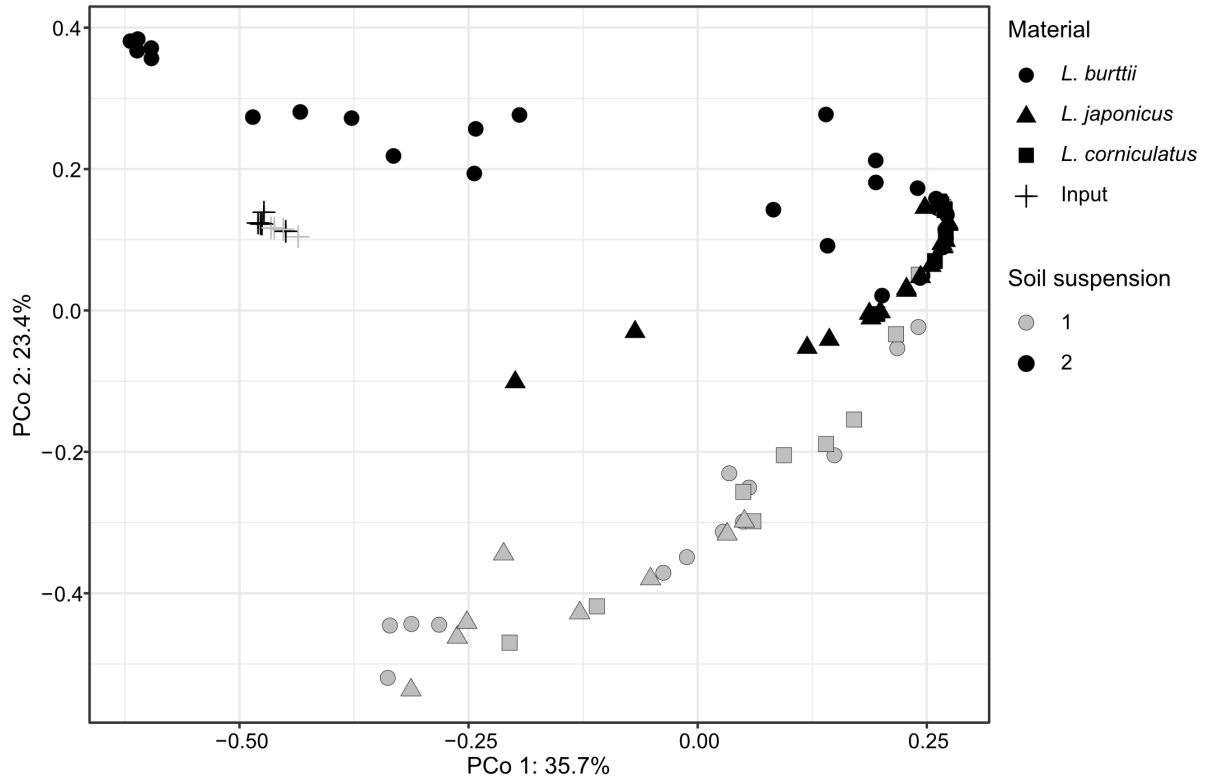
**Fig S2. Number of nodules per plant after inoculation with soil suspensions.** Quantification of pink and white nodules of *Lotus burtii* (a), *Lotus japonicus* (b), and *Lotus corniculatus* (c) plants grown in closed jars for 5 weeks after inoculation with soil suspensions 1 and 2. Each plot consists of results from two independent experiments. Each point represents the number of nodules in one plant. The bold black line and the box depict the median and the interquartile range, respectively. Plants that contained no nodules are not represented. Each sample type contains between 50-150 plants. (d) Percentages of starved plants of *L. burtii* (n=42), *L. corniculatus* (n=27), and *L. japonicus* (n=42) including both pink and white nodules. (e) Percentages of pink and white nodules formed according to species and phenotype after soil suspension 2 inoculation. Plants were inoculated with either soil suspension 1 or 2, grown in closed Weck jars and harvested 5 weeks post inoculation. The plot consists of results from two independent experiments.



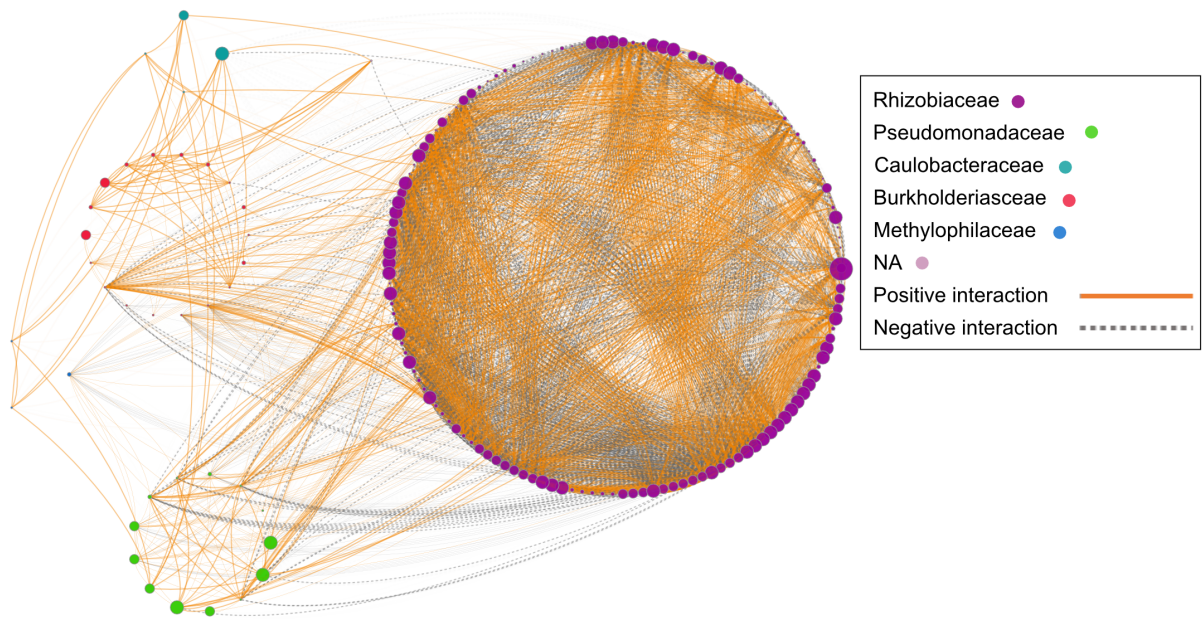
**Fig S3. Rarefaction curves of sequencing data.** Rarefaction curves of nodule samples from soil suspension input (a), *Lotus burttii* (b), *Lotus japonicus* (c), *Lotus corniculatus* (d) showing the number of unique amplicon sequence variants (ASVs) per total reads. Calculated using the *vegan* package in R (Oksanen *et al.*, 2018). Soil suspension input for each sample is discerned by colour.



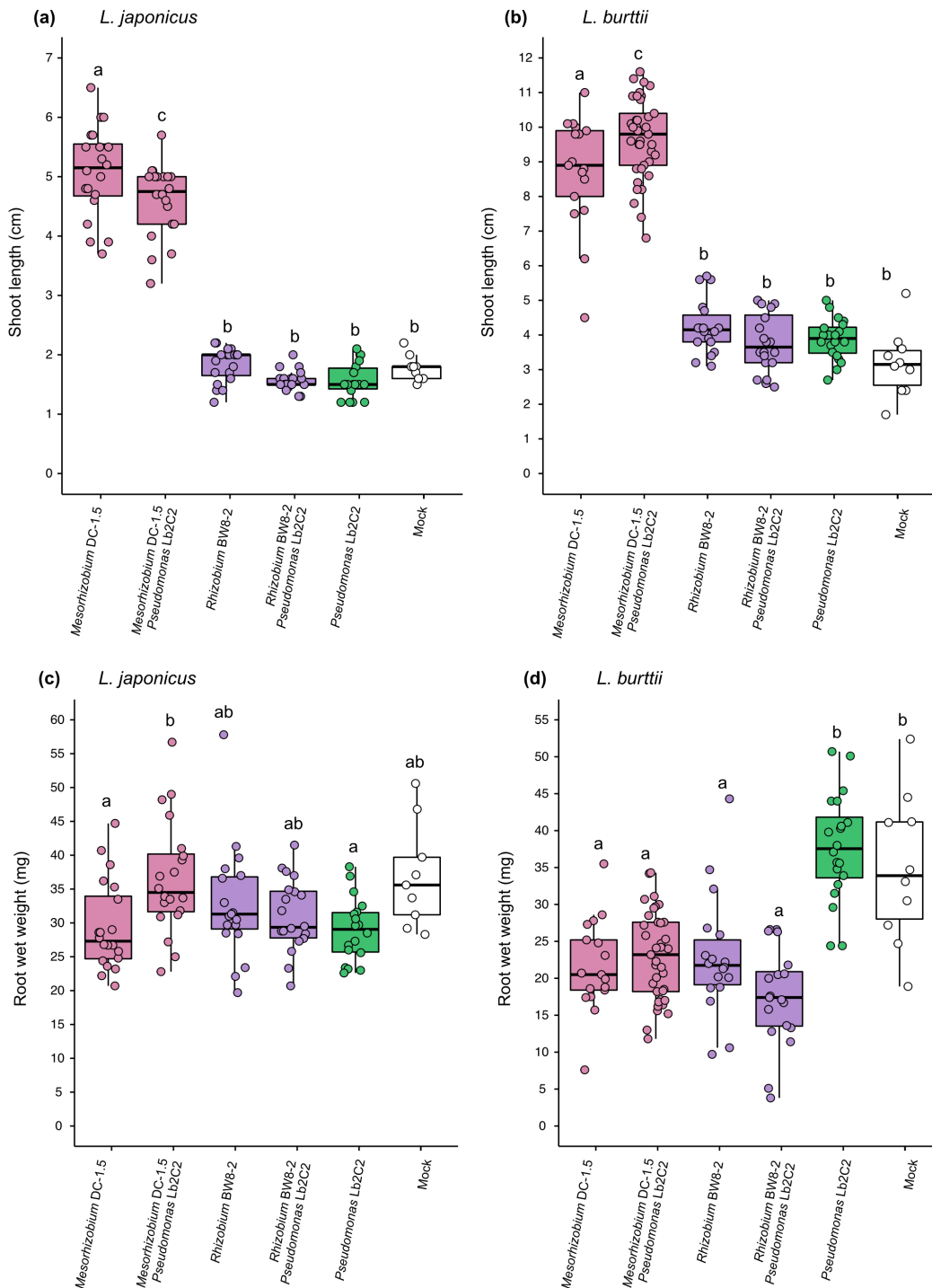
**Fig S4. Nodule microbiome alpha diversity plotted by species and soil suspension input.** (a) Shannon diversity measures and (b) observed amplicon sequence variants (ASVs) of all 99 samples were calculated using unfiltered data. Each point represents a nodule sample or a soil suspension input sample. The bold black line and the box depict the median and the interquartile range, respectively.



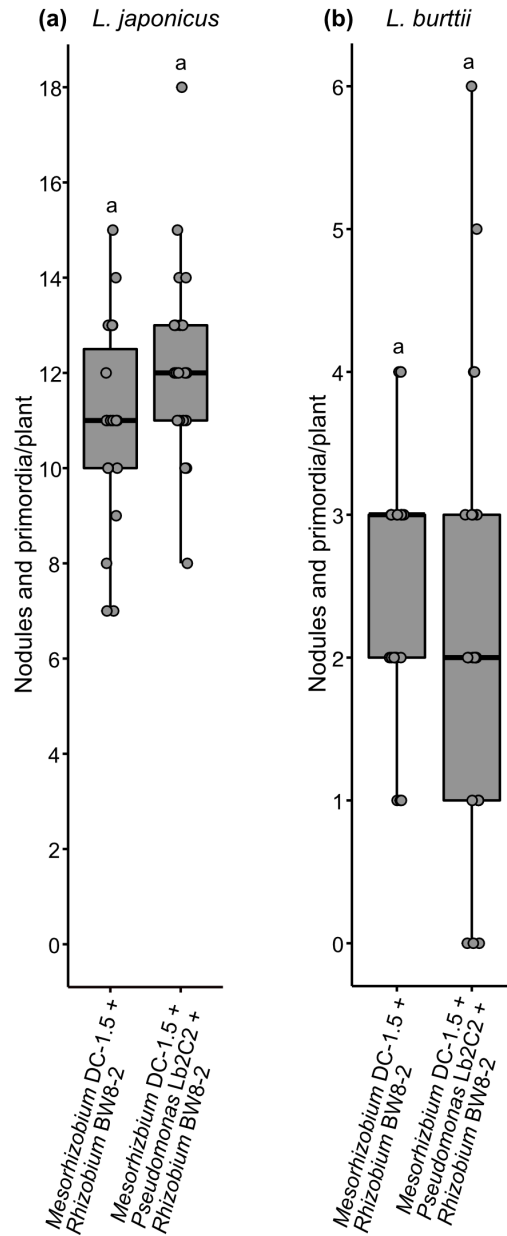
**Fig S5. Global principal coordinate analysis (PCoA) of all samples.** PCoA plot of all soil suspension input and nodule samples based on beta diversity calculated using the Bray-Curtis dissimilarity index (Bray & Curtis, 1957).



**Fig S6. Overview network analysis.** An amplicon sequence variant (ASV) table of *L. burtii* samples inoculated with soil suspension 2 was used to infer a correlation network using the SparCC algorithm (Friedman & Alm, 2012) implemented in FastSpar (Watts *et al.*, 2019). The nodes (dots) of this network corresponding to ASVs are grouped and coloured by Family. Node size indicates the relative abundance. Each edge (line) between two ASVs represents either a positive (orange line) or negative (grey-dashed line) correlation. Significant correlations ( $|R| \geq 0.2$ ,  $P \leq 0.01$ ) are shown in the network. NA indicates taxonomy of ASV could not be assigned at a family level.



**Fig S7. Root weight and shoot length phenotype of *Lotus* plants inoculated with *Rhizobium* sp. BW8-2, *Mesorhizobium* sp. DC-1.5, and *Pseudomonas* sp. Lb2C2.** Box plots of the shoot length and wet root weight of *Lotus japonicus* (a, c) and *Lotus burtii* (b, d) plants. 20 plants were inoculated with *Rhizobium* BW8-2, *Mesorhizobium* sp. DC-1.5, and *Pseudomonas* Lb2C2 nodule isolates. *L. burtii* and *L. japonicus* were harvested at 4 and 5 weeks post inoculation respectively. Each point represents one plant. The bold black line and the box depict the median and the interquartile range, respectively. Significance calculated using ANOVA and Tukey HSD is indicated as lower-case letters.



**Fig S8. Nodule organogenesis phenotype of *Lotus* plants inoculated with *Rhizobium* sp. BW8-2, *Mesorhizobium* sp. DC-1.5, and *Pseudomonas* sp. Lb2C2.** Box plot of the number of nodules and nodule primordia formed on *Lotus japonicus* (a) and *Lotus burttii* (b) roots. 20 plants were inoculated with *Rhizobium* sp. BW8-2, *Mesorhizobium* sp. DC-1.5, or *Pseudomonas* sp. Lb2C2 nodule isolates. *L. burttii* and *L. japonicus* were harvested at 4 and 5 weeks post inoculation, respectively. Each point represents the number of nodules in one plant. The bold black line and the box depict the median and the interquartile range, respectively. Significance calculated using ANOVA and Tukey HSD is indicated as lower-case letters.

**Dataset S1. Metadata of all samples.** All plants were grown in closed jars for 5 weeks. Total reads indicate the number of reads given after MiSeq sequencing. Shannon and Simpson diversity measures were calculated using the phyloseq package in R (McMurdie & Holmes, 2013). NA = not applicable. (See separate file).

## References

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