

Supplementary material

Article

Microbial community investigation of wild brambles with root nodulation from a calcareous nitrogen-deficient soil

Beatrice Farda ^{1,‡}, Alessandro Mattedi ^{1,2,‡}, Rihab Djebaili ^{1,3*}, Loretta Pace ¹, Maddalena Del Gallo ¹, and Marika Pellegrini ^{1,*}

¹ Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio, Coppito, 67100, L'Aquila, Italy.

² Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria, Centro di ricerca Orticoltura e Florovivaismo (CREA-OF), 84098 Pontecagnano SA, Italy

³ National Interuniversity Consortium for Environmental Sciences (CINSA), Parma, Italy.

* Correspondence: marika.pellegrini@univaq.it (M.P.); Tel.: +39-0862-433258 (M.P.); rihab.djebaili@guest.univaq.it (R.D.)

Table S1. Amplicon Sequence Variants (ASVs) relative abundances (%) at the phylum level (no cut-off).

Nodule	Rhizosphere	Domain	Phylum
3.56986101	63.04912244	Bacteria	Actinobacteriota
3.440624238	23.6577007	Bacteria	Bacteroidota
3.089490368	1.290372832	Bacteria	Bacillota
89.5805901	9.396095129	Bacteria	Pseudomonadota

Table S2. Amplicon Sequence Variants (ASVs) relative abundances (%) at the genus level (cut-off 0.5%).

Nodule	Rhizosphere	Domain	Phylum	Class	Order	Family	Genus
2.9	1.4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	ANPR
2.1	1.2	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	<i>Bacillus</i>
0.8	0.0	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Brevundimonas</i>
1.4	0.8	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Caulobacter</i>
1.7	23.3	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	<i>Chitinophaga</i>
1.1	0.0	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	<i>Cupriavidus</i>
0.7	0.0	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	<i>Flavobacterium</i>
0.0	8.8	Bacteria	Actinobacteriota	Actinobacteria	Glycomycetales	Glycomycetaceae	<i>Glycomyces</i>
0.0	0.5	Bacteria	Proteobacteria	Alphaproteobacteria	Azospirillales	Inquilinaceae	<i>Inquilinus</i>
0.0	1.3	Bacteria	Actinobacteriota	Actinobacteria	Propionibacteriales	Nocardiodaceae	<i>Kribbella</i>
0.2	2.7	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Massilia</i>
0.0	1.7	Bacteria	Actinobacteriota	Actinobacteria	Corynebacteriales	Nocardiaceae	<i>Nocardia</i>
0.0	3.0	Bacteria	Actinobacteriota	Actinobacteria	Streptosporangiales	Streptosporangiaceae	<i>Nonomuraea</i>
0.8	0.0	Bacteria	Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae	<i>Paenibacillus</i>
2.7	6.5	Bacteria	Actinobacteriota	Actinobacteria	Micrococcales	Promicromonosporaceae	<i>Promicromonospora</i>
21.3	0.0	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>
1.3	0.0	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>
55.2	0.0	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	<i>Stenotrophomonas</i>
0.0	39.3	Bacteria	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	<i>Streptomyces</i>
0.0	2.4	Bacteria	Patescibacteria	Saccharimonadia	Saccharimonadales	Saccharimonadaceae	TM7a
1.4	1.4	Bacteria	Hydrogenedentes	Hydrogenedentia	Hydrogenedentiales	Hydrogenedensaceae	unknown
1.4	0.7	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	<i>Variovorax</i>

In the Table ANPR refers to *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*.

Table S3. Biochemical traits of the Gram-positive strains with N₂-fixing ability. The green color indicates positive results, while the grey color negative ones.

	N1	N2B	N6A	N6B	N6C	N7
d-xylose						
Arginine Dehydrolase 1						
α-glucosidase						
Ala-Phe-Pro arylamidase						
L-aspartate arylamidase						
Phosphatase						
Leucine arylamidase						
Proline arylamidase						
Pyruvate arylamidase						
Alanine arylamidase						
Tyrosine arylamidase						
Urease						
d-ribose						
L-Lactate alkalization						
sucrose						
d-trealose						
Arginine Dehydrolase 2						

Table S4. Biochemical traits of the Gram-negative strains with N₂-fixing ability. The green color indicates positive results, while the grey color negative ones.

	N2A	N3	N5
L-pyrrolidonyl arylamidase			
β-galattosidase			
d-glucose			
γ-glutamyl-transferase			
glucose fermentation			
β-glucosidase			
d-maltose			
d-mannitol			
d-mannose			
L-proline arylamidase			
Lipase			
Tyrosine arylamidase			
Urease			
Sucrose			
d-Tagatose			
d-trealose			
Malonate			
L-Lactate alkalization			
α-glucosidase			
Succinate alkalization			
β-n-acetyl-galattosaminidase			
Phosphatase			
Glycine arylamidase			
L-histidine assimilation			
Coumarate			
O129 Resistance			
Ellman			

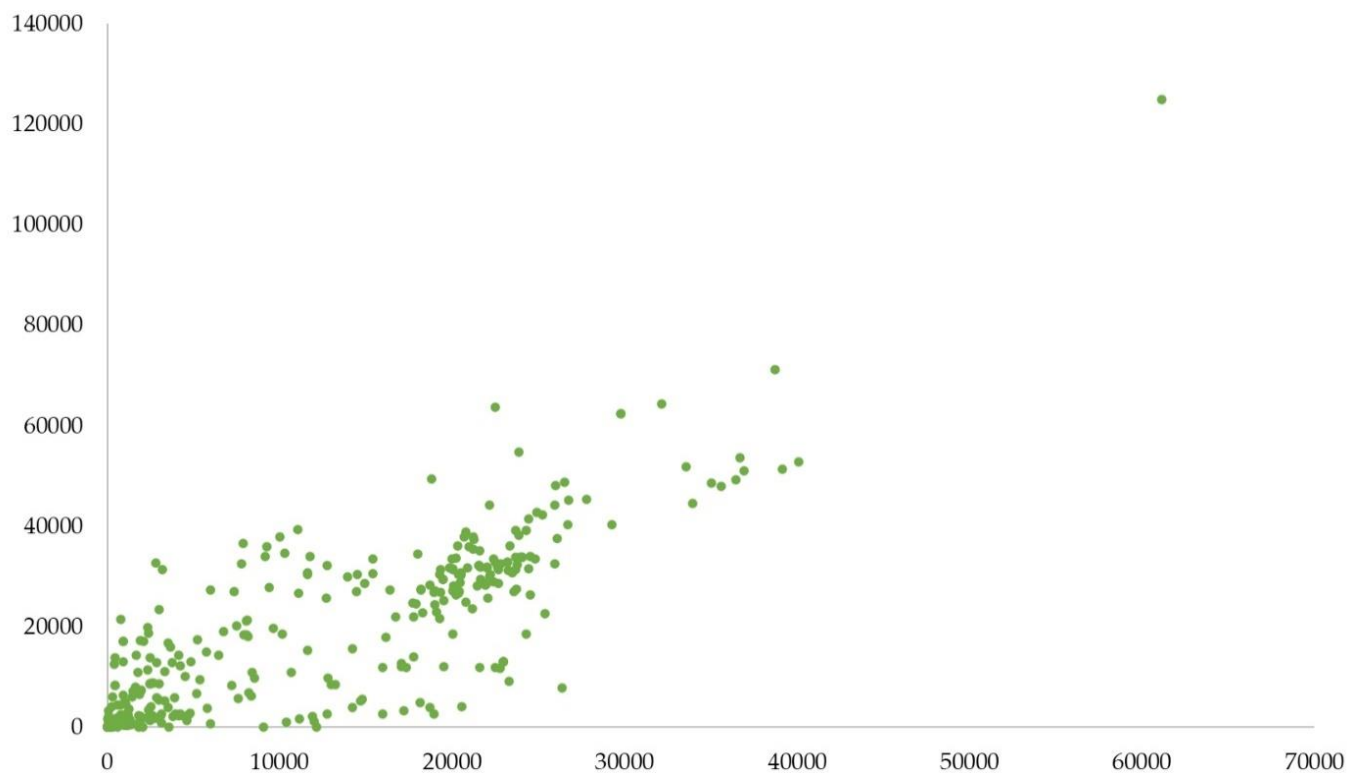


Figure S1. Correlation plot on pathways predicted by PICRUSt 2 software (nodule y axis and rhizosphere x axis). Correlation coefficient: 0.73.