**Supplementary Information**

# Diversity and abundance of diazotrophs and other bacteria associated with legumes in the Succulent Karoo biome in South Africa

Esther K. Muema*, Emma T. Steenkamp, Stephanus N. Venter

Department of Biochemistry, Genetics and Microbiology, DSI-NRF Centre of Excellence in Plant Health Biotechnology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa

***Corresponding author:**

Dr. Esther K. Muema

Phone: +27 (0) 641563314

Email: esther.muema@fabi.up.ac.za

**Table caption**

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**Table S1.** Statistical analysis of soil chemical properties of the study sites showing main effects of different legume species and sites.

| Factors | Geochemical properties [1, 2] | | | | |
|---|---|---|---|---|---|
| | Soil pH | TN [%] | TC [%] | $NH_4^+$ [mg kg$^{-1}$] | $NO_3^-$ [mg kg$^{-1}$] |
| **Legumes** | | | | | |
| *C. sericea* | 5.08 ± 0.28$^a$ | 0.05 ± 0.02$^a$ | 0.76 ± 0.26$^a$ | 8.67 ± 2.64$^a$ | 5.6 ± 2.8$^a$ |
| *L. diffusa* | 5.46 ± 0.37$^{ab}$ | 0.05 ± 0.03$^a$ | 0.61 ± 0.35$^a$ | 12.68 ± 3.54$^a$ | 1.10 ± 0.34$^a$ |
| *V. karroo* | 6.47 ± 0.34$^b$ | 0.16 ± 0.03$^a$ | 1.93 ± 0.32$^b$ | 17.07 ± 3.23$^a$ | 10.87 ±3.65$^a$ |
| *W. monoptera* | 4.00 ± 0.48$^a$ | 0.07 ± 0.04$^a$ | 1.08 ± 0.45$^{ab}$ | 14.39 ± 4.57$^a$ | 0.56 ± 0.22$^a$ |
| **Sites** | | | | | |
| Kamiesberg | 4.67 ± 0.54$^a$ | 0.08 ± 0.04$^a$ | 1.15 ± 0.47$^a$ | 8.97 ± 4.11$^a$ | 3.07 ± 1.57$^a$ |
| Brakputs | 5.64 ± 0.27$^a$ | 0.09 ± 0.02$^a$ | 1.11 ± 0.22$^a$ | 13.92 ± 2.05$^a$ | 4.65 ± 2.30$^a$ |
| Kamieskroon | 4.97 ± 0.62$^a$ | 0.06 ± 0.05$^a$ | 0.80 ± 0.54$^a$ | 9.51 ± 4.74$^a$ | 13.65 ± 5.31$^a$ |
| **Source of variation and statistical significance [3]** | | | | | |
| Legumes (L) | ** | ns | * | ns | ns |
| Site (S) | ns | ns | ns | ns | ns |
| L x S | ns | ns | ns | ns | ns |

[1] Values are means and standard error for the effect of legumes (n=9; *C. sericea*, n=5; *L. diffusa*, n=6; *V. karroo*, n=3; *W. monoptera*) and sites (n=4; Kamiesberg, n=16 Brakputs, n=3; Kamieskroon). Different letters (a-b), against the values indicate legume species with significant differences ($P < 0.05$).

[2] TC = Total carbon, TN = Total nitrogen, $NH_4^+$ = Ammonium, $NO_3^-$ = Nitrates.

[3] Significance levels: ns = not significant at $P > 0.05$, significant at $P < 0.05$ * and $P < 0.01$ ** levels.

**Table S2.** Diverse bacterial phyla in the Succulent Karoo biome in South Africa, their inherent properties and possible impacts on plant communities.

| **Phylum** | **Percent of total sequences** | **Inherent properties** | **Plant impacts** | **References** |
|---|---|---|---|---|
| *Actinobacteria* | 26.7% | Heat and soil acidity tolerant | Carbon nutrient provision through decomposition and mineralization of soil organic matter | Makhalanyane et al., 2015 |
| *Proteobacteria* | 23.5% | Nutrients limited arid soils adapted. | Plant growth promotion through biological nitrogen fixation | Andrews and Andrews, 2017 |
| | | | Antagonistic effects against plant pathogens | Ofek et al., 2012 |
| *Acidobacteria* | 10% | Adapted to acidic and nutrients poor environments | Plant growth promotion through production of indole acetic acids (IAA) and siderophores | Op den Camp et al., 2009; Kielak et al., 2016; |
| *Planctomycetes* | 10% | Adapted to extreme temperatures and pH | Plant growth promotion through provision of carbon via carbon metabolism. | Kovaleva et al., 2015. |
| | | | Plant growth promotion through ammonia oxidation in oxygen limited conditions to nitrates | Strous et al., 1999 |
| *Chloroflexi* | 6.3% | Adapted to extremely hot environments | Provision of carbon nutrient through degradation of complex organic compounds | Xia et al., 2008 |

**Table S2.** Continued.

| | | | | |
|---|---|---|---|---|
| *Bacteroidetes* | 7.6% | Adapted to extreme temperatures | Carbon cycling through degradation of complex organic compounds. | Xia et al., 2008; Jorquera et al., 2012 |
| | | | Promotion of plant growth through nitrogen fixation. | Makhalanyane et al., 2015 |
| *Verrucomicrobia* | 7.3% | Adapted to acidic, high temperature and nutrients poor environments | Plant growth promotion through nitrogen fixation and carbon cycling | Khadem et al., 2010 |
| | | | Provision of organic carbon to plants. | Op dem Camp et al., 2009 |
| *Cyanobacteria* | 0.8% | Can withstand desiccation, high temperatures and unstable pH | Plant growth promotion through fixation of atmospheric nitrogen into ammonium and nitrates | Dojani et al., 2014; |
| *Gemmatimonadetes* | 1.8% | Adapted to extremely hot and moisture limited environments | Plant growth promotion through photosynthesis | Zeng et al., 2017; Makhalanyane et al., 2015 |
| *Firmicutes* | 0.9% | Adapted to extremely hot and dry environments | Plant growth promotion via phosphorus solubilization, nitrogen fixation, production of IAAs, siderophores, photosynthesis and antagonistic effects against plants pathogens | Felestrino et al., 2017; Jorquera et al., 2012 |

**Table S3.** Families containing diazotrophic bacteria (*nifH* gene) in the rhizosphere soils examined and their possible roles in plant growth promotion of legume species in the Succulent Karoo biome in South Africa.

| Family | Percent of total sequences | Legume rhizosphere soil | Role in rhizosphere/soil | References |
|---|---|---|---|---|
| *Bradyrhizobiaceae* | 4.6% | *C. sericea, W. monoptera, V. karroo* | Plant growth promotion through biological nitrogen fixation | Andrews and Andrews, 2017; Moiloa, 2016 |
| *Rhizobiaceae* | 4.2% | *C. sericea, L. diffusa, V. karroo* | Plant growth promotion through biological nitrogen fixation | Andrews and Andrews, 2017; Räsänen et al., 2001; Sankhla et al., 2017 |
| *Nostocaceae* | 24.4% | *C. sericea, W. monoptera, L. diffusa, V. karroo* | Plant growth promotion through biological nitrogen fixation | Dojani et al., 2014 |
| *Comamonadaceae* | 0.18% | *C. sericea, V. karroo* | Promotion of growth through nitrogen fixation and carbon cycling | Schmalenberger et al., 2008 |
| *Phyllobacteriaceae* | 39.0% | *C. sericea, L. diffusa, V. karroo* | Plant growth promotion through biological nitrogen fixation | Andrews and Andrews, 2017; Phalane et al., 2008; Gerding et al., 2012 |
| *Microchaetaceae* | 4.0% | *W. monoptera, V. karroo* | Plant growth promotion through biological nitrogen fixation | Dojani et al., 2014 |
| *Rhodocyclaceae* | 3.7% | *C. sericea, V. karroo* | Free nitrogen fixation | Bae et al., 2007 |

**Table S3.** Continued.

| | | | | |
|---|---|---|---|---|
| *Rhodospirillaceae* | 7.9% | *C. sericea, V. karroo, W. monoptera* | Plant growth promotion through nitrogen fixation | Bao et al., 2013; Selao, 2010 |
| *Methylobacteriaceae* | 3.0% | *C. sericea, V. karroo*, *W. monoptera* | Plant growth promotion through biological nitrogen fixation | Moiloa, 2016 |
| *Acetobacteraceae* | 1.1% | *C. sericea, V. karroo, W. monoptera* | Plant growth promotion through phosphate solubilization, nitrogen fixation and plant pathogen control | Reis and Teixeira, 2015 |
| *Scytonemataceae* | 3.1% | *L. diffusa, W. monoptera* | Plant growth promotion through biological nitrogen fixation | Dojani et al., 2013 |
| *Sphingomonadaceae* | 0.12% | *C. sericea, V. karroo* | Plant growth promotion through production of Indole acetic acids | Tsavkelova et al., 2007; |
| *Enterobacteriaceae* | 0.06% | *W. monoptera* | Plant growth promotion through free-living biological nitrogen fixation | Schmitz et al., 2002 |
| *Geobacteraceae* | 1.78% | *V. karroo* | Plant growth promotion via nitrogen fixation | Berthrong et al., 2014; Holmes et al., 2004 |

**Table S3.** Continued.

| | | | | |
|---|---|---|---|---|
| *Desulfovibrionaceae* | 0.62% | *V. karroo* | Plant growth promotion via nitrogen fixation | Zhang et al., 2017; Bertics et al., 2013 |
| *Ectothiorhodospiraceae* | 0.16% | *V. karroo* | Capacity for plant growth promotion through nitrogen fixation | Tourova et al., 2007 |
| *Lachnospiraceae* | 0.20% | *V. karroo* | Plant growth promotion through nitrogen fixation | Sarria-Guzmán et al., 2016 |
| *Pseudomonadaceae* | 2.7% | *V. karroo* | Plant growth promotion through P solubilization, N fixation, production of siderophores for iron transport to plants and plant pathogens control | Song et al., 2021 |
| *Alcaligenaceae* | 1.2% | *V. karroo* | Plant growth promotion through nitrogen fixation and production of Indole acetic acid | Pedersen et al., 2018; Panke-Buisse et al., 2015. |

**Table S4.** Analysis of variance (ANOVA) and Permutations analysis of variance (PERMANOVA) to test the effects of factors 'legume species', 'sites' and their interactions on the respective alpha and beta diversity matrices, using the 16S rRNA and *nifH* gene barcodes.

| **Diversity measures** | **Effects/sources of variation** [1] | | |
|---|---|---|---|
| | **Site** | **Legumes** | **Site x Legumes** |
| **Alpha diversity (ANOVA)** | | | |
| 16S rRNA | | | |
| Richness (observed taxa) | ns | ** | ns |
| Shannon diversity | ns | *** | ns |
| Shannon evenness | ns | *** | ns |
| *nifH* | | | |
| Richness (observed taxa) | ns | ** | ns |
| Shannon diversity | ns | ** | ns |
| Shannon evenness | ns | * | ns |
| **Beta diversity (PERMANOVA)** | | | |
| 16S rRNA | | | |
| Bray-Curtis | ns | *** | ns |
| Jaccard | ns | *** | ns |
| *nifH* | | | |
| Bray-Curtis | ** | ** | ns |
| Jaccard | *** | *** | ns |

[1] Significance levels: ns = not significant at $P > 0.05$, significant at $P < 0.05$ *; $P < 0.01$ ** and $P < 0.001$ *** levels.