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Supplement of

Microbial biobanking – cyanobacteria-rich topsoil facilitates mine rehabilitation

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Supplementary Tables

Table S1: Descriptions of sources for intact biocrusts used to characterise the cyanobacterial communities: Site vegetation associations: SMU 1 – Red Mallee: *Eucalyptus oleosa* ssp. *oleosa* = open Mallee/Myall woodland; SMU 2 – Chenopod Shrubland: *Maireana sedifolia* and *Atriplex vesicaria*; SMU 3 – Western Myall: *Acacia papyrocarpa Maireana sedifolia* = open Myall woodland Site 1 occurs in a transition between SMU 2 and SMU 3 but was treated as most like SMU 2; Site 6 originated from SMU 3 – *2YO stockpile crust type determined *in situ* also with mosses present; TSF = Tailings storage facility.

Site no.	Primary Vegetation	SMU	Biocrust types	Site identifier and description
1	Western Myall	2	Types 2-5	Edge year 3 clearance
2	Western Myall	3	Types 2-5	Dust mon track
3	Western Myall	3	Types 2-5	Drill track
4	Red Mallee	1	Types 1-3	Drill track - dune
5	Red Mallee	2	Types 2-5	Drill track - gilgai
6	Western Myall	NA	Type 1*	Topsoil stockpile
7	Western Myall	3	Types 2-5	West side TSF
8	Chenopod	2	Types 2-5	Power Station site
9	Red Mallee	1	Types 1-2	Canberra - dune
10	Red Mallee	1	Types 1-3	Nth of pit - slight dune

Table S2: Descriptions of biocrust morphotypes (see Büdel et al., 2009; Doudle et al., 2011)

No.	Crust Type	Identification
1	Light (pale coloured) thin cyanobacterial crust in early stages of development: early-successional	Patchy, brittle, bare patches visible, slight discolouration
2	Cyanobacterial crust, well established, intermediate stages of development	Larger pieces can be easily removed intact, darke discolouration
3	Biocrust, well established, with cyanolichens: late-successional	Larger pieces can be easily removed intact, cyanolichens easily seen with hand lens (10x)
4	Biocrust, well established, with cyanolichens and/or green algal lichens: late-successional	Larger pieces can be easily removed intact, liche easily seen with hand lens (10x)
5	Biocrust (see Type 4) with mosses: late-successional	Larger pieces can be easily removed intact, moss easily seen with hand lens (10x)

Table S3: Topsoil stockpile ages, locations and sampling depths

Stockpile number	Date of topsoil stockpiling	Stockpile Replicate	Latitude	Longitude	Adjacent replicate	Latitude	Longitude	Soil sample depths (cm)	Date sampled
10	31/10/2009	1	0234527	6578956	1	0234433	6578944	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
10	31/10/2009	2	0234542	6578921	2	0234422	6578933	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
10	31/10/2009	3	0234557	6578881	3	0234436	6578913	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
12	6/11/2009	1	0233396	2578200	1	0233414	6578180	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
12	6/11/2009	2	0233377	6578206	2	0233437	6578227	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
12	6/11/2009	3	0233362	6578218	3	0233431	6578263	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12
18	13/07/2010	1	0234870	6578033	1	0234878	6577787	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
18	13/07/2010	2	0234852	6578023	2	0234873	6577776	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
18	13/07/2010	3	0234860	6578007	3	0234853	6577782	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
19	29/06/2010	1	0234999	6578800	1	0234922	6578851	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
19	29/06/2010	2	0235000	6578820	2	0234918	6578856	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
19	29/06/2010	3	0235003	6578834	3	0234913	6578849	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	9/3/12
20	2011	1	023457	6577150	1	0234938	6577286	0-2, 2-4, 4-6, 10, 25, 50, 50 (sterilised control)	8/3/12

Supplementary Figures

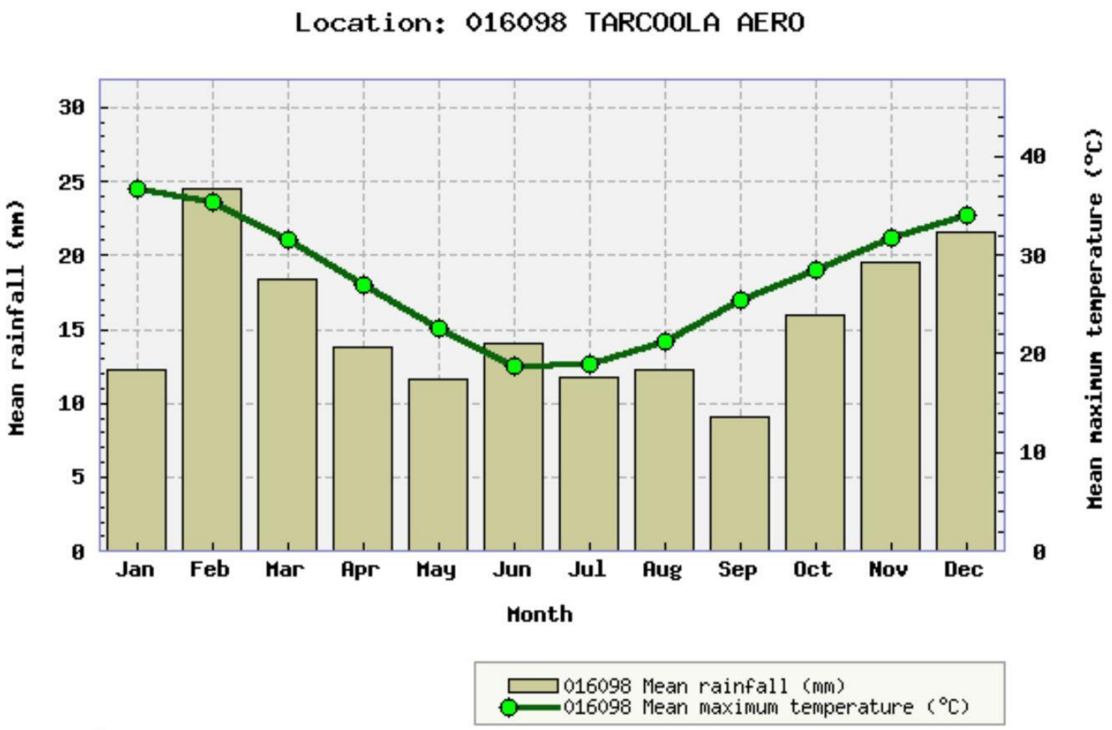

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Figure S1: Mean monthly maximum temperature and rainfall data from nearby Tarcoola aerodrome for the past 20 years (source: bom.gov.au)

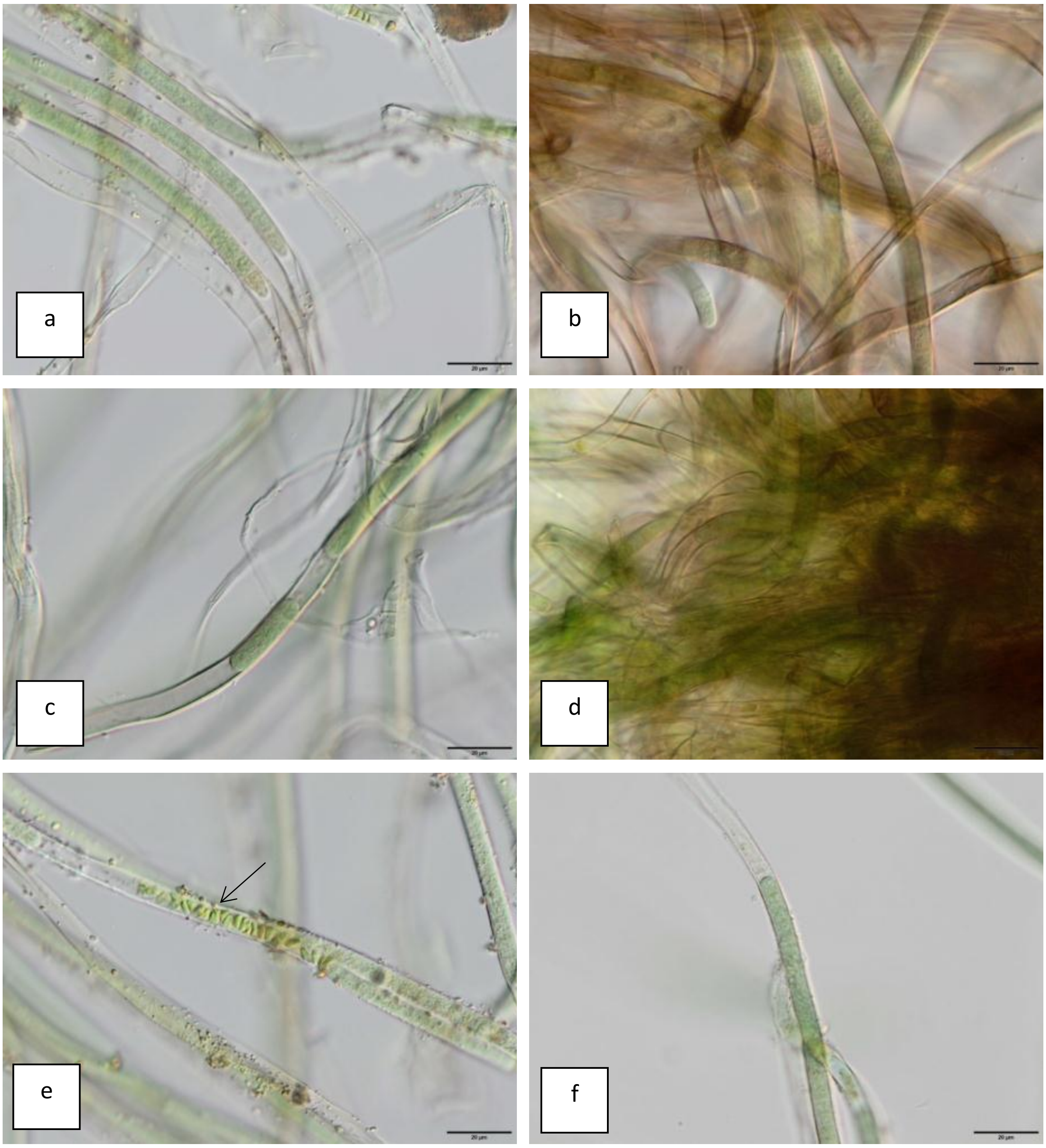

Figure S2: (a-f) The predominant *Symploca* species found across all sites; **(b)** illustrates distinctive colour found in those with UV pigmentation; **(c)** thin outer sheath; **(d)** masses of filaments that form mats; **(e)** desiccated cells inside sheath following period of time without moisture (arrow) and other cells already rehydrated; **(f)** filament with typical elongated sock-like sheath; (Scale bars 20 µm).

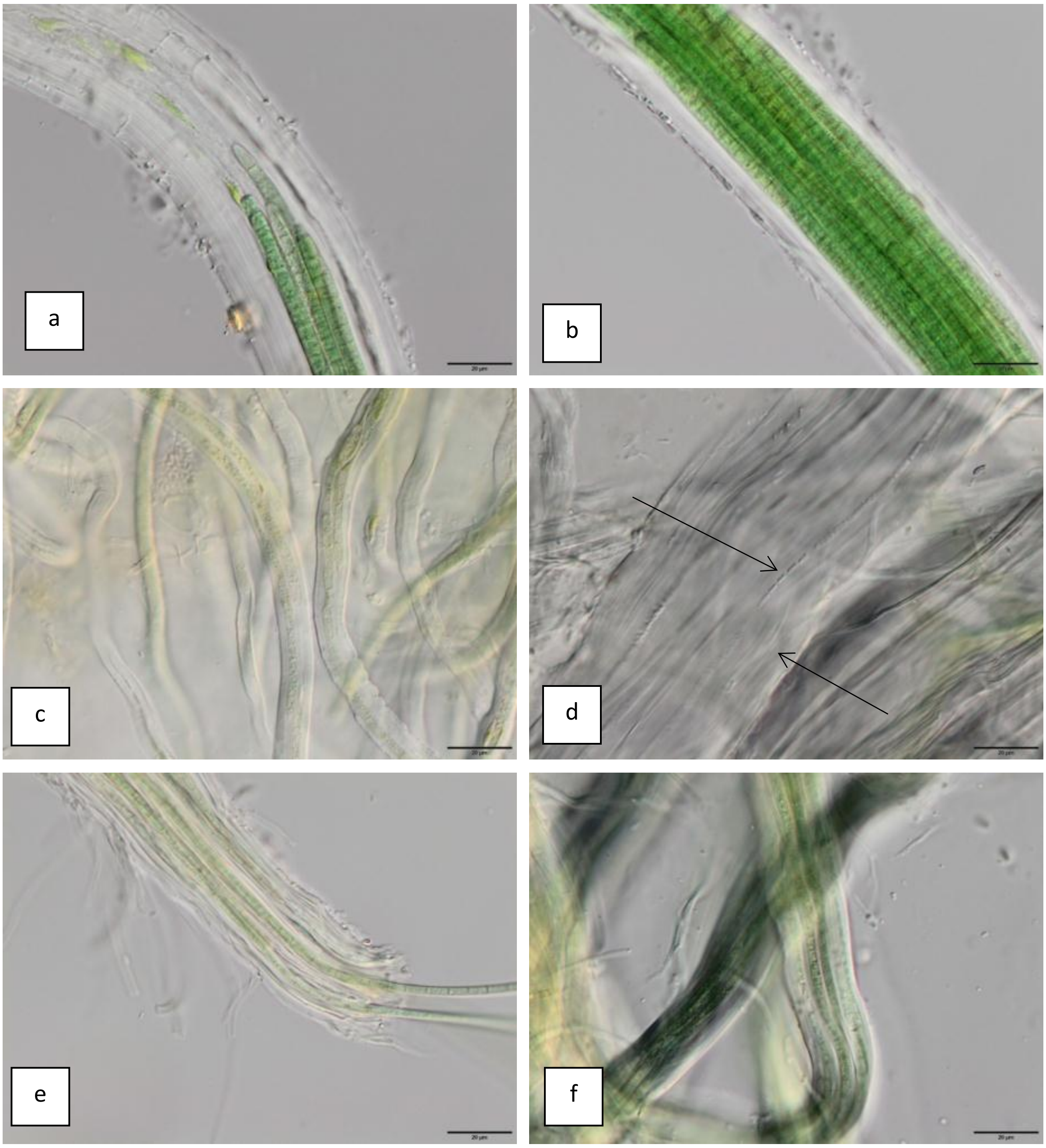

Figure S3: (a-b) *Microcoleus paludosus* with multiple bright trichomes encased in a common clear gelatinous sheath; **(c-f)** *Schizothrix*; three species were observed with varying morphological structure that included long entangled filaments with enclosed ends; *Schizothrix* was a subsurface species but was often found on the surface during a growth phase; **(d)** *Schizothrix* had thick, sticky, gelatinous sheaths (arrow), often with fine, almost invisible trichomes (arrow), and was hard to separate from the soil particles; **(e-f)** typically one or two trichomes encased in gelatinous sheaths clumped together to form rope-like filaments; (scale bars 20 µm).

Figure S4: *Scytonema* species **(a-f)** Illustrations of three (of four) apparently different species of *Scytonema* that were identified; morphological attributes that differ between species included cell size and shape, size of heterocysts (straight arrows) and type of branching (curvy arrows); *Scytonema* sp. 2 **(c-d)** is heavily granulated, golden coloured and larger than the others, most common at Lake Ifould but also found elsewhere; *Scytonema* formed tufted or prostrate colonies on the surface and were rarely seen without UV pigmentation; note (b, e-f) are cultured specimens (scale bars 20 µm).

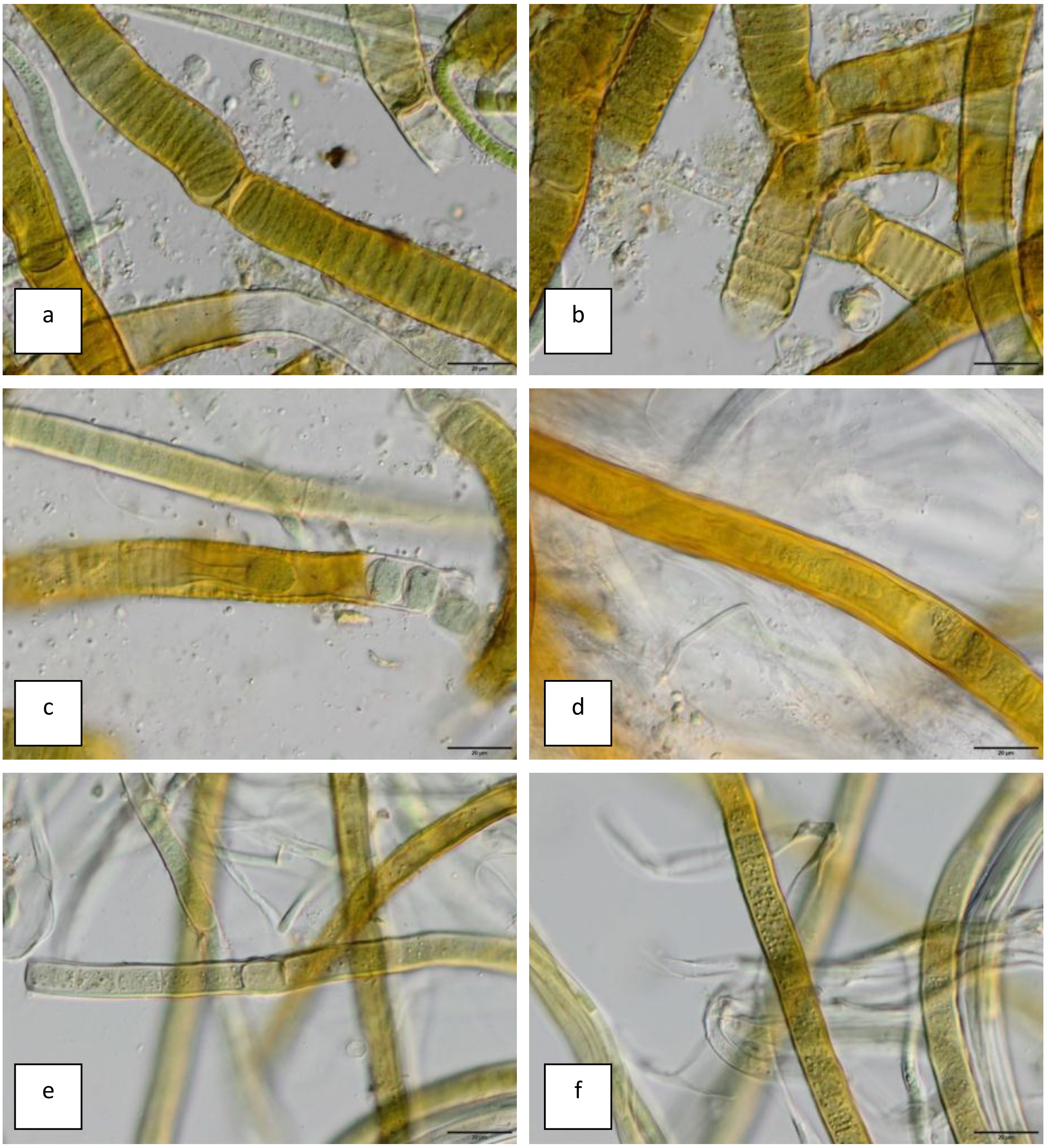

Figure S5: *Brasilonema* – *Scytonema* complex: **(a-d)** In these studies this species was identified as most like *Brasilonema* however it contained variable morphology, yet its identity was supported by molecular analysis. *Brasilonema* was almost twice the size of most of the *Scytonema* species in 4.2, contained occasional to numerous heterocytes, a range of cell shapes, false branching; **(e-f)** *Scytonema* species; **(e)** with longer cells than wide, similar to 4.2a and; **(f)** with squarish cells and heavy granulation similar to 4.2d; (scale bars 20 µm).

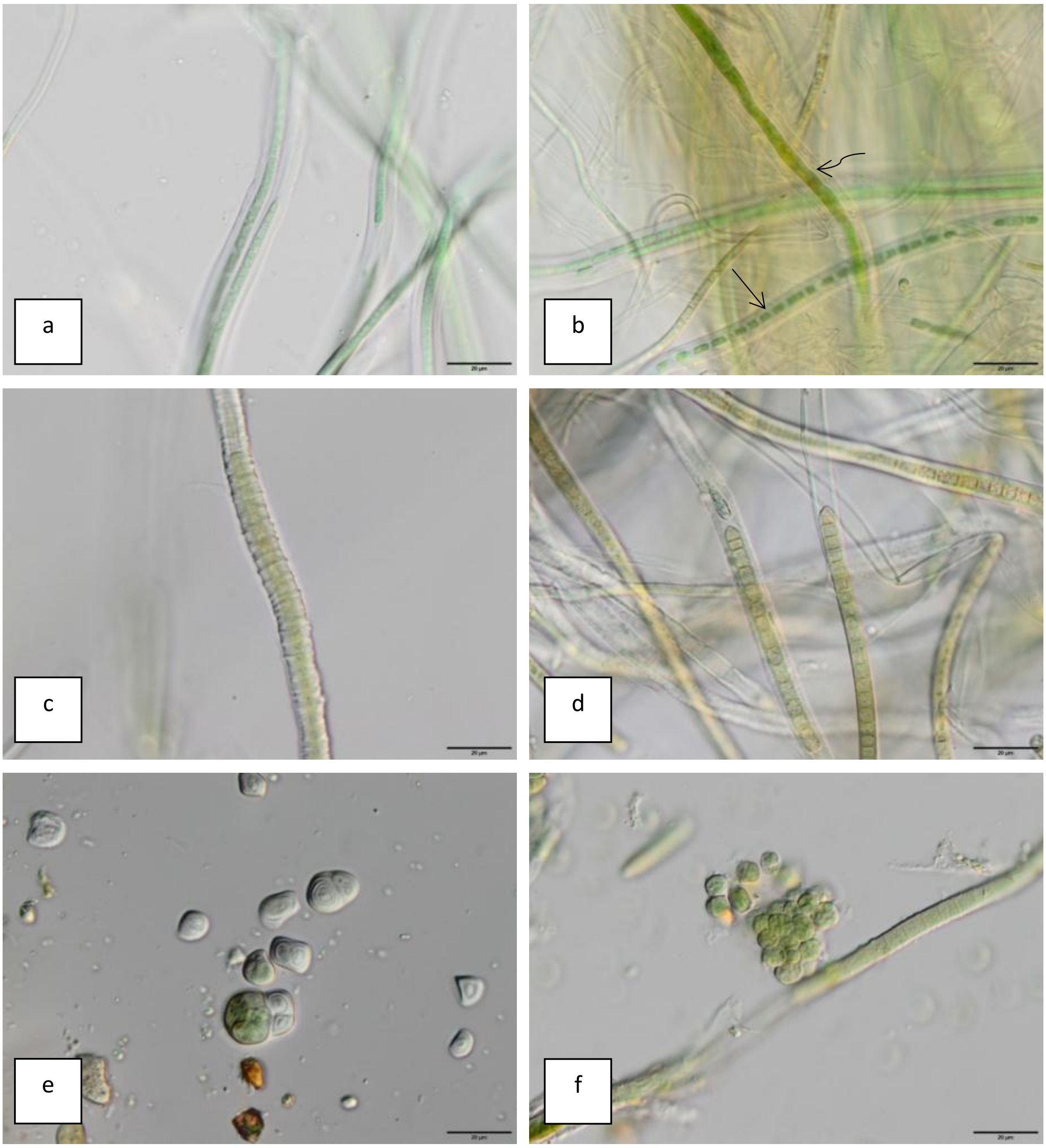

Figure S6: (a) *Schizothrix* species; **(b)** *Leptolyngbya*-like species (arrow) with *Microcoleus* (curvy arrow) and *Schizothrix* (background) intermingled with other cyanobacteria illustrating complex diversity within the micro-scale; **(c-d)** *Porphyrosiphon* species (1) that was most commonly found that when mature had a crinkled outer sheath **(c)**; and was in very long filaments with an unusual pointed terminal cell; **(e)** *Gloeocapsa* sp. (unicellular) cyanobacterium encased in thick lamellated EPS layered envelopes; **(f)** unidentified unicellular Chroococcales; (scale bars 20 µm).

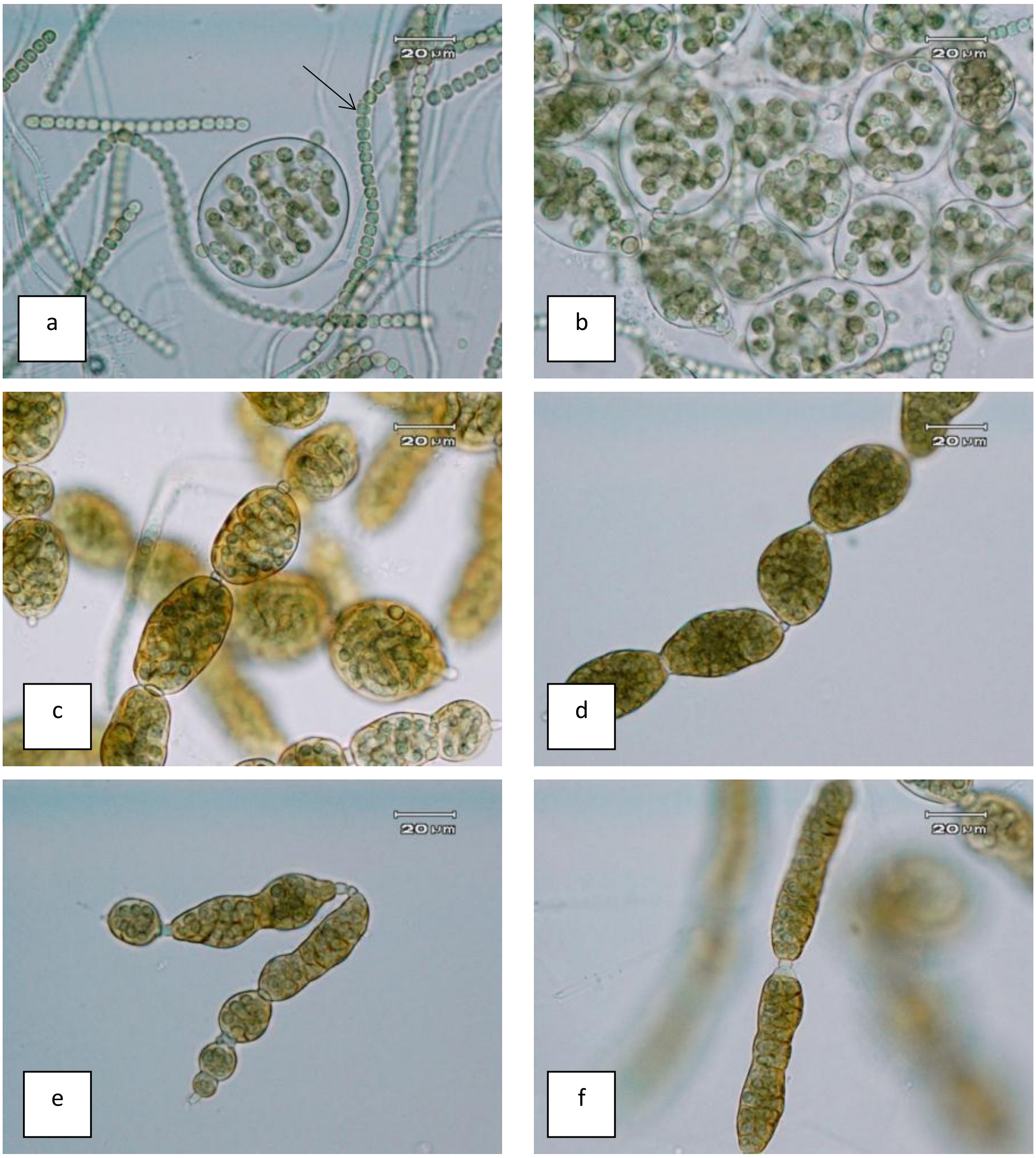

Figure S7: (a-f) Early stages and different morphs of *Nostoc* life cycle (cultured); **(a-b)** illustrating long *Nostoc* cf. commune chains (arrows) and sacks containing chains of *Nostoc* cells, about the third phase of its life cycle; **(c-d)** pigmentation of these cell sacks provides UV protection; **(d-e)** changing morphs with **(e)** most likely *Nostoc flagelliforme* (see Aboal et al., 2014); (scale bars 20 µm).

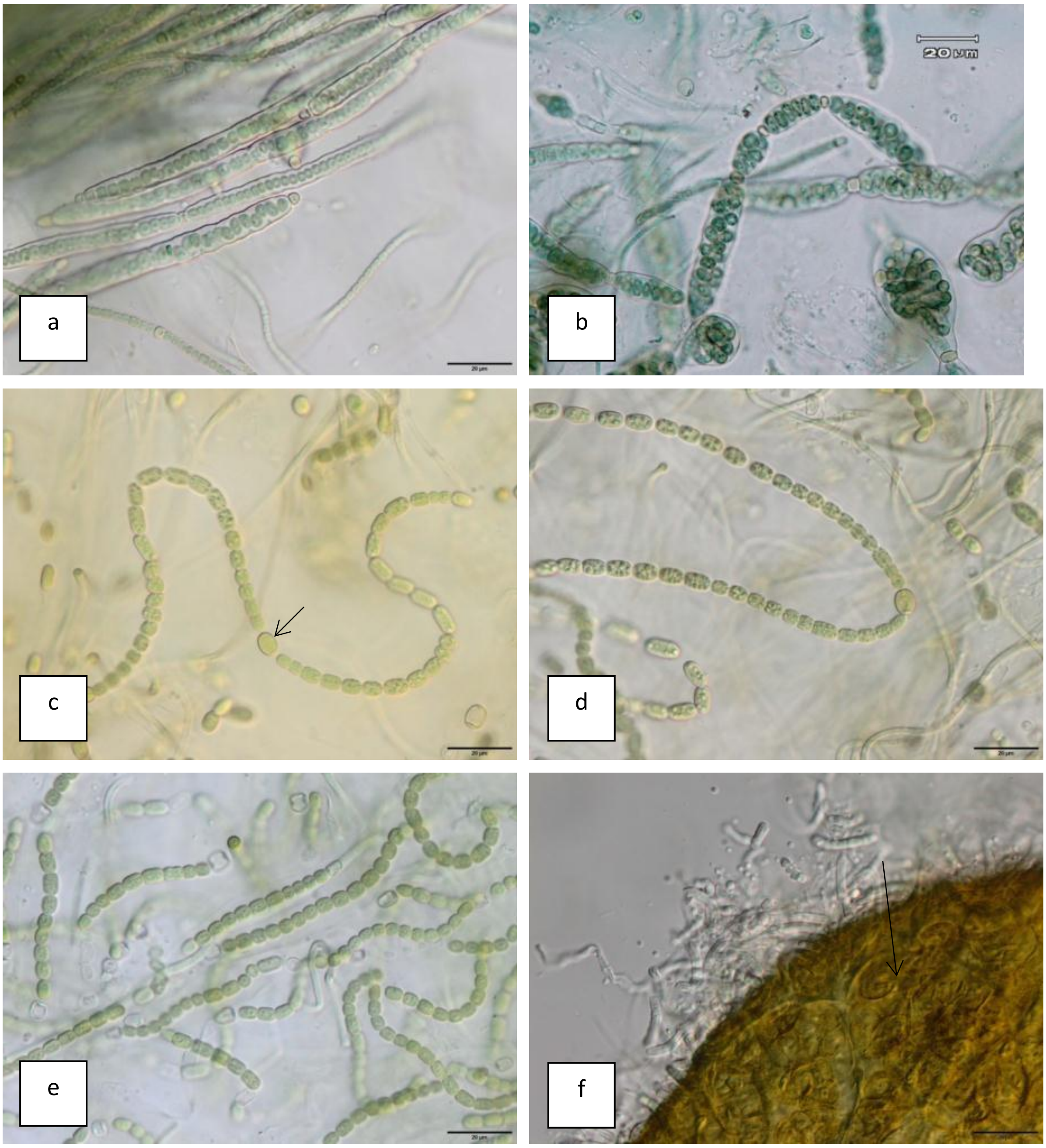

Figure S8: *Nostoc* species (cultured); **(a-b)** illustrating elongated parallel filaments of *Nostoc flagelliforme* possibly about the third phase of its life cycle; **(c-d)** long chains *Nostoc* cf. *pruniforme* with linking akinetes (arrow); **(e-f)** Nostoc changing morphs with **(e)** most likely tightly packed colonies of *Nostoc commune* in a thick, highly pigmented EPS envelope (arrow); (scale bars 20 μm).

Figure S9: (a-c) *Symplocastrum* sp. (from Lake Ifould perimeter) with heavily granulated cells and golden to rust coloured sheaths shown forming a tuft-like peak **(b)** and; **(c)** in a partially desiccated state; **(d)** tightly entwined bundles of *Leptolyngbya* like filaments; **(e-f)** *Chroococcus* sp. from Lake Ifould perimeter in golden pigmented spherical colony **(f)**; (scale bars 20 µm).

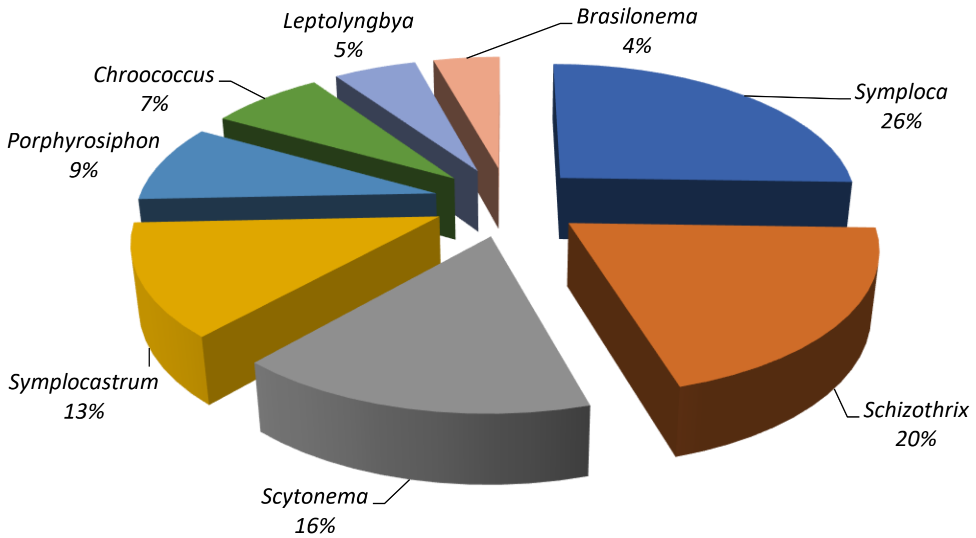

Figure S10: Cyanobacterial species richness in SMU 1 shows *Symploca* is dominant while sub-surface cyanobacterium *Schizothrix* is important for binding soil together with EPS. *Symploca*, *Scytonema*, *Porphyrosiphon* and *Brasilonema* contribute to N-fixation.

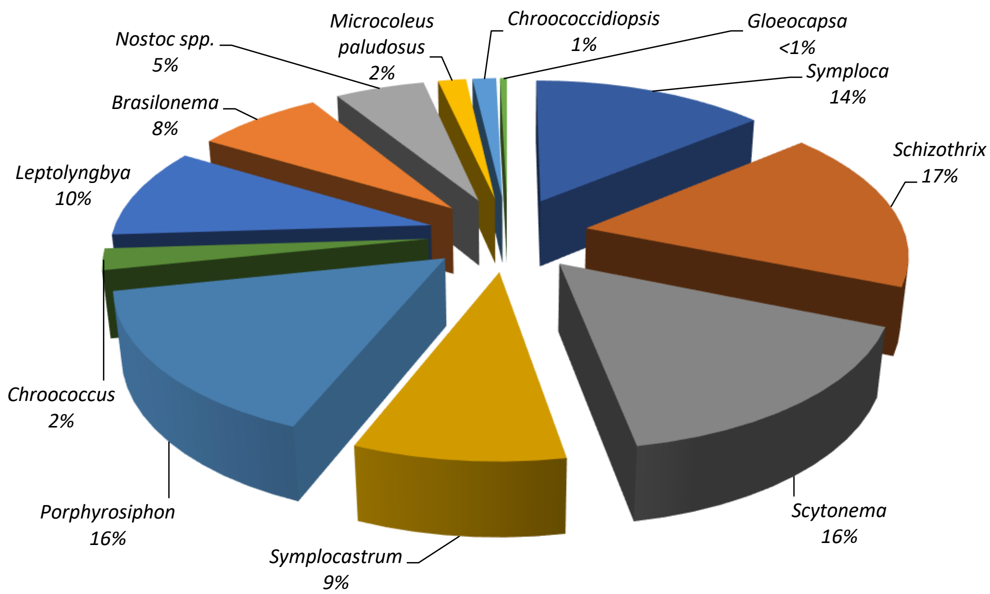

Figure S11: Cyanobacterial species richness in SMU 2 shows *Schizothrix, Porphyrosiphon, Scytonema and Symploca* share dominance.

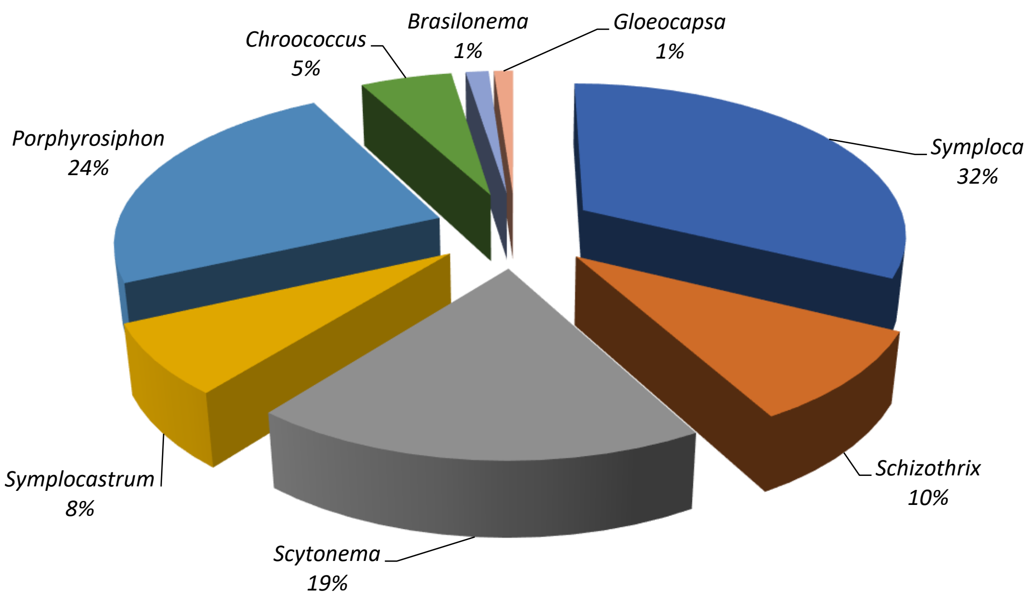

Figure S12: Cyanobacterial species richness in SMU 3 shows *Symploca* is clearly dominant with *Porphyrosiphon* and *Scytonema* abundant.

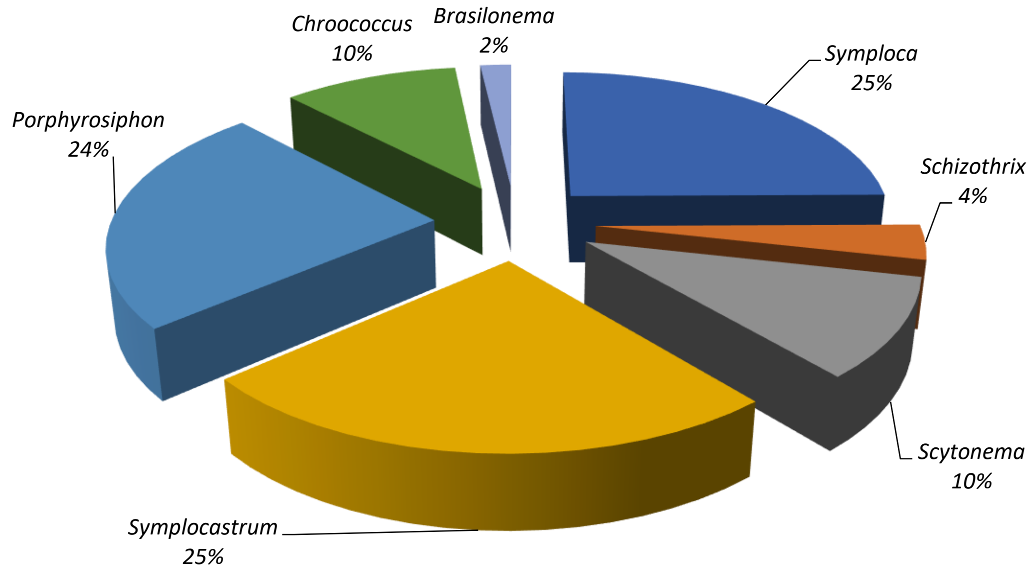

Figure S13: Cyanobacterial species richness in Site 6, the 2YO topsoil stockpile (T2) had originated from SMU 3 and mostly reflects the same dominant genera as SMU 3 (*Symploca*) however; *Symploca* and *Symplocastrum* are co-dominant and *Porphyrosiphon* abundant. N-fixing species were *Symploca*, *Porphyrosiphon*, *Scytonema* and *Brasilonema*.