Supplementary material

qPCR & PCR amplification

Primers 515F (5′-GTGYCAGCMGCCGCGGTA-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′) (Tamaki et al. 2011), gITS7 (5′-GTGARTCATCGARTCTTTG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Ihrmark et al. 2012) were used for determining the absolute abundances of the 16S rRNA and ITS genes by quantitative polymerase chain reaction (qPCR). Each qPCR reaction mixture (10 μl) contained: 5 μl of SsoFast^{TM} EVaGreen$^{®}$ Supermix (Bio-Rad Laboratories Inc., CA, USA), 0.5 μl each primer (10μM), 2 μl DNA template (10ng $μl^{-1}$) and 2.5 μL ddH_2O.

For 16S rRNA gene, the qPCR thermocycler was programmed with an initial denaturation step at 98 °C for 2 min, followed by 40 cycles of 98 °C for 10s, 58 °C for 1 min, and 72 °C for 30s; for ITS, the initial denaturation was still 98 °C for 2 min followed by 35 cycles of 98 °C for 10s, 56 °C for 30s, and 68 °C for 30s. Amplification efficiency was higher than 90%, and the R^2 value of qPCR standard curves were higher than 0.997.

Primer set nifH2 (5'-TGY GAY CCN AAR GCN GA-3') and nifH1 (5'- GCC ATC ATY TCN CC-3') were used for qPCR of *nifH* gene (Zehr and McREYNOLDS, 1989). The qPCR thermocycler was programmed with an initial denaturation step at 98 ℃ for 2 min, followed by 35 cycles of 98 ℃ for 10s, 50 ℃ for 1 min, and 72 ℃ for 1 min. Amplification efficiency was 92%, and the R^2 value of qPCR standard curve was 0.999.

The primers nifH2 and nifH1 were used for PCR amplification as well. For each sample, primer nifH1 was linked with 12 unique barcodes at its 5'-end. The 25 μL reaction mixture for amplification including 1 μL of each primer (10 μM), 2 μL template DNA (10 ng·$μl^{-1}$), 8.5 μL ddH_2O, and 12.5 μL of MasterMix containing Taq DNA Polymerase, PCR Buffer, Mg^{2+} and dNTPs (CWBIO, China). The PCR reaction procedure was conducted at 95 °C for 3 min, then 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Negative controls were performed to verify contamination. Triplicate PCR reactions were performed per sample and pooled for purification using 1% (w/v) agarose gel electrophoresis in 1.0× TAE buffer, and the bands with about 360 bp size were excised and purified using the AxyPrep DNA Gel Extraction Kit (AP-GX-250, Axygen, USA). PCR product quality was assessed by $A_{260/280}$ and $A_{260/230}$ ratios using a NanoDrop ND-2000 Spectrophotometer (Nano-Drop Technologies Inc., Wilmington, DE). Then, libraries were constructed with equimolar mixed amplicons using TruSeq DNA kit and sequenced using an Illumina Hiseq platform.

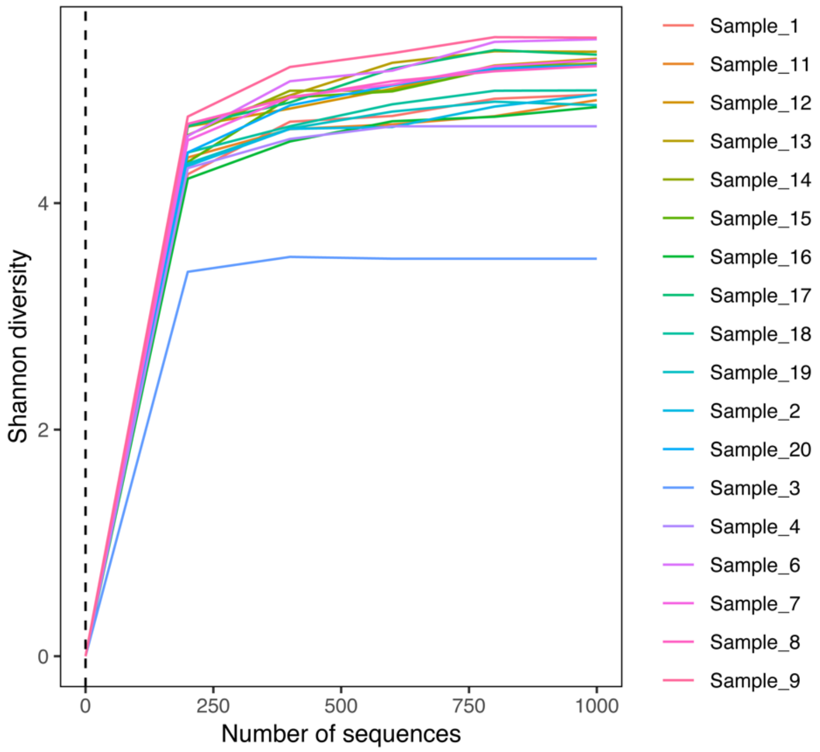

Fig. S1 Shannon diversity based rarefaction curves for diazotrophic *nifH* gene sequences from biocrust samples

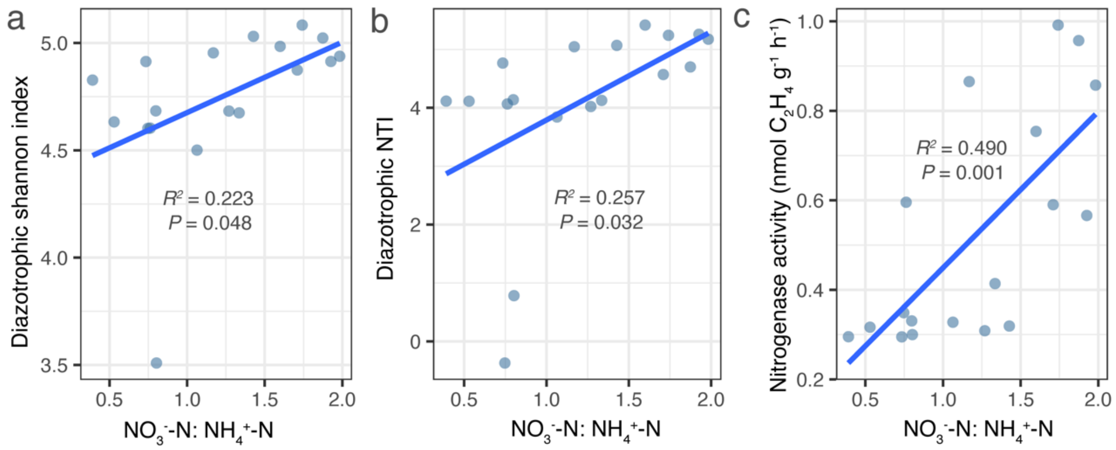

Fig. S2 Relationships between exchangeable NO_3^--N/NH_4^+-N and diazotrophic Shannon and NTI indices and nitrogenase activity during biocrust succession.

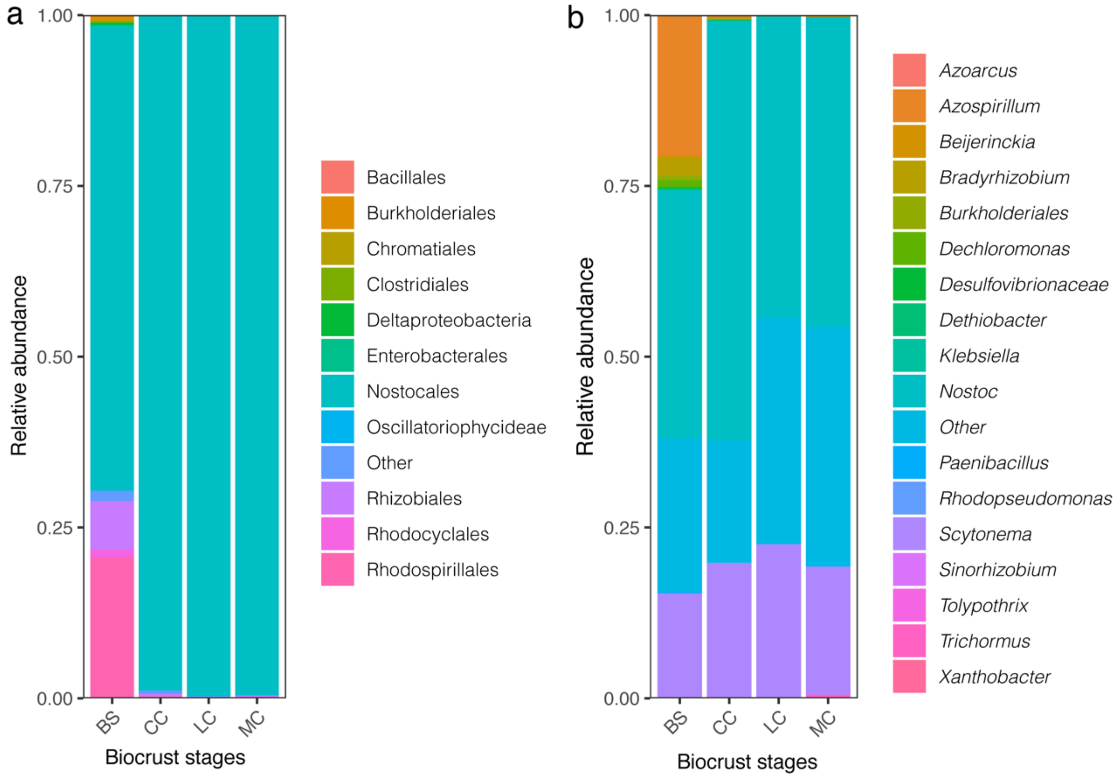

Fig. S3 The compositions of diazotrophic communities at order (a) and genus (b) levels in bare soil (BS), cyanobacterial crust (CC), lichen crust (LC) and moss crust (MC).

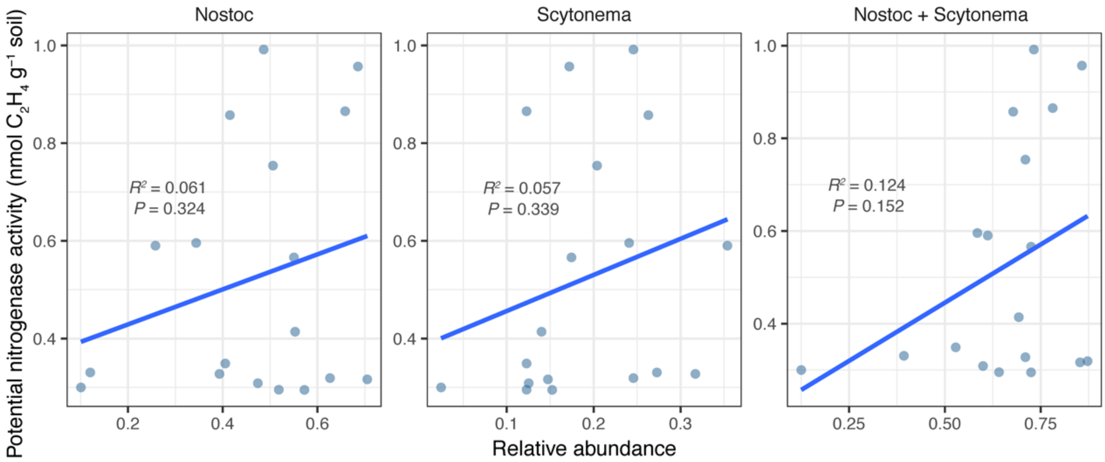

Fig. S4 Relationships between the relative abundances of *Nostoc* and *Scytonema* and nitrogenase activity during the biocrust succession.

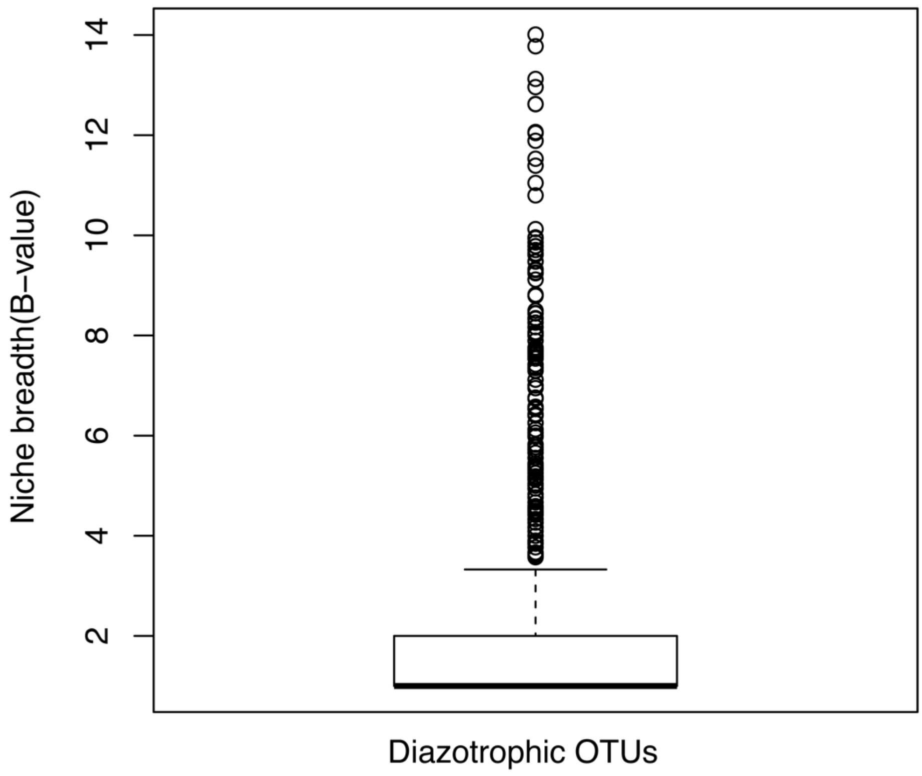

Fig. S5 B-value distribution of diazotrophic OTUs. Generalists OTUs were those with B-values lied above the largest outlier area (B > 3.237), while specialists were those with B-values equal to 1, and the remaining OTUs were regarded as intermediate taxa (Logares et al. 2013)..

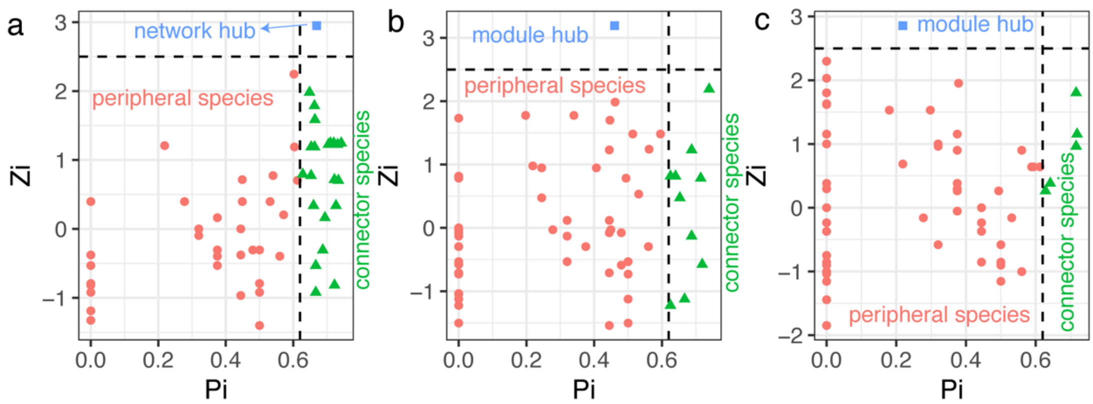

Fig. S6 The distributions of OTUs according to within-module (Zi) and among-module (Pi) connectivity, (a) meta network, (b) sub-network-early-stage, (c) sub-network-later-stage. Peripheral species (Zi < 2.5 and Pi < 0.62, red circles), own few links with both their own module and other modules; connector species (Zi < 2.5 and Pi > 0.62, green triangles) connect many modules together (important to network coherence); module hubs (Zi > 2.5 and Pi < 0.62, blue squares) have more interactions with those nodes within same module; network hubs (Zi > 2.5 and Pi > 0.62, blue squares) act as both connector species and module hubs.

Table S1 Environmental variables in each biocrust stages.

Environmental variables	Bare soil	Cyanobacterial crust	Lichen crust	Moss crust
AK (mg kg^{-1})	143.2.00±58.316^{a}	179.000±26.315^{a}	198.800±37.659^{a}	205.000±23.917^{a}
AP (mg kg^{-1})	2.135±0.582^{c}	4.431±0.578^{b}	3.405±0.464^{bc}	7.934±1.831^{a}
NA (nmol C_2H_4 g^{-1} soil h^{-1})	0.333±0.054$^{a(B)}$	0.675±0.364$^{a(A)}$	0.615±0.209$^{a(A)}$	0.367±0.129$^{a(B)}$
Bacterial abundance （copies g^{-1} soil log_{10} scale）	9.075±0.781^{b}	10.473±0.463^{a}	10.493±0.184^{a}	10.481±0.203^{a}
Chlorophyll- a (μg g^{-1} soil)	0.900±0.786^{c}	5.179±2.435^{b}	6.473±1.935^{b}	14.291±1.760^{a}
CO_2 flux (μmol m^{-2} s^{-1})	0.090±0.109^{d}	1.131±0.497^{c}	1.879±0.557^{b}	3.552±0.545^{a}
Fungal abundance （copies g^{-1} soil log_{10} scale）	7.274±0.853^{c}	7.757±0.292^{bc}	8.191±0.372^{ab}	8.936±0.748^{a}
nifH abundance (log_{10} (copies g^{-1} soil))	9.098±0.327^{c}	9.595±0.211^{a}	9.152±0.260^{bc}	9.470±0.108^{ab}
NH_4^+-N (mg kg^{-1})	15.449±3.245^{a}	13.972±1.607^{a}	17.372±3.523^{a}	17.927±1.654^{a}
NO_3^--N (mg kg^{-1})	13.668±8.55^{bc}	21.227±2.253^{ab}	28.736±2.163^{a}	11.353±2.575^{c}
pH	8.728±0.106^{a}	8.276±0.109^{b}	8.386±0.024^{b}	8.114±0.084^{c}
Sand (%)	96.681±1.329^{a}	97.038±1.894^{a}	94.400±1.518^{b}	94.378±0.621^{b}
Silt (%)	2.738±0.832^{b}	2.3±1.364^{b}	4.178±1.132^{a}	4.184±0.499^{a}
Clay (%)	0.581±0.537^{b}	0.662±0.602^{b}	1.422±0.398^{a}	1.438±0.123^{a}
TK (g kg^{-1})	21.369±0.459^{a}	21.562±0.344^{a}	21.67±0.334^{a}	21.11±0.225^{a}
TN (g kg^{-1})	0.085±0.027^{c}	0.244±0.026^{b}	0.279±0.046^{b}	0.486±0.061^{a}
TOC (g kg^{-1})	1.12±0.213^{c}	2.206±0.231^{b}	2.569±0.267^{b}	4.547±0.661^{a}
TP (g kg^{-1})	0.347±0.044^{a}	0.391±0.043^{a}	0.389±0.038^{a}	0.404±0.042^{a}
F/B ratio	0.801±0.058^{ab}	0.742±0.05^{b}	0.781±0.036^{ab}	0.853±0.071^{a}
NO_3^--N/NH_4^+-N	0.849±0.387^{b}	1.541±0.276^{a}	1.697±0.286^{a}	0.643±0.176^{b}
TOC/TN	13.554±1.994^{a}	9.066±0.832^{b}	9.301±0.942^{b}	9.354±0.636^{b}
TOC/TP	3.211±0.348^{d}	5.656±0.461^{c}	6.619±0.410^{b}	11.256±1.039^{a}

TN, total nitrogen; TP, total phosphorus; TK, total potassium; AP, available phosphorus; AK, available potassium; NA, potential nitrogenase activity. Different lower-case letters indicate significant differences at $P < 0.05$ tested by one-way ANOVA, and different capital letters indicate significances at $P < 0.1$.

Table S2 Correlations between diazotrophic diversity indices and environmental variables

Environmental Variables [b]	Diazotrophic α diversity [a]				Diazotrophic β diversity [a]					
	Shannon		NTI		Bray-Curtis		Jaccard		βMNTD	
	r [c]	*P*	*r* [c]	*P*	*r* [c]	*P*	*r* [c]	*P*	*r* [c]	*P*
NO_3^--N/NH_4^+-N	*0.610***	0.007	*0.647***	0.004	0.026	0.336	0.026	0.357	0.027	0.376
TOC/TN	**-0.515***	0.029	**-0.519***	0.027	**0.353***	0.027	**0.353***	0.023	**0.454****	0.008
TOC/TP	0.096	0.705	0.187	0.458	0.345*	0.013	0.345**	0.005	0.323*	0.016
TK	0.28	0.261	*0.401ᴬ*	0.099	-0.057	0.678	-0.057	0.698	-0.059	0.692
AP	0.267	0.284	0.243	0.332	0.141	0.158	0.141	0.165	0.139	0.172
AK	0.201	0.423	*0.402ᴬ*	0.098	0.098	0.215	0.098	0.179	0.125	0.19
pH	-0.353	0.15	-0.255	0.307	0.259ᴬ	0.055	0.259*	0.046	0.344*	0.018
Clay	0.036	0.887	0.212	0.399	0.296*	0.012	0.296*	0.017	0.32**	0.009
Silt	-0.007	0.977	0.189	0.453	0.107	0.258	0.107	0.225	0.162	0.16
Sand	-0.011	0.964	-0.195	0.438	0.198	0.115	0.198	0.111	0.247ᴬ	0.072
Chlorophyll-a	0.129	0.61	0.162	0.521	0.284*	0.012	0.284*	0.016	0.229*	0.03
CO_2 flux	0.245	0.328	0.375	0.126	0.357**	0.005	0.357***	0.001	0.300*	0.009
F/B	-0.34	0.168	-0.1	0.693	0.149	0.165	0.149	0.17	0.134	0.216
nifH abundance	0.348	0.157	0.261	0.295	-0.053	0.601	-0.053	0.615	-0.052	0.608

[a] Spearman correlations between diazotrophic α diversity and environmental variables, whilst Mantel test was used to test the relationships between diazotrophic β diversity and environmental variables using Spearman's rho

[b] AK: available potassium, AP, available phosphorus, NA, potential nitrogenase activity, CO_2 flux: CO_2 flux net photosynthetic CO_2, TK, total potassium, F/B, Fungi to prokaryotes ratio. **Bold fonts** indicate variables showing significant (or marginal significant) correlations with both α and β diversity; *italic fonts* indicate significant (or marginal significant) variables only with α diversity; underline fonts indicate significant (or marginal significant) variables only with β diversity. Only significant variables were shown

[c] Significance codes: ᴬ *P < 0.1*, **P < 0.05*, ***P < 0.01*

Table S3 Mean nearest taxon index (NTI) in each biocrust stage

	Bare soil	Cyanobacterial crust	Lichen crust	Moss crust
NTI	2.096±2.235	5.014±0.227	4.889±0.581	4.240±0.296

Table S4 Relative abundances (%) of diazotrophic genera in each biocrust stage.

Genera	Bare soil	Cyanobacterial crust	Lichen crust	Moss crust
Azoarcus	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.049±0.049[a]
Azospirillum	**20.516±15.802[a]**	**0.307±0.155[a]**	**0.049±0.049[a]**	**0.147±0.06[a]**
Beijerinckia	0.369±0.159[a]	0.000±0.000[b]	0.000±0.000[b]	0.000±0.000[b]
Bradyrhizobium	**2.641±0.974[a]**	**0.307±0.184[b]**	**0.098±0.06[b]**	**0.098±0.06[b]**
Burkholderiales	0.614±0.123[a]	0.061±0.061[b]	0.049±0.049[b]	0.000±0.000[b]
Dechloromonas	1.044±1.044[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]
Desulfovibrionaceae	0.184±0.118[a]	0.123±0.071[a]	0.000±0.000[a]	0.049±0.049[a]
Dethiobacter	0.123±0.123[a]	0.000±0.000[a]	0.000±0.000a	0.000±0.000[a]
Klebsiella	0.184±0.118[a(A)]	0.000±0.000[a(B)]	0.000±0.000[a(B)]	0.000±0.000[a(B)]
Nostoc	**36.302±9.466[a]**	**61.425±4.426[a]**	**44.079±5.073[a]**	**45.209±10.116[a]**
Paenibacillus	0.184±0.118[a]	0.000±0.000[a]	0.000±0.000[a]	0.049±0.049[a]
Scytonema	**15.111±6.085[a]**	**19.656±3.009[a]**	**22.408±3.935[a]**	**18.722±2.926[a]**
Rhodopseudomonas	0.123±0.123[a]	0.000±0.000[a]	0.000±0.000[a]	0.049±0.049[a]
Sinorhizobium	0.123±0.123[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]
Tolypothrix	0.000±0.000[a]	0.061±0.061[a]	0.049±0.049[a]	0.049±0.049[a]
Trichormus	0.000±0.000[a]	0.061±0.061[a]	0.098±0.098[a]	0.491±0.433[a]
Xanthobacter	0.061±0.061[a]	0.061±0.061[a]	0.000±0.000[a]	0.000±0.000s[a]
Unclassified Nostocales	22.42±3.751[a(AB)]	17.936±3.478[a(B)]	33.17±2.417[a(AB)]	35.086±7.882[a(A)]

Different lower-case letters indicate significant differences at $P < 0.05$ tested by one-way ANOVA, and capital letters indicate marginal significance at $P < 0.1$

Table S5 Diazotrophic keystone species identified by Co-MENs in meta-network

OTUID	Order	Family	Genus	Species	Type of core species
OTU_59	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_128	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_144	Nostocales	Nostocaceae			Connector species
OTU_146	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_305	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_321	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_336	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_378	Nostocales	Scytonemataceae	Scytonema	Scytonema hofmannii	Connector species
OTU_424	Nostocales	Scytonemataceae	Scytonema	Scytonema hofmannii	Connector species
OTU_466	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_484	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_494	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_530	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_659	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_791	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_924	Nostocales	Scytonemataceae	Scytonema		Connector species
OTU_1101	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_2192	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_2360	Nostocales	Nostocales			Connector species
OTU_2603	Nostocales	Nostocales			Connector species
OTU_2746	Nostocales	Scytonemataceae	Scytonema	Scytonema hofmannii	Connector species
OTU_5352	Nostocales	Nostocaceae	Nostoc	Nostoc punctiforme	Connector species
OTU_2533	Nostocales	Nostocales			Network hub

Table S6 Diazotrophic keystone species identified by Co-MENs in early stage sub-network

OTUID	Order	Family	Genus	Species	Type of core species
OTU_47	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_80	Nostocales	Nostocaceae	*Nostoc*	*Nosto cpunctiforme*	Connector species
OTU_336	Nostocales	Nostocaceae	*Nostoc*	*Nosto cpunctiforme*	Connector species
OTU_495	Nostocales	Nostocales			Connectors pecies
OTU_856	Nostocales	Nostocales			Connectors pecies
OTU_1101	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_1487	Nostocales	Nostocaceae	*Nostoc*	*Nosto cpunctiforme*	Connector species
OTU_1818	Nostocales	Scytonemataceae	*Scytonema*	*Scytonema sp.*	Connector species
OTU_1860	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_4614	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_938	Nostocales	Nostocales			Module hub

Table S7 Diazotrophic keystone species identified by Co-MENs in later stage sub-network

OTU ID	Order	Family	Genus	Species	Type of core species
OTU_102	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_613	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_2360	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_2549	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_5489	Nostocales	Nostocaceae	*Nostoc*	*Nostoc punctiforme*	Connector species
OTU_3237	Nostocales	Scytonemataceae	*Scytonema*	*Scytonema hofmannii*	Module hub