

Table S1. Primers used for PCR amplification

Name	Primer Sequence (5'-3')	Gene target	Taxon target	Reference
First PCR round DGGE analysis				
FGPH19	TACGGCAARGGTGGNATHG	<i>nifH</i>	Diazotrophic	(Simonet et al. 1991)
POLR	ATSGCCATCATYTCRCCGGA	<i>nifH</i>	Diazotrophic	(Poly et al. 2001)
799F	AACMGGATTAGATACCCKG	16S rRNA	Bacteria	(Chelius and Triplett 2001)
1492R	TACGGYTACCTTGTTACGACTT	16S rRNA	Bacteria	(Chelius and Triplett 2001)
F203 α	CCGCATACGCCCTACGGGGGAAAGATTTAT	16S rRNA	Alphaproteobacteria	(Gomes et al. 2001)
F948 β	CGCACAAGCGGTGGATGA	16S rRNA	Betaproteobacteria	(Gomes et al. 2001)
F243HCG	GGATGAGCCCGCGGCCTA	16S rRNA	Actinobacteria	(Heuer et al. 1997)
BACF	GGGAAACCGGGGCTAATACCGGAT	16S rRNA	Firmicutes	(Garbeva et al. 2003)
Second PCR round DGGE analysis				
POLF-GC	CGCCCGCCGCGCCCCGCGCCCGGCCCGCCCCCG CCCCTGCGAYCCSAARGCBGACTC	<i>nifH</i>	Diazotrophic	(Poly et al. 2001)
AQER	GACGATGTAGATITCCTG	<i>nifH</i>	Diazotrophic	(Poly et al. 2001)
F968-GC	CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGC ACGGGGGGAACGAAGAACCTTAC	<i>16S rRNA</i>	Bacteria	(Heuer et al. 1999)
R1401	CGGTGTGTACAAGACCC	<i>16S rRNA</i>	Bacteria	(Heuer et al. 1997)
qPCR analysis				
POLR	ATSGCCATCATYTCRCCGGA	<i>nifH</i>	Diazotrophic	(Poly et al. 2001)
POLF	TGCGAYCCSAARGCBGACTC	<i>nifH</i>	Diazotrophic	(Poly et al. 2001)
6S-27F	AGAGTTTGATCCTGGCTCAG	16S rRNA	Bacteria	Bulgari et al., 2014
338R	GCTGCCTCCCGTAGGAGT	16S rRNA	Bacteria	Bulgari et al., 2014

Table 2. Primers used for Ion Torrent pyrosequencing analysis.

Primer	Primer sequence (5'-3')	Reference
967F-PP	CNACGCGAAGAACCTTANC	(Jünemann et al. 2012)
967F-UC1	CAACGCGAAAAACCTTACC	(Jünemann et al. 2012)
967F-UC2	CAACGCGCAGAACCTTACC	(Jünemann et al. 2012)
967F-UC3	ATACGCGARGAACCTTACC	(Jünemann et al. 2012)
967F-AQ	CTAACCGANGAACCTYACC	(Jünemann et al. 2012)
1046R	CGACAGCCATGCANACCT	(Jünemann et al. 2012)
1046R-PP	CGACAACCATGCANACCT	(Jünemann et al. 2012)
1046R-AQ1	CGACGGCCATGCANACCT	(Jünemann et al. 2012)
1046R-AQ2	CGACGACCATGCANACCT	(Jünemann et al. 2012)

Table S3. Alpha diversity indices. Statistical analysis of the total endophytic and diazotrophic endophytic bacterial community associated with sweet sorghum cv. M81E, grown under different N-fertilization levels (+/-N).

Treatment/ N- fertilization level*	Endophytic bacteria			Diazotrophic endophytic bacteria		
	Chao-1 (richness)	Simpson 1-D	Shannon H	Chao-1 (richness)	Simpson 1-D	Shannon H
R -N	21.55	0.91a	2.69a	5.67	0.41a	0.96a
R +N	16.41	0.87a	2.33a	8.33	0.71a	1.51a
LS -N	17.16	0.84a	2.25a	7.66	0.78a	1.71a
LS +N	20.33	0.88a	2.54a	9.66	0.82a	1.95a
US -N	20.66	0.90a	2.63a	9.33	0.73a	1.66a
US +N	20.33	0.89a	2.55a	9.66	0.81a	1.91a

*R: root, LS: low stem, US: upper stem, +/-N: high and low N-fertilization level. Means within two treatments that have the same letter are not significantly different by Fisher LSD test with a $P < 0.05$

Table S4. Identification by NCBI BlastN of 16S rRNA sequences retrieved from DGGE bands.

	Bands	Accession number NCBI	Closest match	% Identity
From the general 16S RNAr DGGE gel				
Betaproteobacteria	B7	NR_025770.1	<i>Duganella violaceinigra</i> YIM 31327	99%
	B14	NR_108739.1	<i>Aquabacterium limnoticum</i> ABP-4	97%
	B20	NR_027537.1	<i>Bordetella hinzii</i> R-39474	97%
	B21	NR_137346.1	<i>Massilia arvi</i> THG-RS20	96%
Gammaproteobacteria	B8, B10	NR_103936.1	<i>Klebsiella pneumoniae</i> DMS 30104	99%
	B23	NR_112008.1	<i>Serratia liquefaciens</i> JCM1245	100%
	B9, B24, B33	NR_043883.1	<i>Pantoea dispersa</i> LMG 2603	99%
	B1, B2, B3, B29, B30	NR_041973.1	<i>Pantoea cypripedii</i> DSM 3873	99%
	B4, B5, B31	NR_074910.1	<i>Salmonella enterica</i> serovar Typhimurium LT21	99%
	B25	NR_116403.1	<i>Kosakonia radicincitans</i> Ah-143	99%
	B26	NR_137380.1	<i>Pantoea intestinalis</i> strain 29Y89B	90%
	B27, B38	NR_116748.1	<i>Pantoea brenneri</i> LMG 5343	99%
	B28	NR_114481.1	<i>Pseudomonas tolaasii</i> ATCC 33618	94%
	B32	NR_114507.1	<i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i> ATCC 13884	99%
	B34, B37	NR_026045.1	<i>Pantoea ananatis</i> LMG 1846	99%
	B36	NR_113648.1	<i>Stenotrophomonas maltophilia</i> NBRC 14161	98%
From the Alphaproteobacteria DGGE gel				
	B12a	NR_026519.1	<i>Agrobacterium larrymoorei</i> AF3.10	99%
	B13a	NR_044393.1	<i>Ancylobacter oerskovii</i> NS05	94%
	B17a	NR_037106.1	<i>Brevundimonas variabilis</i> CB17	99%
	B18a, B19a	NR_114056.1	<i>Pleomorphomonas oryzae</i> NBRC 102288	98%
From the Betaproteobacteria DGGE gel				
	B1b, B2b, B4b	NR_043308.1	<i>Massilia albidiflava</i> 45	98%
	B5b, B8b, B9b, B11b	NR_137346.1	<i>Massilia arvi</i> THG-RS20	99%

B7b	NR_136517.1	<i>Methyloversatilis discipulorum</i> FAM1	97%
From the Actinobacteria DGGE gel			
B7ac	NR_114322.1	<i>Sediminihabitans luteus</i> H97-3	96%
B9ac	NR_104839.1	<i>Curtobacterium oceanosedimentum</i> ATCC31317	99%
B13ac	NR_135869.1	<i>Microbacterium proteolyticum</i>	99%
B15ac	NR_116334.1	<i>Nocardia mikamii</i> W8061	100%
From the Firmicutes DGGE gel			
B1f	NR_118445.1	<i>Bacillus circulans</i> ATCC 4513	97%
B2f	NR_117050.1	<i>Bacillus kochii</i> WCC 4582	98%
B3f, B10f	NR_108906.1	<i>Bacillus eiseniae</i> A1-2	98%
B4f	NR_036847.1	<i>Macrococcus brunensis</i> CCM 4811	100%
B5f	NR_118534.1	<i>Exiguobacterium himgiriensis</i> K22-26	99%
B6f	NR_036904.1	<i>Staphylococcus epidermidis</i> Fussel	99%
B8f	NR_113585.1	<i>Exiguobacterium acetylicum</i> NBRC 12146	99%
B11f	NR_036828.1	<i>Staphylococcus capitis</i> JCM 2420	100%

Table S5. Identification by NCBI Blastx of *nifH* sequences retrieved from DGGE bands.

Bands	Accession number NCBI	Closest match	% identity	Accession number NCBI	Closest match (culturable)	% identity	Phylogenetic position (Class ^a /Order)
B1n	GU056207.1	<i>Oscillatoria</i> sp. MMG-2	96%				Cyanobacteria, Occilatoriales
B4n	FJ593869.1	<i>Pantoea</i> sp. EC080527_02	97%				γ/Enterobacteriales
B5n	KC989924.1	<i>Enterobacter</i> sp. R4-368	98%				γ/Enterobacteriales
B6n, B23n, B30n	HQ204226.1	<i>Enterobacter sacchari</i> HX148S	98%-99%				γ/Enterobacteriales
B7n	KF872961.1	Uncultured bacterium clone IFRpool-70	99%	HQ204232.1	<i>Enterobacter</i> sp. PG132S	94%	γ/Enterobacteriales
B8n	KF872888.1	Uncultured bacterium clone ABFRpool-29	100%	HQ204226.1	<i>Enterobacter sacchari</i> HX148S	99%	γ/Enterobacteriales
B9n	LC049050.1	<i>Klebsiella variicola</i>	100%				γ/Enterobacteriales
B10n	KF732646.1	<i>Klebsiella variicola</i> DX120E	99%				γ/Enterobacteriales
B11n, B15n	HQ404304.1	<i>Klebsiella pneumoniae</i> NG14	99%				γ/Enterobacteriales
B12n	FJ593869.1	<i>Pantoea</i> sp. EC080527_02	99%				γ/Enterobacteriales
B13n, B17n, B21n	HQ204258.1	<i>Enterobacter sacchari</i> NN143E	99%				γ/Enterobacteriales
B14n	HQ204222.1	<i>Klebsiella</i> sp. CZ150S	99%				γ/Enterobacteriales
B16n	HQ204258.1	<i>Enterobacter sacchari</i> NN143E	76%				γ/Enterobacteriales
B18n	EF438102.1	Uncultured marine microorganism clone C11-16	88%	CP001614.2	<i>Teredinibacter turnerae</i> T7901	80%	γ/Alteromonadales
B19n	AY231580.1	<i>Ideonella</i> sp. Long 7	98%				β/Burkholderiales
B20n	AF331982.1	Uncultured bacterium clone g1(102)	85%	CP010415.1	<i>Azotobacter chroococcum</i> NCIMB 8003	84%	γ/ Pseudomonadales
B22n	KC989924.1	<i>Enterobacter</i> sp. R4-368	99%				γ/ Enterobacteriales

B24n	KF872888.1	Uncultured bacterium clone ABFRpool-29	96%	HQ204258.1	<i>Enterobacter sacchari</i> NN143E	95%	γ/ Enterobacteriales
B25n	HQ204233.1	<i>Enterobacter</i> sp. QZ25S	99%				γ/ Enterobacteriales
B27n	CP011636.1	<i>Klebsiella oxytoca</i> CAV1374	99%				γ/ Enterobacteriales
B31n	CP007215.2	<i>Enterobacter sacchari</i> SP1	97%				γ/ Enterobacteriales
B32n	KM885223.1	Uncultured microorganism clone L- 49	99%	FJ593868.1	<i>Enterobacter</i> sp. MTP_05051217	99%	γ/ Enterobacteriales

^aβ: Betaprotebacteria; γ: Gammaprotebacteria.

Figure S1. Rarefactions curves of sweet sorghum samples treated with high and low N-fertilization levels (+N and -N respectively).

