soil systems

MDPI

Supplementary material

Table S1. Details of gBlock qPCR standards, Dilution range had 1 order of magnitude apart between each of 5 standards.

Target gene	Microbial source for sequence included in the gBlock standards	Sequence used	Dilution range of the standards having the targeted gene (copies/2 µl)
phoD	*Sinorhizobium meliloti*	CTGGGCGATCACGTTCCAGGTGGCGCTGGACGCCGCAAGCTCGCGCTTCAGCCAGT CCGCCTGCCGCCAGCGAAGAGGCCGCCCCCCCCCTCTTCCTGGTTGGGGCCGCGAT AGGAGCGCAGATCCACGAAGAAGACGTCGAGCAGCGGACCATAGGCGATCTTGCG GAAAATGCGGCCGGGCTCCGTCGGCAGCGTGCGGATCGCCGTCATCTCGTGAAACG CGCGGGCCGCGCGGGCGGCATAGACGGCGACATCCTTCTCCGGATAGCGCGGGTC GTCGCTGAGATCGGTCGAGGCCGACCAGTTGTTCAGGACCTCGTGATCGTCCCA	$10^6 – 10^2$
16S rRNA	*Pseudomonas denitrificans*	CCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGAAACCCTGATCCAGC CATGCCGCGTGTGTTAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGA AGGGTTGTTGGCTAATATCCAGCAATTTTGACGTTACCGACAGAATAAGCACCGGC TAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTA CTGGGCGTAAAGCGCGCGTAGGTGGTTCAGTAAGATGGGTGTGAAATCCCCGGGCT TAACCTGGGAACTGCTTTCATAACTGCTGAGCTGGAGTACGGTAGAGGGTAGTGGA ATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAG GCGACTACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAG GATTAGATACCCTGGTAGTCC	$10^7 – 10^3$
AMF-18S rRNA	*Glomus intraradices*	AAGCTCGTAGTTGAATTTCGGGACCAATATGTCGGTCGTACCTCGGTACGTACTGG CATCGTTGGTTTCTCCCTTCTGACGAACCATGATGTCATTTATTTGGTGTCATGGGG AATCAGGACTGTTACTTTGAAAAAATTAGAGTGTTTAAAGCAGGCTCACGCTTGAA TACATTAGCATGGAATAATGAAATAGGACGTTTGATTCTATTTTGTTGGTTCCTAGG ATCGACGTAATGATTAATAGGGATAGTTGGG	$10^6 – 10^2$

ITS	*Rhizopus microsporus*	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCG GAAGGATCATTAACTAATGTATTGGCACTTTACTGGGATTTACTTCTCAGTATTGTT TGCTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAACTGACCTTCGGGAGAGA CTCAGGACATATAGGCTATAATGGGTAGGCCTGTTCTGGGGTTTGATCGATGCCAA TCAGGATTACCTTTCTTCCTTTGGGAAGGAAGGCGCCTGGTACCCTTTACCATATAC CATGAATTCAGAATTGAAAGTATAATATAATAACAACTTTTAACAATGGATCTCTT GGTTCTCGCATCGATGAAGAACGCAGCG	$10^7 – 10^3$

Table S2. Quality control details of the qPCR runs.

Target microbial group	Positive control	Negative control	R2 value of standard curve	Reaction efficiency
phoD harboring microbes	*Sinorhizobium meliloti* 1021	*Escherichia coli* K-12	0.99	1.07
16S rRNA	*Escherichia coli* K-12	*Methanospirillum hungatei* JF-1	0.99	1.03
AMF 18S rRNA	*Glomus intraradices*	*Escherichia coli* K-12	0.987	1.00
ITS	*Rhizopus microsporus*	*Escherichia coli* K-12	0.99	1.06

Table S3. Primers and conditions used for the qPCR assays in this study.

Target microbial group	Primers and sequences	qPCR reaction mixture	Thermal profile	Reference
phoD-harboring microbes	*phoD*-F733(5'-TGGGAYGATCAYGARGT-3')/ *phoD*-R1083 (5'-CTGSGCSAKSACRTTCCA-3')	7.5 µl SYBR Green (2x) Master Mix, 1.5 µl each primer (5 µM), 2 µl DNA template, 2.5 nuclease free H_2O.	5 min at 98°C for initial denaturation; 35 cycles of 30 s at 98°C, 30 s at 58°C, extension for 30 s at 72°C, and acquisition for 10 s at 82°C. Melt curve produced at 55-98°C (1° and 5 s/cycle melt).	Modified after Ragot et al. [1]
Total bacteria (16S rRNA)	341f-(5'-CCTACGGGAGGCAGCAG-3')/ 797r-(5'-GGACTACCAGGGTATCTAATCCTGTT-3')	7.5 µl SYBR Green (2x) Master Mix, 0.225 µl F primer (0.3 µM), 0.675 µl R primer (0.9 µM), 2 µl DNA template, 4.6 nuclease free H_2O.	3 min at 98°C for initial denaturation; 40 cycles of 30 s at 98°C, 30 s at 61.5°C, extension for 20 s at 72°C, and acquisition for 10 s at 82°C. Melt curve produced at 50-99°C (1° and 5 s/cycle melt) after a pre-melt conditioning for 90 s at 50°C.	Modified after Harter et al. [2]

Table S3. Continued.

Target microbial group	Primers and sequences	qPCR reaction mixture	Thermal profile	Reference
Total AMF (18S rRNA)	GC-AMV4.5NF- (5'-CGC CCG CCG CGC GCG GCGGGCGGG GCG GGG GCA CGG GGG G [GC clamp] AAG CTC GTA GTT GAA TTT CG-3′)/ AMDGR-(5′-CCC AAC TAT CCC TAT TAA TCA T-3′)	7.5 µl SYBR Green (2x) Master Mix, 1.5 µl each primer (5 µM), 2 µl DNA template, 2.5 nuclease free H_2O.	10 min at 98°C for initial denaturation; 35 cycles of 30 s at 98°C, 30 s at 55°C, extension for 45 s at 72°C, and acquisition for 10 s at 82°C. Melt curve produced at 50-98°C (1° and 5 s/cycle melt).	Modified after Sato et al. [3]
Total fungi (ITS)	ITS1f-(5'-TCC GTA GGT GAA CCT GCG G-3')/5.8s-(5'-CGC TGC GTT CTT CAT CG-3')	7.5 µl SYBR Green (2x) Master Mix, 1.5 µl each primer (5 µM), 2 µl DNA template, 2.5 nuclease free H_2O.	10 min at 98°C for initial denaturation; 35 cycles of 60 s at 98°C, 30 s at 53°C, extension for 45 s at 72°C, and acquisition for 10 s at 82°C. Melt curve produced at 48-98°C (1° and 5 s/cycle melt).	Modified after Fierer et al. [4]

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