

Soil biochar amendment affects the diversity of *nosZ* transcripts: Implications for N₂O formation

Johannes Harter ¹, Mohamed El-Hadidi ², Daniel H. Huson ², Andreas Kappler ¹,
Sebastian Behrens ^{4,5,*}

¹ Geomicrobiology & Microbial Ecology, Center for Applied Geosciences, University of Tuebingen, Tuebingen, Germany

² Algorithms in Bioinformatics, Center for Bioinformatics, University of Tuebingen, Tuebingen, Germany

³ Bioinformatics, Center for Informatics Science (CIS), Nile University, Giza, Egypt

⁴ Department of Civil, Environmental, and Geo-Engineering, University of Minnesota, Minneapolis, MN, USA

⁵ BioTechnology Institute, University of Minnesota, St. Paul, MN, USA

Supplementary information

* Corresponding author (Tel.: +1-612-626-8225 and +1-612-624-8454. Email: sbehrens@umn.edu).

Table S1: Reaction mixtures and thermal profiles of the PCRs used to amplify *nirK*, typical *nosZ*, and atypical *nosZ* transcripts.

target gene	reaction mixture	volume (μl)	thermal profile
<i>nirK</i>	FastStart HiFi Polymerase (5 U/μl)	0.25	94°C – 5 min
	FastStart 10 x Buffer (with 18 mM MgCl ₂)	2.5	(1 cycle)
	dNTP mix (10 mM each)	0.5	94°C – 30 s
	F1aCu_OA (10 μM)	1	56°C – 45 s
	R3Cu_OA (10 μM)	1	72°C – 60 s
	DMSO	0.5	(40 cycles)
	BSA	0.25	72°C – 8 min
	PCR water	16	(1 cycle)
	cDNA sample	3	
typical <i>nosZ</i>	FastStart HiFi Polymerase (5 U/μl)	0.25	94°C – 5 min
	FastStart 10 x Buffer (without MgCl ₂)	2.5	(1 cycle)
	MgCl ₂ (25 mM)	3.5	94°C – 30 s
	dNTP mix (10 mM each)	0.5	56°C – 45 s
	nosZ2F_OA (10 μM)	2	72°C – 60 s
	nosZ2R_OA (10 μM)	2	(40 cycles)
	DMSO	1	72°C – 8 min
	BSA	0.25	(1 cycle)
	PCR water	10	
	cDNA sample	3	
atypical <i>nosZ</i>	FastStart HiFi Polymerase (5 U/μl)	0.25	94°C – 8 min
	FastStart 10 x Buffer (without MgCl ₂)	2.5	(1 cycle)
	MgCl ₂ (25 mM)	3.5	94°C – 45 s
	dNTP mix (10 mM each)	0.5	56°C – 45 s
	nosZ-II-F_OA (10 μM)	2	72°C – 60 s
	nosZ-II-R_OA (10 μM)	2	(40 cycles)
	DMSO	2	72°C – 8 min
	BSA	0.5	(1 cycle)
	PCR water	8.75	
	cDNA sample	3	

Primers consist of **target-specific primers** (F1aCu/R3Cu, nosZ2F/nosZ2R, and nosZ-II-F/nosZ-II-R) with overhang adapters.

F1aCu_OA: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATCATGGTCTGCCGCG**-3'

R3Cu_OA: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GCCTCGATCAGRTTGTGGTT**-3'

nosZ2F_OA: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CGCRACGGCAASAAGGTSMSST**-3'

nosZ2R_OA: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CAKRTGCAKSGCRTGGCAGAA**-3'

nosZ-II-F_OA: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CTNGGNCCNYTKCAYAC**-3'

nosZ-II-R_OA: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GCNGARCARAANTCBGTRC**-3'

Table S2: Relative sequence abundances of all *nirK*-expressing microbial species.

<i>nirK</i> -expressing species	rel. seq. abundance [%]
<i>Mesorhizobium ciceri</i>	47.0
<i>Rhodopseudomonas palustris</i>	13.3
<i>Mesorhizobium opportunistum</i>	6.12
<i>Mesorhizobium australicum</i>	4.02
<i>Chelativorans</i> sp. BNC1	2.05
<i>Bradyrhizobium</i> BTAi1	0.48
<i>Ensifer fredii</i>	0.44
<i>Ochrobactrum anthropi</i>	0.39
<i>Ensifer medicae</i>	0.35
<i>Bradyrhizobium oligotrophicum</i>	0.08
<i>Burkholderia multivorans</i>	0.06
<i>Nitrosomonas</i> sp. AL212	0.06
<i>Hyphomicrobium nitrativorans</i>	0.03
<i>Shewanella loihica</i>	0.03
<i>Bradyrhizobium</i> ORS-278	0.02
<i>Rhizobium etli</i>	0.01
<i>Achromobacter xylosoxidans</i>	<0.01
<i>Phaeobacter gallaeciensis</i>	<0.01
<i>Pseudomonas mendocina</i>	<0.01
<i>Rhodobacter sphaeroides</i>	<0.01

Table S3: Relative sequence abundances of all typical *nosZ*-expressing microbial species.

typical <i>nosZ</i> -expressing species	rel. seq. abundance [%]
<i>Oligotropha carboxidovorans</i>	31.8
<i>Bradyrhizobium diazoefficiens</i>	15.1
<i>Hyphomicrobium nitratorans</i>	12.4
<i>Ensifer meliloti</i>	10.6
<i>Azoarcus</i> sp. KH32C	6.57
<i>Ensifer fredii</i>	4.68
<i>Polymorphum gilvum</i>	2.45
<i>Azoarcus</i> sp. BH72	1.24
<i>Rhodobacter sphaeroides</i>	0.86
<i>Bradyrhizobium</i> sp. BTAi1	0.81
<i>Rubrivivax gelatinosus</i>	0.76
<i>Rhodospirillum centenum</i>	0.41
<i>Pseudomonas stutzeri</i>	0.39
<i>Thiobacillus denitrificans</i>	0.39
<i>Azospirillum lipoferum</i>	0.21
<i>Pseudomonas brassicacearum</i>	0.17
<i>Pseudomonas fluorescens</i>	0.17
<i>Anaeromyxobacter dehalogenans</i>	0.16
<i>Dinoroseobacter shibae</i>	0.16
<i>Ralstonia pickettii</i>	0.15
<i>Pseudomonas aeruginosa</i>	0.14
<i>Ochrobactrum anthropi</i>	0.10
<i>Gamma-proteobacterium</i> HdN1	0.09
<i>Pseudogulbenkiania</i> sp. NH8B	0.07
<i>Rhodoferrax ferrireducens</i>	0.07
<i>Paracoccus denitrificans</i>	0.06
<i>Leptothrix cholodnii</i>	0.04
<i>Hyphomicrobium denitrificans</i>	0.03
<i>Rhodopseudomonas palustris</i>	0.03
<i>Roseobacter denitrificans</i>	0.03
<i>Bordetella petrii</i>	0.02
<i>Anaeromyxobacter</i> sp. Fw109-5	0.01
<i>Aromatoleum aromaticum</i>	0.01
<i>Methylobacterium</i> sp. 4-46	0.01
<i>Ralstonia solanacearum</i>	0.01
<i>Alicyciphilus denitrificans</i>	<0.01
<i>Bradyrhizobium oligotrophicum</i>	<0.01
<i>Burkholderia multivorans</i>	<0.01
<i>Janthinobacterium</i> sp. Marseille	<0.01
<i>Legionella pneumophila</i>	<0.01
<i>Rhodospirillum photometricum</i>	<0.01
<i>Sphingomonas wittichii</i>	<0.01
<i>Stigmatella aurantiaca</i>	<0.01

Table S4: Relative sequence abundances of all atypical *nosZ*-expressing microbial species.

atypical <i>nosZ</i> -expressing species	rel. seq. abundance [%]
<i>Melioribacter roseus</i>	35.3
<i>Caldilinea aerophila</i>	12.6
<i>Taylorella asinigenitalis</i>	7.08
<i>Burkholderia multivorans</i>	4.43
<i>Owenweeksia hongkongensis</i>	3.75
<i>Pedobacter saltans</i>	3.01
<i>Belliella baltica</i>	2.72
<i>Mesorhizobium ciceri</i>	2.44
<i>Clostridium botulinum</i>	2.23
<i>Gemmatimonas aurantiaca</i>	1.92
<i>Opitutus terrae</i>	1.66
<i>Thermomicrobium roseum</i>	1.62
<i>Ignavibacterium album</i>	1.50
<i>Runella slithyformis</i>	1.36
<i>Flavobacteriaceae bacterium 3519-10</i>	1.17
<i>Legionella pneumophila</i>	0.80
<i>Azospirillum lipoferum</i>	0.77
<i>Carnobacterium maltaromaticum</i>	0.77
<i>Lactobacillus crispatus</i>	0.71
<i>Rhodospirillum photometricum</i>	0.63
<i>Rhodopseudomonas palustris</i>	0.59
<i>Chelativorans</i> sp. BNC1	0.46
<i>Niastella koreensis</i>	0.44
<i>Clostridium tetani</i>	0.42
<i>Heliobacterium modesticaldum</i>	0.36
<i>Corynebacterium efficiens</i>	0.34
<i>Persephonella marina</i>	0.28
<i>Enterococcus mundtii</i>	0.27
<i>Shewanella piezotolerans</i>	0.26
<i>Blattabacterium</i> sp. (Nauphoeta cinerea)	0.24
<i>Anaeromyxobacter</i> sp. Fw109-5	0.20
<i>Mesorhizobium australicum</i>	0.20
<i>Nocardia cyriacigeorgica</i>	0.18
<i>Mesorhizobium opportunistum</i>	0.16
<i>Streptococcus dysgalactiae</i>	0.14
<i>Bacillus subtilis</i>	0.11
<i>Solitalea canadensis</i>	0.07
<i>Ensifer fredii</i>	0.06
<i>Hydrogenobacter thermophilus</i>	0.05
<i>Bradyrhizobium oligotrophicum</i>	0.04
<i>Flavobacterium columnare</i>	0.04
<i>Desulfotomaculum ruminis</i>	0.03
<i>Mycobacterium rhodesiae</i>	0.03
<i>Dyadobacter fermentans</i>	0.02
<i>Ensifer medicae</i>	0.02
<i>Helicobacter felis</i>	0.02
<i>Rhodothermus marinus</i>	0.02
<i>Azoarcus</i> sp. KH32C	0.01
<i>Bacillus coagulans</i>	0.01
<i>Bradyrhizobium</i> sp. ORS 278	0.01
<i>Haliscomenobacter hydrossis</i>	0.01
<i>Muricauda ruestringensis</i>	0.01
<i>Ochrobactrum anthropi</i>	0.01
<i>Riemerella anatipestifer</i>	0.01
<i>Sphaerobacter thermophilus</i>	0.01
<i>Sulfuricella denitrificans</i>	0.01