

Supplemented information

Mobile incubator for iron(III) reduction in the gut of soil-feeding earthworm *Pheretima guillelmi* and interaction with denitrification

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Number of figures: 7.

Number of tables: 3.

Number of pages: 17.

1 **Supporting Methods**

2 **Quantitative PCR**

3 Determination of the abundances of genes (including the bacterial 16S *rRNA*, *nar G*,
4 *nirK*, *nirS* and *nosZ*) was performed with a real-time PCR Detection System (Roche
5 480, Roche, Indianapolis, IN, USA). Primers of these genes and thermal cycling
6 condition were listed in the Table S4. The reaction mixture contained 2 μL DNA (as
7 template), 0.8 μL of each primer, 10 μL of SYBR 2 Premix EX *Taq*, 0.6 μL BSA (20
8 mg ml^{-1}) and 5.8 μL of dd H_2O . Standard plasmids carrying these genes were
9 prepared by cloning these genes from samples. A standard curve was carried out
10 using 10-fold serial dilutions of standard plasmids DNA. The melting curve
11 displayed only one peak at a melting temperature (T_m). Only the Reaction of PCR
12 with efficiencies between 90% and 110% were accepted.

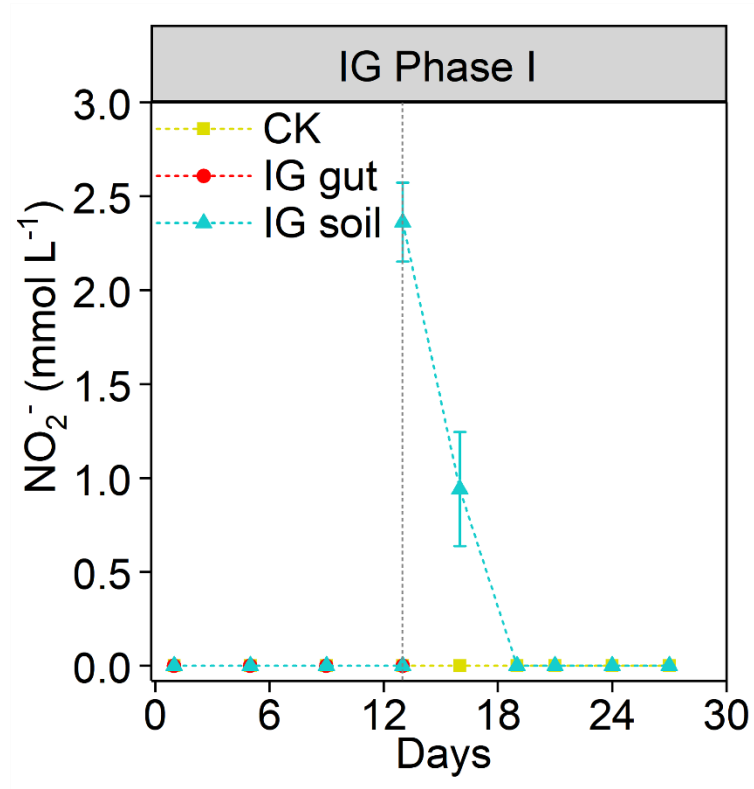
13 **Gut content microcosm in the second generation**

14 To further investigate the order of nitrate reduction and iron(III) reduction occurred
15 in the gut content. The IG gut incubations were transferred (10%, v/v) to fresh
16 anoxic iron(III) reduction medium (assigned as generation II) after the ferrihydrite in
17 the treatment of IG gut was completely reduced. The anoxic iron(III) reduction
18 medium was prepared as described in the text. Generation II was divided into two
19 phases, namely phase I and phase II. The stage without exogenous organic carbon
20 was divided as phase I, and the phase II began when acetate was added to the
21 medium after the iron(III) reduction ceased. Two treatments were set up during the
22 second generation incubation: (1) abiotic treatment inoculated with sterile deionized
23 water (CK); (2) biotic treatment inoculated with IG gut incubation (IIG gut).

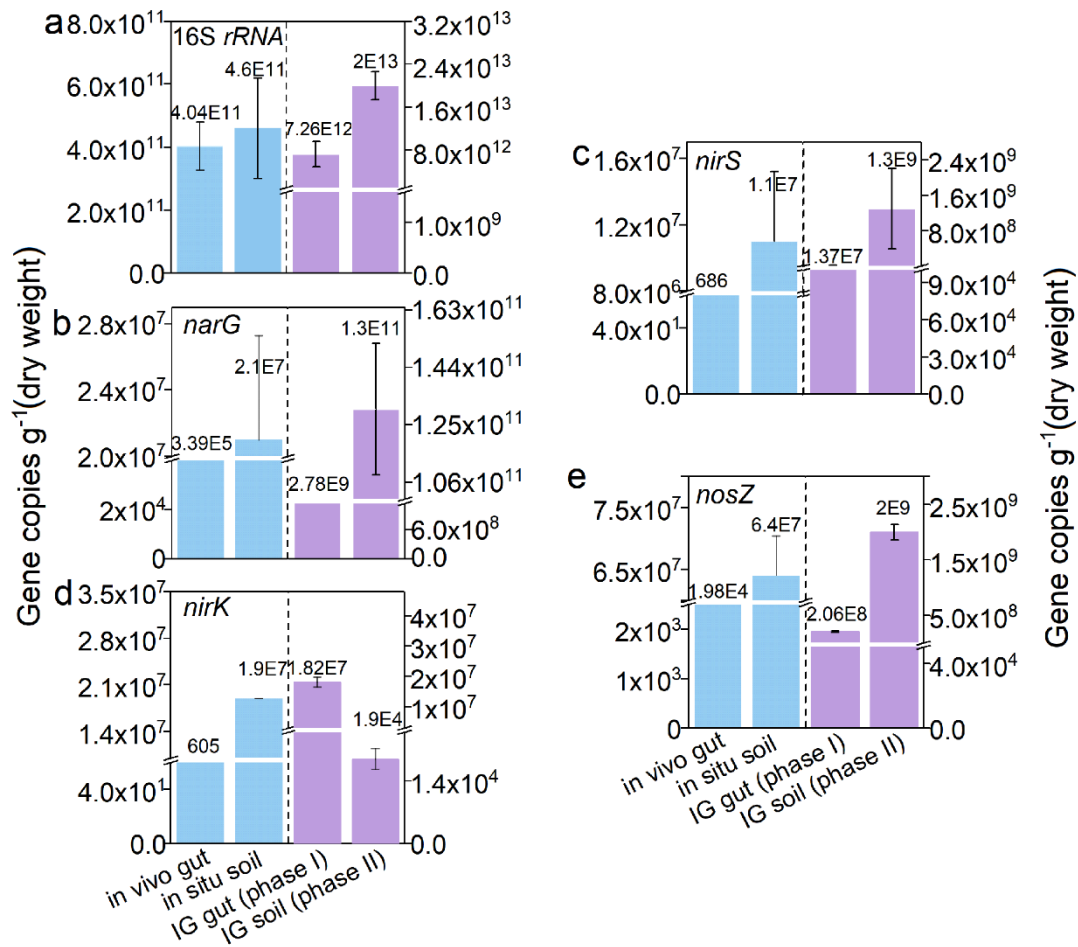
24 **Supporting Results**

25 **Turnover of acetate, nitrate, Fe(II) and total iron in the microcosm experiment** 26 **incubated with IIG gut**

27 During microcosmic experiment of generation II, nitrate reduction happened earlier
28 than iron(III) reduction during the incubation, and no iron(III) reduction was
29 observed until the addition of acetate during the phase II of IIG gut (Figure 6). The
30 remaining acetate contained in the medium was completely consumed with a rate of
31 $0.068 \text{ mmol L}^{-1} \text{ day}^{-1}$ during the phase I in the treatment of IIG gut, and the 8 mmol
32 L^{-1} acetate added at the beginning of phase II was used up within 16 days (Figure
33 S6b). NO_3^- reduction, with a rate of $0.46 \text{ mmol L}^{-1} \text{ day}^{-1}$, stopped after 6 days of
34 incubation and then continued after the addition of acetate in the treatment of IIG gut
35 (Figure S6b). NO_2^- accumulation was substantially higher at the beginning of phase
36 II ($1.18 \pm 0.14 \text{ mmol L}^{-1}$) than that of phase I ($0.30 \pm 0.12 \text{ mmol L}^{-1}$) and then
37 decreased promptly in the treatment of IIG gut (Figure S6c). After nitrate exhausted
38 in the system, rate of iron(III) reduction increased sharply and all ferrihydrite was
39 consumed up with 24 days during the phase II in the treatment of IIG gut (Figure
40 S6a). Total extractable iron in the treatment of IIG gut remained constant at around
41 27 mmol L^{-1} throughout the incubation (Figure S6a).



42 **Figure S1.** Development of NO_2^- in treatments of IG gut and IG soil during the
43 microcosmic incubation. The error bars indicate the standard deviations of three
44 replications.



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46 **Figure S2.** Gene copy numbers of 16S *rRNA* (a), *narG* (b), *nirK* (c), *nirS* (d) and

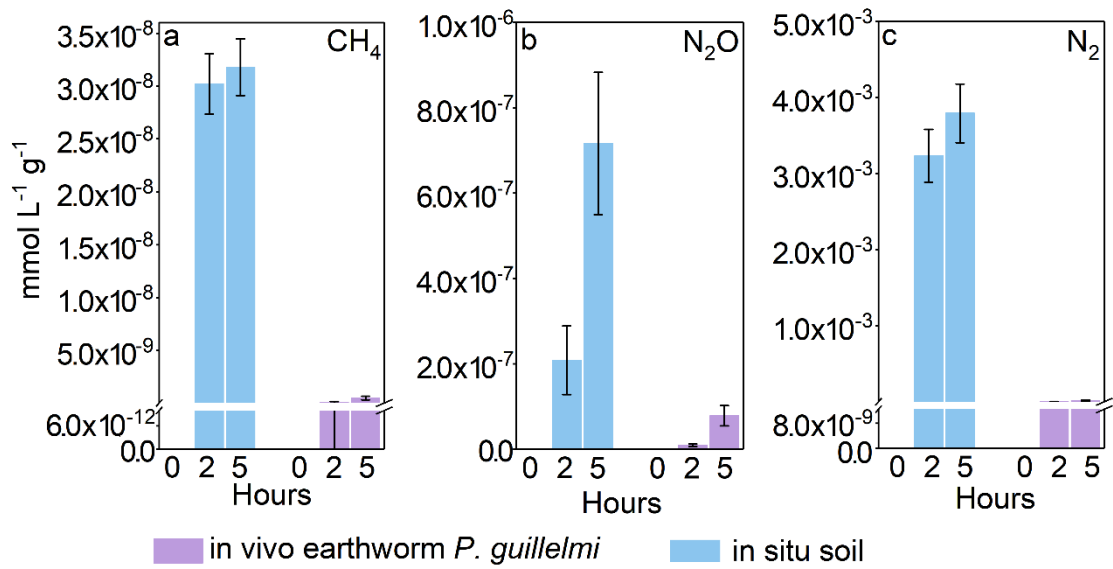
47 *nosZ* (e) in setups of *in situ* soil, *in vivo* gut, IG soil (phase II) and IG gut (phase I).

48 The error bars indicate the standard deviations of three replications.



49

50 **Figure S3.** Heatmap of log relative abundance of the taxa at the family level in
 51 setups of *in situ* soil, *in vivo* gut, IG soil (phase II) and IG gut (phase I). Each
 52 treatment contained three lanes, which represented three replicates. The color
 53 intensity of the heatmap in each cell denotes the average relative abundance
 54 (calculated from the three replications) of a genus in a treatment with the legend
 55 shown at the top of the figure.



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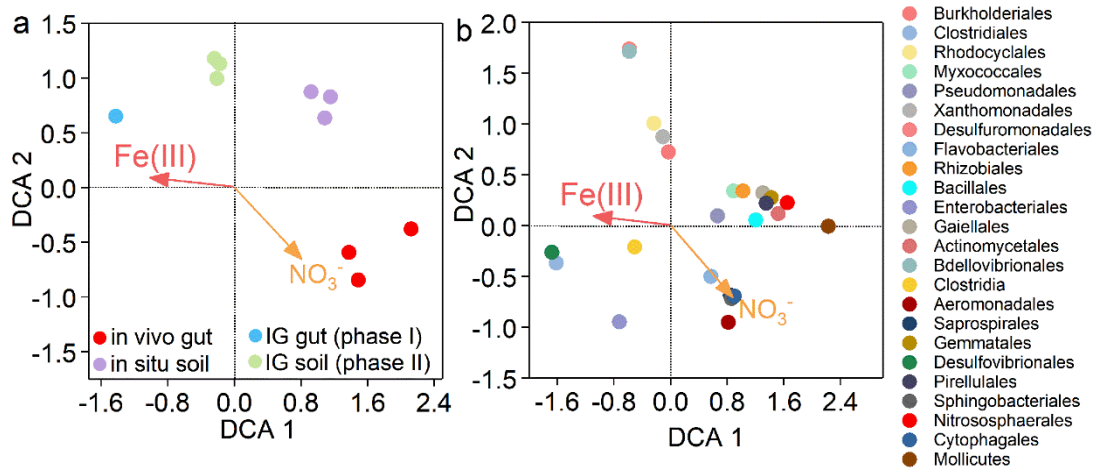
57 **Figure S4.** Emission of CH₄ (a), N₂O (b) and N₂ (c) in the in vivo gut of earthworm

58 *P. guillelmi* and in situ soil during 5-hour incubation. The detection was based on the

59 fresh weight of in vivo earthworm and in situ soil. The error bars indicate the

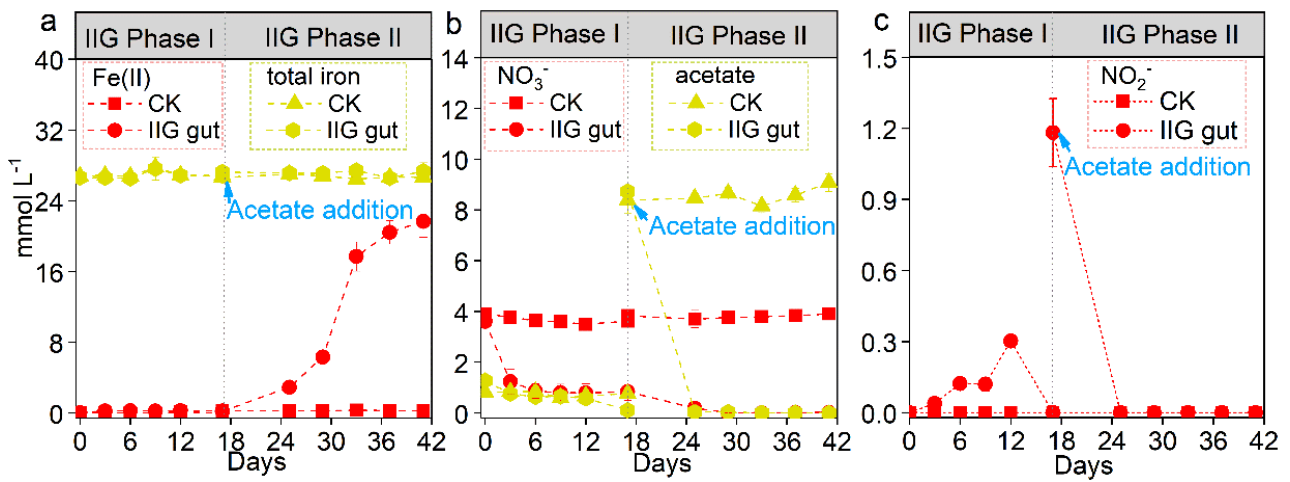
60 standard deviations of three replications.

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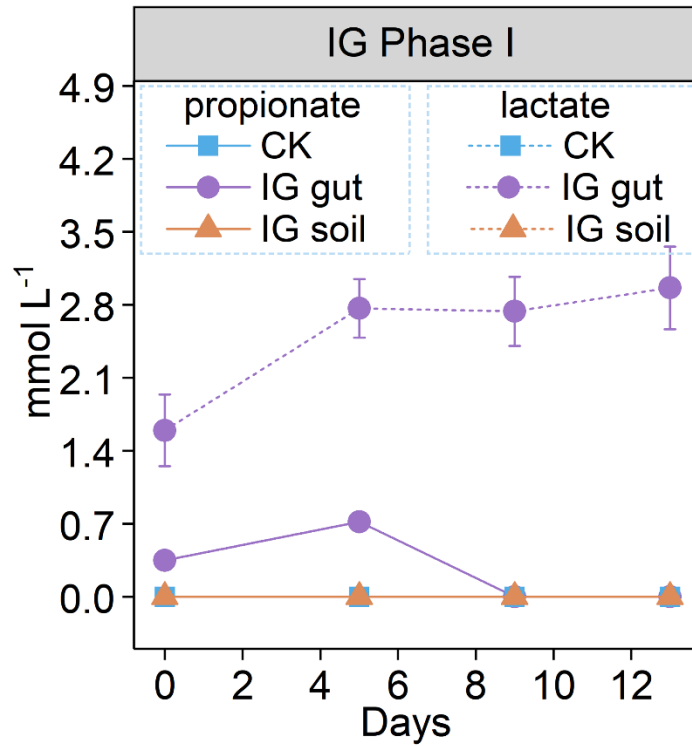


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63 **Figure S5.** RDA (redundancy analysis) of the bacterial communities at phylum level
64 (top 24) from all the setups of *in situ* soil, in vivo gut, IG soil (phase II) and IG gut
65 (phase I). The concentrations of Fe(III) ($p < 0.001$) and nitrate ($p = 0.002$) were
66 tested to significantly influence the bacterial community patterns of all setups.



67 **Figure S6.** Development of Fe(II) (a), total iron (a), NO₃⁻ (b), acetate (b) and NO₂⁻ (c)
 68 concentrations in treatment of IIG gut during the microcosmic incubation. The error
 69 bars indicate the standard deviations of three replications.



70

71 **Figure S7.** Development of lactate and propionate in treatments of IG gut and IG
 72 soil during the microcosmic incubation. The error bars indicate the standard
 73 deviations of three replications.

74 **Table S1.** The average numbers of OTUs and sequences for each sample of all
75 treatments.

Sample	Average number of sequences	Average number of OTUs
in situ soil	165548	7091
in vivo gut	126118	9242
IG soil (phase II)	136512	7983
IG gut (phase I)	133834	3500

76

77 **Table S2.** List of taxonomy at the family level corresponding to the heatmap of all
 78 the setup of *in situ* soil, *in vivo* gut, IG soil (phase II) and IG gut (phase I).

ID	Family
A100	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Oxalobacteraceae
A101	k_Bacteria; p_Planctomycetes; c_Planctomycetia; o_Gemmatales; f_Isosphaeraceae
A102	k_Bacteria; p_Firmicutes; c_Bacilli; o_Bacillales; f_Bacillaceae
A103	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Enterobacteriales; f_Enterobacteriaceae
A104	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Microbacteriaceae
A105	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; f_Xanthobacteraceae
A106	k_Bacteria; p_Firmicutes; c_Bacilli; o_Bacillales; f_Paenibacillaceae
A107	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Comamonadaceae
A108	k_Bacteria; p_Bacteroidetes; c_Cytophagia; o_Cytophagales; f_Cytophagaceae
A109	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; f_Rhizobiaceae
A110	Unassigned; Other; Other; Other; Other
A111	k_Archaea; p_Crenarchaeota; c_Thaumarchaeota; o_Nitrososphaerales; f_Nitrososphaeraceae
A112	k_Bacteria; p_Bacteroidetes; c_Flavobacteriia; o_Flavobacteriales; f_[Weeksellaceae]
A113	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Burkholderiaceae
A114	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; f_Methylobacteriaceae
A115	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pseudomonadales; f_Pseudomonadaceae
A116	k_Bacteria; p_Bacteroidetes; c_Sphingobacteriia; o_Sphingobacteriales; f_Sphingobacteriaceae
A117	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Pseudomonadales; f_Moraxellaceae
A118	k_Bacteria; p_Planctomycetes; c_Planctomycetia; o_Gemmatales; f_Gemmataceae
A119	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Methylophilales; f_Methylophilaceae
A120	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Caulobacterales; f_Caulobacteraceae
A121	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; f_
A122	k_Bacteria; p_Planctomycetes; c_Planctomycetia; o_Pirellulales; f_Pirellulaceae
A123	k_Bacteria; p_Bacteroidetes; c_Flavobacteriia; o_Flavobacteriales; f_Flavobacteriaceae
A124	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Sphingomonadales; f_Sphingomonadaceae

A125	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Burkholderiales; f_Alcaligenaceae
A126	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; f_Hyphomicrobiaceae
A128	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; f_Bradyrhizobiaceae
A130	k_Bacteria; p_Planctomycetes; c_Planctomycetia; o_Planctomycetales; f_Planctomycetaceae
A131	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhodospirillales; f_Rhodospirillaceae
A132	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Legionellales; f_Legionellaceae
A133	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhizobiales; Other
A137	k_Bacteria; p_Chloroflexi; c_Anaerolineae; o_envOPS12; f_
A138	k_Bacteria; p_Firmicutes; c_Bacilli; o_Bacillales; f_Planococcaceae
A139	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Clostridiaceae
A140	k_Bacteria; p_Acidobacteria; c_DA052; o_Ellin6513; f_
A142	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Micrococcaceae
A143	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Aeromonadales; f_Aeromonadaceae
A145	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Streptomycetaceae
A146	k_Bacteria; p_Actinobacteria; c_Thermoleophilia; o_Gaiellales; f_Gaiellaceae
A147	k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Syntrophobacterales; f_Syntrophobacteraceae
A151	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Xanthomonadales; f_Sinobacteraceae
A152	k_Bacteria; p_Acidobacteria; c_Solibacteres; o_Solibacterales; f_Solibacteraceae
A153	k_Bacteria; p_Bacteroidetes; c_[Saprosirae]; o_[Saprosirales]; f_Chitinophagaceae
A157	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Peptococcaceae
A158	k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Myxococcales; f_
A162	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_[Tissierellaceae]
A164	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhodospirillales; f_Acetobacteraceae
A166	k_Bacteria; p_Firmicutes; c_Bacilli; o_Bacillales; f_Thermoactinomycetaceae
A168	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Nocardioideaceae
A169	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Ruminococcaceae
A170	k_Bacteria; p_Acidobacteria; c_Acidobacteria-6; o_iii1-15; f_
A171	k_Bacteria; p_Nitrospirae; c_Nitrospira; o_Nitrospirales; f_0319-6A21

A173	k_Bacteria; p_Acidobacteria; c_Solibacteres; o_Solibacterales; f_
A177	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_
A179	k_Bacteria; p_Acidobacteria; c_Acidobacteriia; o_Acidobacteriales; f_Acidobacteriaceae
A184	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Intrasporangiaceae
A186	k_Bacteria; p_Chloroflexi; c_Ellin6529; o_; f_
A189	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Syntrophomonadaceae
A190	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Rhodocyclales; f_Rhodocyclaceae
A192	k_Bacteria; p_Nitrospirae; c_Nitrospira; o_Nitrospirales; f_Nitrospiraceae
A193	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Micromonosporaceae
A197	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Nocardiaceae
A199	k_Bacteria; p_Actinobacteria; c_Thermoleophilia; o_Solirubrobacterales; f_
A202	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Ellin6067; f_
A205	k_Bacteria; p_Actinobacteria; c_Actinobacteria; o_Actinomycetales; f_Mycobacteriaceae
A206	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_MND1; f_
A208	k_Bacteria; p_Chloroflexi; c_Thermomicrobia; o_JG30-KF-CM45; f_
A212	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Peptostreptococcaceae
A215	k_Bacteria; p_Acidobacteria; c_Acidobacteriia; o_Acidobacteriales; f_Koribacteraceae
A221	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_
A231	k_Bacteria; p_Actinobacteria; c_MB-A2-108; o_0319-7L14; f_
A233	k_Bacteria; p_Tenericutes; c_Mollicutes; o_; f_
A234	k_Bacteria; p_Actinobacteria; c_Thermoleophilia; o_Gaiellales; f_
A236	k_Bacteria; p_Proteobacteria; c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae
A237	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Dehalobacteriaceae
A240	k_Bacteria; p_Firmicutes; c_Bacilli; o_Bacillales; f_Alicyclobacillaceae
A241	k_Bacteria; p_Gemmatimonadetes; c_Gemm-1; o_; f_
A247	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae
A248	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_; f_
A250	k_Bacteria; p_Acidobacteria; c_[Chloracidobacteria]; o_RB41; f_
A251	k_Bacteria; p_Actinobacteria; c_Acidimicrobiia; o_Acidimicrobiales; f_

A258	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Christensenellaceae
A259	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_[Mogibacteriaceae]
A260	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Caldicoprobacteraceae
A262	k_Bacteria; p_Firmicutes; c_Clostridia; o_OPB54; f_
A275	k_Bacteria; p_WS3; c_PRR-12; o_Sediment-1; f_PRR-10
A282	k_Bacteria; p_Tenericutes; c_Mollicutes; o_RF39; f_
A285	k_Bacteria; p_Acidobacteria; c_Acidobacteria-6; o_iii1-15; f_RB40
A289	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Neisseriales; f_Neisseriaceae
A290	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Gracilibacteraceae
A299	k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Desulfuromonadales; Other
A301	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Thiotrichales; f_Piscirickettsiaceae
A302	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_SC-I-84; f_
A308	k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Desulfuromonadales; f_Geobacteraceae
A310	k_Bacteria; p_Plactomycetes; c_Phycisphaerae; o_WD2101; f_
A313	k_Bacteria; p_Actinobacteria; c_Acidimicrobiia; o_Acidimicrobiales; f_C111
A314	k_Bacteria; p_Firmicutes; c_Clostridia; o_; f_
A315	k_Bacteria; p_Tenericutes; c_RF3; o_ML615J-28; f_
A323	k_Bacteria; p_Proteobacteria; c_Gammaproteobacteria; o_Legionellales; f_Coxiellaceae
A324	k_Bacteria; p_Firmicutes; c_Clostridia; o_Thermoanaerobacterales; f_
A326	k_Bacteria; p_Actinobacteria; c_Thermoleophilia; o_Solirubrobacterales; f_Solirubrobacteraceae
A331	k_Bacteria; p_Proteobacteria; c_Betaproteobacteria; o_Nitrosomonadales; f_Nitrosomonadaceae
A338	k_Bacteria; p_Verrucomicrobia; c_Opitutae; o_Opitutales; f_Opitutaceae
A346	k_Bacteria; p_Actinobacteria; c_Acidimicrobiia; o_Acidimicrobiales; f_EB1017
A353	k_Bacteria; p_Acidobacteria; c_Acidobacteria-6; o_iii1-15; f_mb2424
A356	k_Bacteria; p_Acidobacteria; c_Holophagae; o_Holophagales; f_Holophagaceae
A357	k_Bacteria; p_Bacteroidetes; c_Flavobacteriia; o_Flavobacteriales; f_Cryomorphaceae
A360	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; Other
A363	k_Bacteria; p_Bacteroidetes; c_Sphingobacteriia; o_Sphingobacteriales; f_
A376	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_Symbiobacteriaceae

A377	k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Desulfovibrionales; f_Desulfovibrionaceae
A378	k_Bacteria; p_Firmicutes; c_Clostridia; o_Clostridiales; f_EtOH8
A381	k_Bacteria; p_Firmicutes; c_Clostridia; o_MBA08; f_
A383	k_Bacteria; p_Firmicutes; c_OPB54; o_; f_
A397	k_Bacteria; p_Chloroflexi; c_Anaerolineae; o_Caldilineales; f_Caldilineaceae
A399	k_Bacteria; p_Cyanobacteria; c_ML635J-21; o_; f_
A416	k_Bacteria; p_Proteobacteria; c_Deltaproteobacteria; o_Bdellovibrionales; f_Bacteriovoraceae
A440	k_Bacteria; p_Bacteroidetes; c_[Saprosirae]; o_[Saprosirales]; f_Saprosiraceae
A492	k_Bacteria; p_Verrucomicrobia; c_Opitutae; o_; f_

79 **Table S3.** Primers of denitrification genes and thermal cycling conditions.

Genes	Primers	Thermal cycling conditions	References
<i>16S rRNA</i>	515F GTGCCAGCMGCCGCGG	95 °C 5 min 95 °C 30 s 58 °C 30 s 72 °C 30 s 72 °C 10 min 4 °C ∞	30 cycles 1
	907R CCGTCAATTCMTTTRAGTTT		
<i>narG</i>	1960F TAYGTSGGSCARGARAA	95 °C 5 min 95 °C 30 s 55 °C 40 s 72 °C 40 s 72 °C 10 min 4 °C ∞	30 cycles 2
	2650R TTYTCRTACCABGTAGC		
<i>nirK</i>	nirK1F GGMATGGTKCCSTGGCA	95 °C 5 min 95 °C 30 s 57 °C 40 s 72 °C 40 s 72 °C 10 min 4 °C ∞	30 cycles 3,4
	nirK5R AACTTGCCGTVGYCCAGAC		
<i>nirS</i>	nirScd3Af TAYCACCCSGARCCGCGCGT	95 °C 5 min 95 °C 5 s 54 °C 40 s 72 °C 35 s 72 °C 10 min 4 °C ∞	30 cycles 3,4
	nirSR3cd AGKCGTTGACTTKCCGGTTCG		
<i>nosZ</i>	nosZF CCCGCTGCACACCRCTTCGA	95 °C 5 min 95 °C 15 s 60 °C 15 s 72 °C 30 s 72 °C 10 min 4 °C ∞	30 cycles 3,4
	nosZR CGTCGCCSGAGATGTCGATCA		

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