



Effects of Nitrate Addition on Rumen Fermentation, Bacterial Biodiversity and Abundance

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- Supplementary Data -

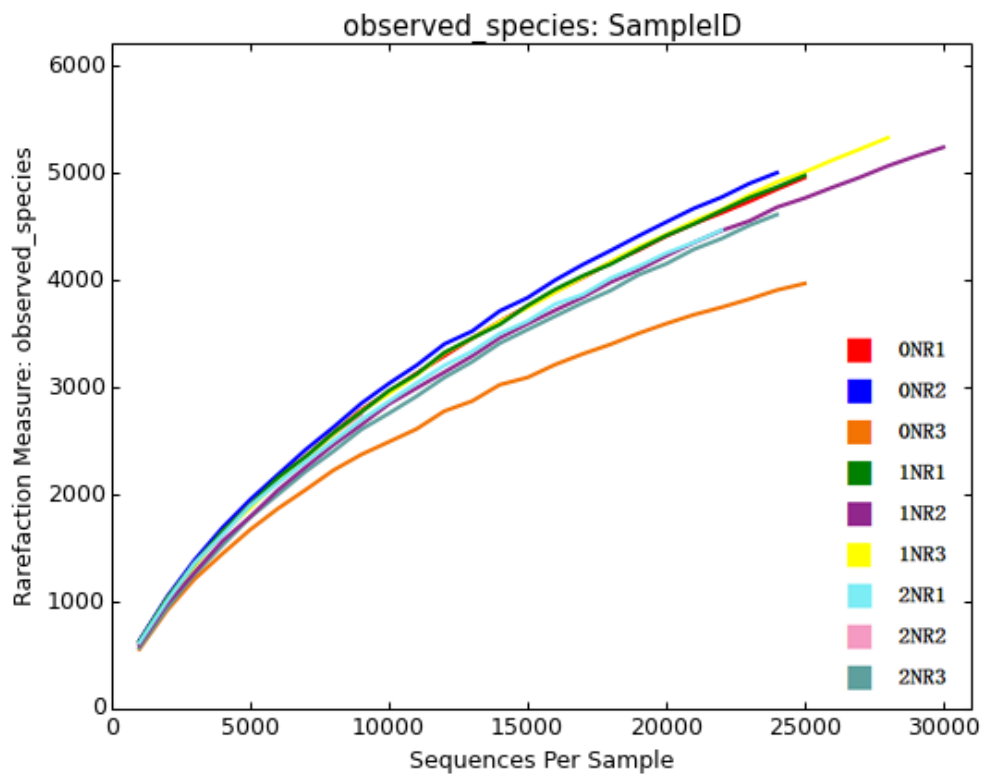
Supplementary Table S1. Primers used in real-time PCR

	F/R	Premier sequence	References
<i>S. ruminantium</i>	F	CGCCGATGCGGTACTAGGT	Lin et al (2013)
	R	CCAGAGCTTCCAGGCTTTG	
<i>V. parvula</i>	F	TCCTCTTCTTCGGAAGCCAGA	Lin et al. (2013)
	R	AGAGAGTGTTTCTCGGGTTTGC	
<i>W. succinogenes</i>	F	CCCGAGGGAACGCTCAA	Lin et al. (2013)
	R	CCTCAAGGTCTCGCATGA	
<i>C. fetus</i>	F	GATAAGAGAGTGCTAGCTTGCTAGAA	Lin et al. (2013)
	R	GTTAGCAACTAAATACGTGGGTTG	
<i>M. succiniciproducens</i>	F	TCGGTGACGAGGAAGGTGTT	Lin et al. (2013)
	R	CTGTGATTAACGTCAATTGCTTGTC	
<i>F. succinogenes</i>	F	GTTCGGAATTACTGGGCGTAAA	Denman and McSweeney (2006)
	R	CGCCTGCCCTGAACTATC	
<i>R. ablus</i>	F	TGTTAACAGAGGGAAGCAAAGCA	Stevenson and Weimer (2007)
	R	TGCAGCCTACAATCCGAACTAA	
<i>R. flavefaciens</i>	F	TGGCGGACGGGTGAGTAA	Stevenson and Weimer (2007)
	R	TTACCATCCGTTTCCAGAAGCT	

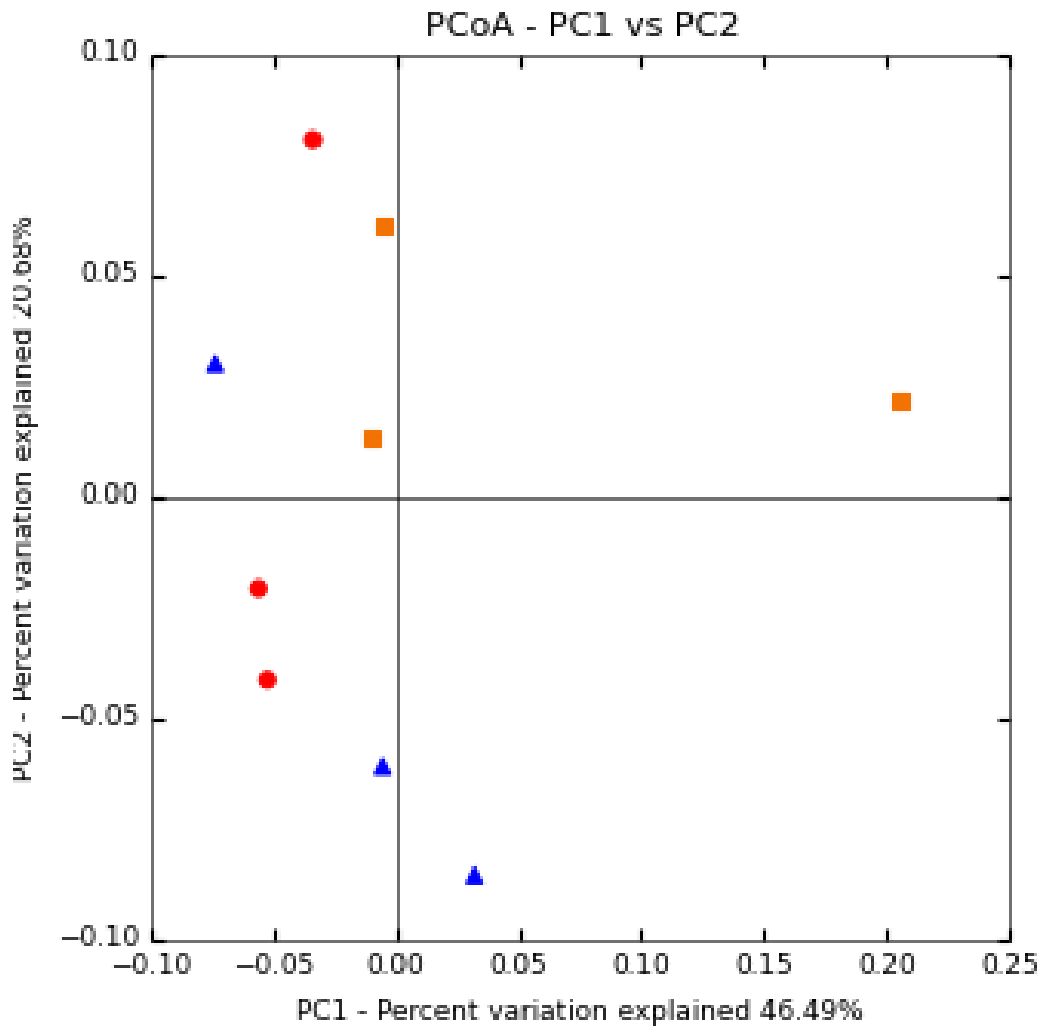
Supplementary Table S2. Comparison of reads and OTUs abundance and alpha diversity of 16S rRNA gene libraries from Ion Torrent sequencing analysis

Sample ID	Sequence count	OTUs count	Goods' Coverage
0NR1	25195	2865	0.8169
0NR2	24794	2972	0.8073
0NR3	25122	2417	0.8588
1NR1	25510	2847	0.8183
1NR2	30822	2729	0.8208
1NR3	28430	2830	0.8178
2NR1	22843	2810	0.8257
2NR2	9573	2837	0.8276
2NR3	24154	2694	0.8266

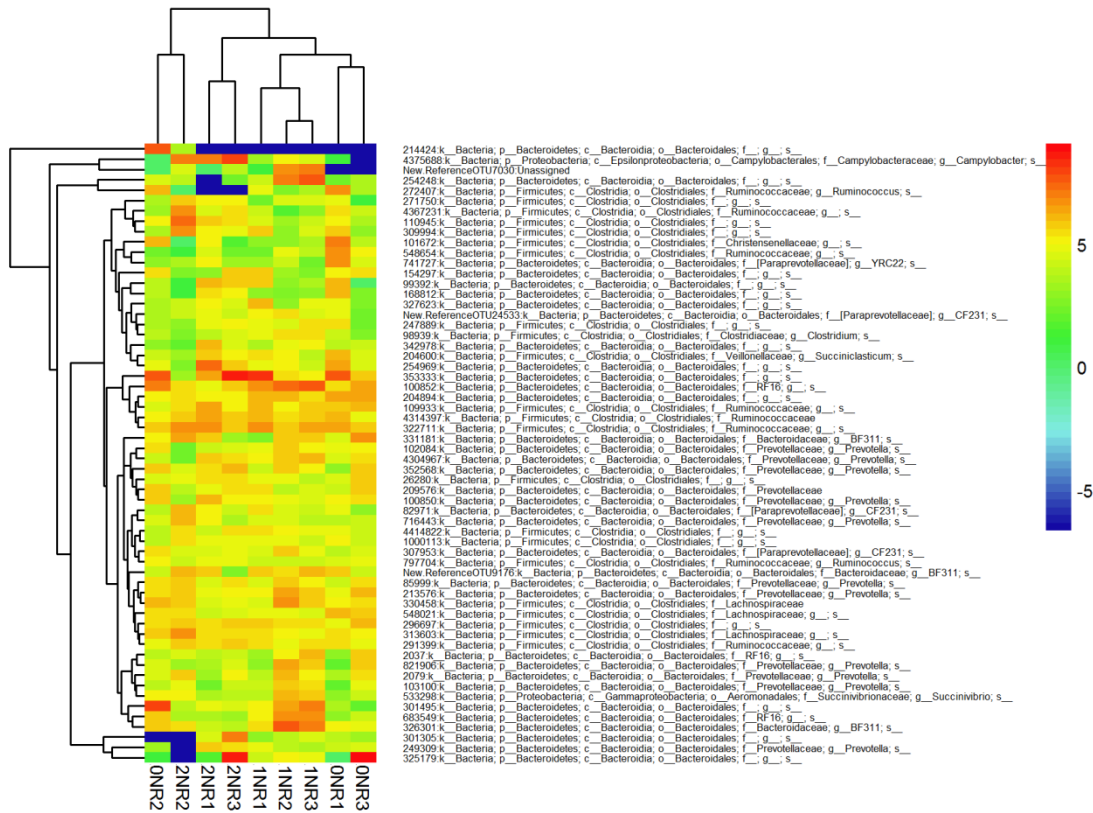
0NR: control; 1NR: 1% nitrate; 2NR: 2% nitrate



Supplementary Figure S1. Figure 1 Rarefaction curve for each sample with a cutoff level of 0.03.



Supplementary Figure S2. Principal coordinates analysis showing relationships among bacterial populations in rumen of steers fed different nitrate levels. The PCoA plot was generated using the weighted UniFrac method. ■ Samples from 0NR; ● samples from 1NR; ▲ samples from 2NR.



Supplementary Figure S3. Double dendrogram of bacteria present in the rumen of steers fed different nitrate levels.