Appendix A

Table A1 Oligonucleotide primers for PCR amplification via quantitative polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE) techniques

Techniques	Target gene	Primer	Sequence (5′–3′)	Annealing Temp. (℃)	Reference
qPCR	All bacteria	338F	ACTCCTACGGGAGGCAGC	55	Lane (1991)
		518R	TACCGCGGCTGCTGGCAC		Muyzer et al. (1993)
	amoA-AOB	amoA-1F	GGGGTTTCTACTGGTGGT	56	Rotthauwe et al. (1997)
		amoA-2R	CCCCTCKGSAAAGCCTTCTTC		
	Nitrobacter	Nb1000F	TGCGACCGGTCATGG	55	Wang et al. (2012)
	16S rDNA	1387R	GGGCGGWGTGTACAAGGC		
	Nitrospira	NSR1113F	CCTGCTTTCAGTTGCTACCG	55	Wang et al. (2012)
	16S rDNA	NSR1264R	GTTTGCAGCGCTTTGTACCG		Yapsakli et al. (2011)
	nirS	cd3aF	GTSAACGTSAAGGARACSGG	59	Michotey et al. (2000)
		R3cd	GASTTCGGRTGSGTCTTGA		Throbäck et al. (2004)
	nirK	F1aCu	ATYGGCGGVCAYGGCGA	59	Hallin and Lindgren (1999)
		R3Cu	GCCTCGATCAGRTTRTGGTT		
DGGE	*AOB	1st step: CTO189fABC	GGAGRAAAGYAGGGGATCG	57	Kowalchuk et al. (1997)
	16S rRNA		CTAGCYTTGTAGTTTCAAACGC		
		CTO654r			
		2nd step: 357f-GC	CCTACGGGAGGCAGCAG	55	Muyzer et al. (1993)
		518r	ATTACCGCGGCTGCTGG		
	nirS	cd3aF	GTSAACGTSAAGGARACSGG	60	Throbäck et al. (2004)
		R3cd-GC	GASTTCGGRTGSGTCTTGA		
	nirK	F1aCu	ATYGGCGGVCAYGGCGA	60	Throbäck et al. (2004)
		R3Cu-GC	GCCTCGATCAGRTTRTGGTT		

Notes: * nested PCR protocol.

The GC clamp sequence: 5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG-3′

Appendix B

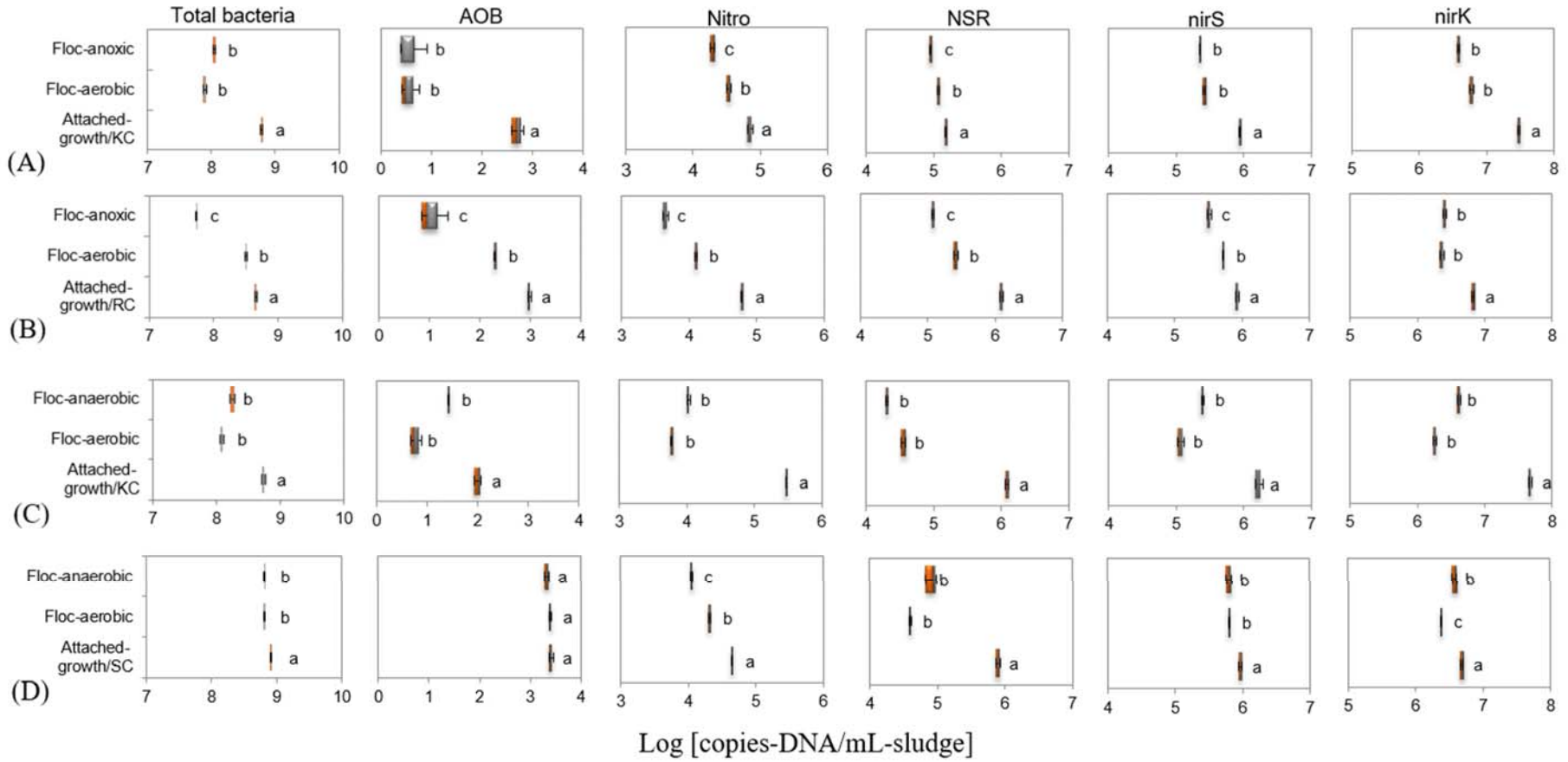

Fig. B1 Means ± SE for total bacteria, AOB, NOB (*Nitro* and NSR genes), and DNB (*nirS* and *nirK* genes) at Broomfield (A), Fukuoka (B), South Adams County (C), and Saga (D) WRRFs. Letters *a*, *b* and *c* within each sample reflect significant differences based on one-way ANOVA and Fisher's LSD Method, significance level $\alpha = 0.05$

Appendix references

Hallin S, Lidgren R E (1999). PCR detection of genes encoding nitrite reductase in denitrifying bacteria. Applied and Environmental Microbiology, 65(4): 1652–1657

Kowalchuk G A, Stephen R J, Boer W D, Prosser J I, Embley T M, Woldendorp J W (1997). Analysis of ammonia-oxidizing bacteria of the β subdivision of the class *Proteobacteria* in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. Applied and Environmental Microbiology, 63(4): 1489–1497

Lane D (1991). 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, editors. Nucleic Acid Techniques in Bacterial Systematics. 1st ed. New York: John Wiley and Sons, 115–175

Michotey V, Mejean V, Bonin P (2000). Comparison of methods for quantification of cytochrome cd_1–denitrifying bacteria in environmental marine samples. Applied and Environmental Microbiology, 66(4): 1564–1571

Muyzer G, de Waal E C, Uitterlinden A G (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Applied and Environmental Microbiology, 59(3): 695–700

Rotthauwe J H, Witzel K P, Liesack W (1997). The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Applied and Environmental Microbiology, 63(12): 4704–4712

Throbäck I N, Enwall K, Jarvis A, Hallin S (2004). Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiology Ecology, 49(3): 401–417

Wang F, Liu Y, Wang J H, Zhang Y L, Yang H Z (2012). Influence of growth manner on nitrifying bacterial communities and nitrification kinetics in three lab-scale bioreactors. Journal of Industrial Microbiology and Biotechnology, 39: 595–604

Yapsakli K, Aliyazicioglu C, Mertoglu B (2011). Identification and quantitative evaluation of nitrogen-converting organisms in a full-scale leachate treatment plant. Journal of Environmental Management, 92(3): 714–723