

Abundance and diversity of *Planctomycetes* in a Tyrrhenian coastal system of central Italy

Ilaria Pizzetti^{1,2}, Angélique Gobet³, Bernhard M. Fuchs¹, Rudolf Amann¹, Stefano Fazi^{2,*}

¹Max Planck Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany

²Water Research Institute (IRSA-CNR), via Salaria km 29.300, 00015 Monterotondo, Roma, Italy

³Station Biologique de Roscoff, UMR 7144, CNRS et Université Pierre et Marie Curie (UPMC), Place Georges Teissier, 29680 Roscoff, France

*Corresponding author. Email: fazi@irsa.cnr.it

Aquatic Microbial Ecology 65: 129–141 (2011)

Supplementary material

Fig. S1. Comparison of samples fixed with formaldehyde (FA; 2% final concentration) in 2003 with samples from 2008 fixed with paraformaldehyde (PFA; 1% final concentration). *Planctomycetes* absolute counts after fluorescence *in situ* hybridization (FISH) and catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH). Each bar represents the mean of counts at site SAB2 in the surface and bottom of the lake (see Fig. 1). Only in 2008 FISH samples were false-positive signals detected by the NON338 probe. Probe cell counts from the negative control were subtracted from counts with the probe PLA46

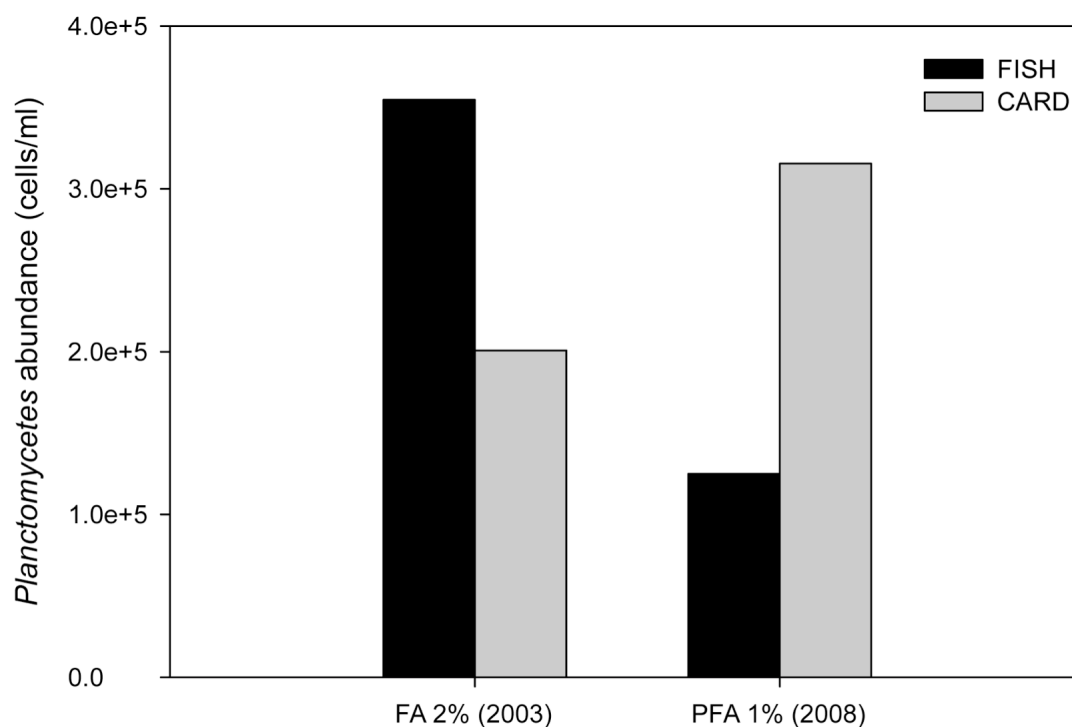


Fig. S2. Partial least-square regression (PLSR) model represented by the first 2 latent variables (LV) as 2 axes. (A) Scores of individual samples, (B) loadings for the *X*-descriptor (environmental) variables and the *Y*-response (*Planctomycetes* absolute abundance) variables. Chl *a*: chlorophyll *a*; DO: dissolved oxygen; DOC: dissolved organic carbon. Samples: 1: SAB1; 2: SAB2; 3: SAB3; 4: SAB4; J1: 8 July; J2: 29 July; A: August; S: September; O: October; N: November; D: December

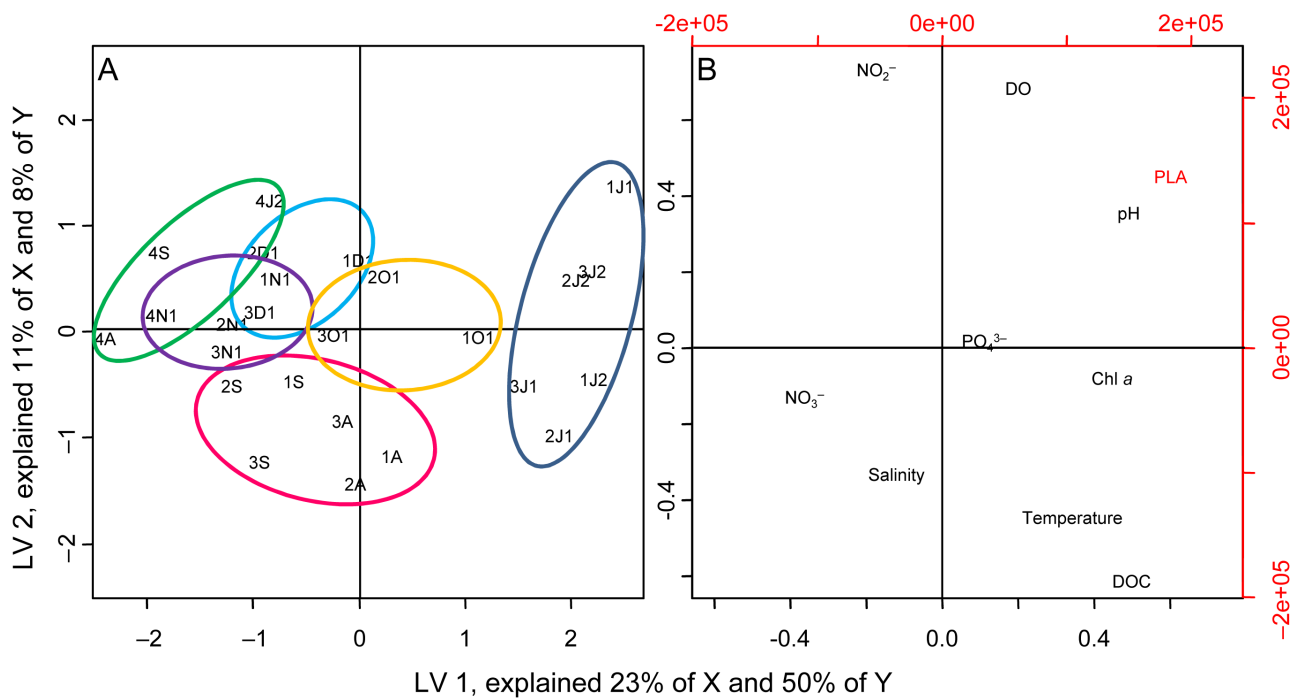


Fig. S3. Rarefaction curves of almost full length 16S rRNA sequences (>1200 nucleotides) recovered from the surface and bottom of the lake on 15 September 2008. The expected numbers of operational taxonomic units, OTUs, was calculated using cut-off values of sequence identity for species (97%) and genus (95%) levels

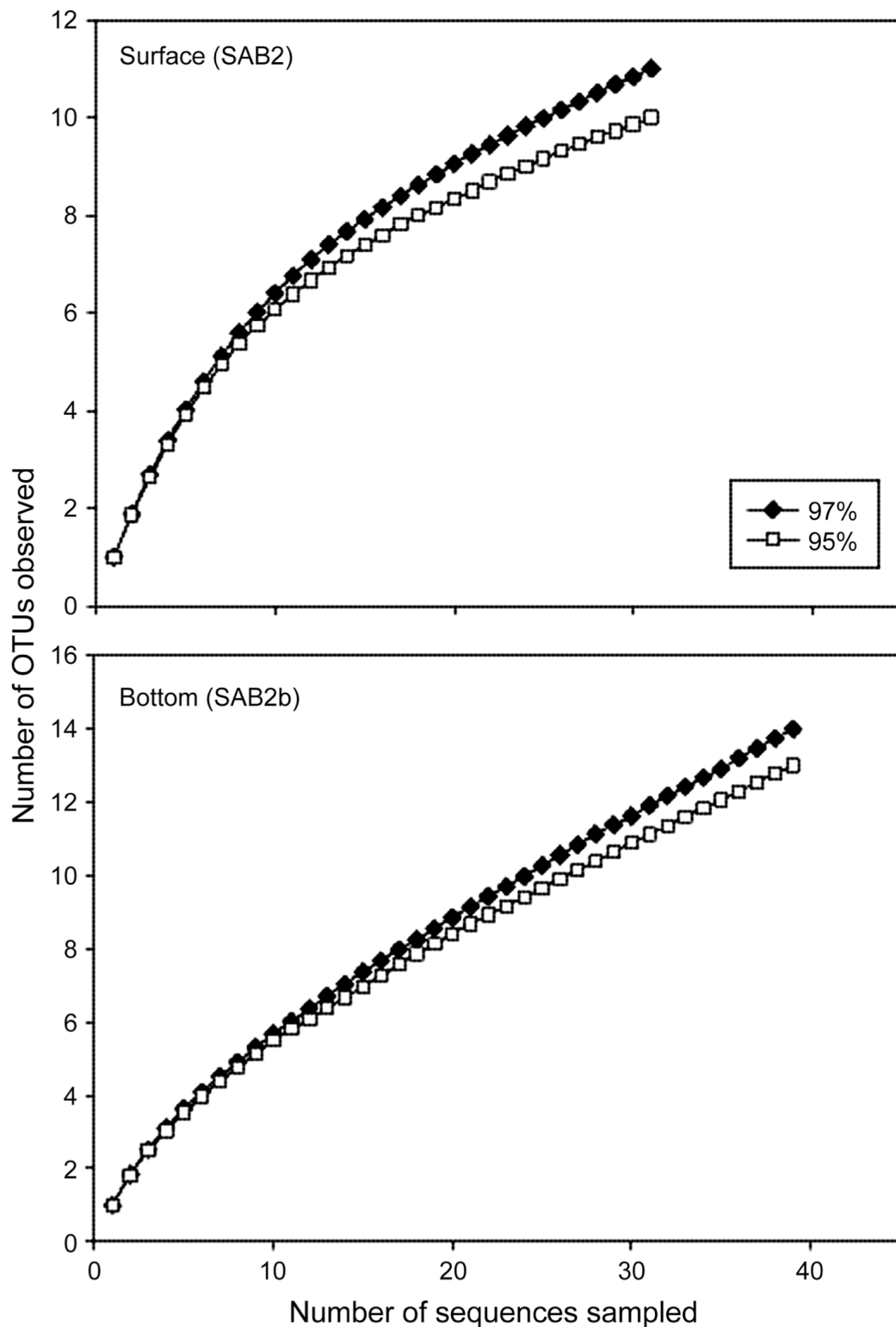


Fig. S4. Epifluorescence micrographs of bacteria from the surface of Lago di Paola. Blue: DAPI signals of DNA containing cells; green: signals of catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH)-positive cells using the probes: (a,b) PLA46 (10 ml), (c) PirF85 (50 ml), (d) PlaS2114 (50 ml), (e) PirE62 (50 ml) and (f,g) uPlaB440 (50 ml). Scale bar = 5 μ m

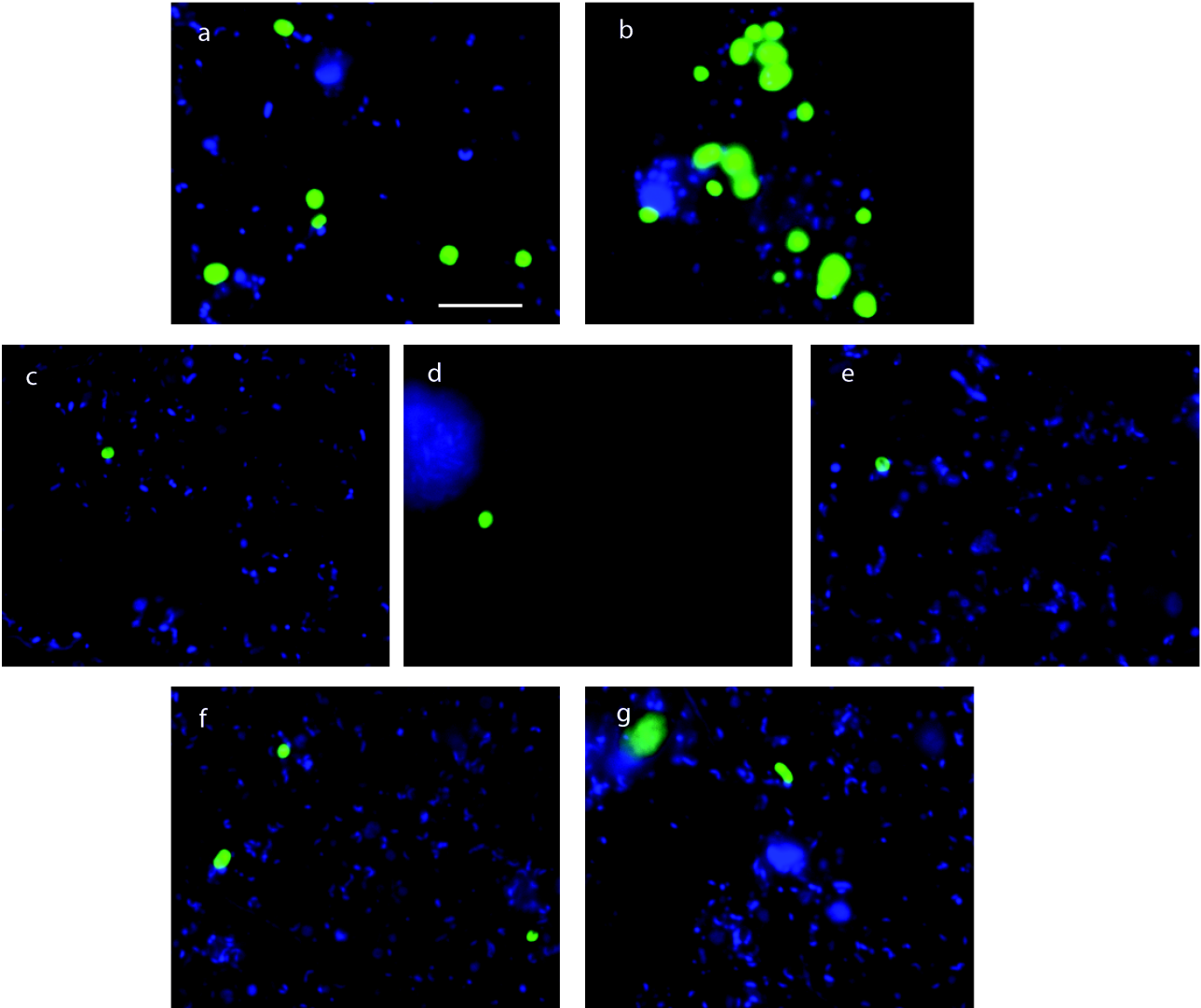


Table S1. Specificity of probes PirF85 (*Pirellula*-related group F), PirE62 (*Pirellula*-related group E), PlaS2114 (*Planctomyces*-related group A) and uPlaB440 (uncultured *Planctomyces* group B). Dots indicate full identity to the probe target sequence. Uppercase letters indicate nucleotide changes leading to strong mismatches; lowercase letters indicate changes leading to weak mismatches. The isolation source and the weighted mismatches of non-target organisms are represented

Probe	Isolation source	Weighted mismatches	Probe (3'–5')	Target (5'–3')
PirF85			Probe (3'–5')	ACGTAATTCTGCCTGTCG
<i>Pirellula</i> -related group F			Target (5'–3')	UGCAUUAAGACGGACAGC
Uncultured <i>Chloroflexi</i>	Rhizosphere biofilm (reactor)	1.1		g..g..gg.g.....
Uncultured <i>Chloroflexi</i>	Sediment	1.4		C..g.....g...ug..
Eukarya-Brachiopoda	Seawater	1.9		C..Cg.....
PirE62			Probe (3'–5')	AGCACGCTCTTCGAAGGA
<i>Pirellula</i> -related group E			Target (5'–3')	UCGUGCGAGAAGCUCCU
Uncultured <i>Planctomycetales</i>	Sediment	0.3	C
Uncultured <i>Planctomycetales</i>	Seafloor lavas	0.7	uu.
Uncultured <i>Planctomycetales</i>	Microbial mat/ sediment/seafloor lavas	1.0		..A.....
Uncultured <i>Planctomycetales</i>	Karst spring water	1.3	C.u.
Uncultured <i>Firmicutes/Acidobacteria</i>	Animal feces/wastewater sludge/coral	1.5		..A.....ug
PlaS2114			Probe (3'–5')	CATCATTCGGTAGCCTTG
<i>Planctomyces</i> -related group A			Target (5'–3')	GUAGUAAGGCAUCGGAAC
Uncultured <i>Planctomycetales</i>	Soil/asphalt seep/ ergovaline treatment gut	0.3	g.....
Uncultured <i>Gammaproteobacteria</i>	Acid mine drainage/acidic environment	0.9		Ug.....g...
Uncultured <i>Gammaproteobacteria</i>	Acid mine drainage/wetland/mine tailings	0.9		Ug.....u
<i>Kodamaea ohmeri</i> (Eukarya-Fungi)	Fish gut	1.0		U....g.....g.u
Uncultured <i>Planctomycetales</i>	Wastewater treatment plant	1.9	U.....
uPlaB440			Probe (3'–5')	GCAACCCCTCCTTTACGT
Uncultured <i>Planctomyces</i> group B			Target (5'–3')	CGUUGGGGAGGAAAUGCA
Uncultured <i>Planctomycetales</i>	Termite gut	0.3	u.
Uncultured <i>Planctomycetales</i>	Wastewater sludge	0.8		..A.....g
Uncultured <i>Treponema</i> sp. (Spirochetes)	Termite gut	0.9		a.....g.....C
Uncultured <i>Planctomycetales</i>	Freshwater lake/sediment	1.0		..A.....
Uncultured <i>Pseudomonas</i> sp. (<i>Gammaproteobacteria</i>)	Spacecraft room	1.0		a.....gg...
Uncultured <i>Planctomycetales</i>	Mangrove forest/wastewater	1.2		.Ag.....
Uncultured <i>Treponema</i> sp. (Spirochetes)	Termite gut	1.3		a.g.....g.....C

Table S2. *Planctomycetes*-specific oligonucleotide probes. FA: formamide concentration (v/v) in CARD-FISH hybridization buffer

Probe	Target organism	Sequence (5' → 3')	FA (%)	Source
PLA46	<i>Planctomycetes</i>	GACTTGCATGCCTAATCC	30	Neef et al. (1998)
uPlaB440	Uncultured <i>Planctomycetes</i> group B	TGCATTTCTCCCAACG	30	Pizzetti et al. (2011)
PirF85	<i>Pirellula</i> -related group F	GCTGTCCGTCTTAATGCA	45	Present study
PirE62	<i>Pirellula</i> -related group E	AGGAAGCTTCTCGCACGA	20	Present study
PlaS2114	<i>Planctomycetes</i> -related group A	GTTCCGATGCCTTACTAC	30	Present study

Table S3. *Planctomycetes* relative and absolute abundances (means ± SD of 3 replicates) and total microbial abundances between July and December 2003

Date	Station	<i>Planctomycetes</i> relative abundance (%)	<i>Planctomycetes</i> absolute abundance ($\times 10^5$ cells ml ⁻¹)	Total cell abundance ($\times 10^7$ cells ml ⁻¹)
08/07/03	SAB1	4.7 ± 0.4	15.4 ± 1.4	3.3
	SAB2	1.5 ± 0.4	5.2 ± 1.4	3.4
	SAB2b	1.5 ± 0.1	3.1 ± 0.3	2.1
	SAB3	1.8 ± 0.2	5.1 ± 0.7	2.9
	SAB4	0.8 ± 0.3	0.37 ± 0.1	0.47
29/07/03	SAB1	1.7 ± 1.3	2.9 ± 2.3	1.7
	SAB2	5.4 ± 2.1	12.6 ± 4.9	2.3
	SAB3	3.4 ± 3.4	5.9 ± 5.8	1.7
	SAB4	1.1 ± 0.2	0.35 ± 0.06	0.32
31/08/03	SAB1	1.2 ± 0.5	1.7 ± 0.7	1.4
	SAB2	1.5 ± 0.7	1.7 ± 0.7	1.1
	SAB2b	2.1 ± 0.4	2.8 ± 0.6	1.4
	SAB3	0.9 ± 0.6	1.1 ± 0.7	1.3
	SAB4	1.6 ± 0.9	0.56 ± 0.3	0.35
23/09/03	SAB1	0.8 ± 0.5	1.2 ± 0.8	1.5
	SAB2	0.8 ± 0.2	1.4 ± 0.3	1.7
	SAB3	2.3 ± 0.9	4.0 ± 1.5	1.7
	SAB4	2.0 ± 0.8	0.18 ± 0.07	0.091
13/10/03	SAB1	1.9 ± 0.2	3.3 ± 0.3	1.7
	SAB2	2.8 ± 0.7	5.9 ± 1.6	2.1
	SAB2b	1.4 ± 0.9	1.9 ± 1.3	1.4
	SAB3	3.2 ± 1.4	6.2 ± 2.7	2.0
	SAB4	1.8 ± 1.1	0.23 ± 0.1	0.13
10/11/03	SAB1	1.4 ± 0.6	1.9 ± 0.9	1.4
	SAB2	2.0 ± 0.8	2.8 ± 1.1	1.4
	SAB2b	1.7 ± 0.3	2.2 ± 0.4	1.3
	SAB3	2.2 ± 1.4	2.8 ± 1.8	1.3
	SAB4	1.0 ± 0.8	0.098 ± 0.09	0.10
15/12/03	SAB1	1.8 ± 1.1	2.1 ± 1.3	1.2
	SAB2	1.5 ± 0.7	1.7 ± 0.7	1.1
	SAB2b	1.5 ± 0.4	1.4 ± 0.3	0.89
	SAB3	2.0 ± 0.3	1.2 ± 0.2	0.62
	SAB4	0.9 ± 0.2	0.12 ± 0.02	0.13

Table S4. Spearman rank correlation between *Planctomycetes* absolute abundance and environmental parameters measured at SAB1–SAB4 at all 28 sampling dates between January and December 2003. Chl *a*: chlorophyll *a*; DO: dissolved oxygen; DOC: dissolved organic carbon; PLA46: probe targeting *Planctomycetes*. *: $p \leq 0.05$; **: $p \leq 0.01$. **Bold** values indicate statistically significant r_s at the Bonferroni-corrected level $p \leq 0.001$ ($n = 45$ simultaneous independent comparisons)

	PLA46	Temperature	Salinity	DO	pH	Chl <i>a</i>	DOC	PO ₄ ³⁻	NO ₂ ⁻	NO ₃ ⁻
PLA46										
Temperature										
Salinity	-0.56**									
DO	0.48*									
pH	0.38*			0.71						
Chl <i>a</i>	0.65		-0.70	0.52**						
DOC	0.62	0.62	-0.42*			0.60				
PO ₄ ³⁻				-0.44*						
NO ₂ ⁻		-0.86					-0.49**			
NO ₃ ⁻		-0.64	-0.43*						0.77	