The ISME Journal (2014) 8, 1476–1491

www.nature.com/ismej

ORIGINAL ARTICLE

A microarray for assessing transcription from pelagic marine microbial taxa

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Metagenomic approaches have revealed unprecedented genetic diversity within microbial communities across vast expanses of the world's oceans. Linking this genetic diversity with key metabolic and cellular activities of microbial assemblages is a fundamental challenge. Here we report on a collaborative effort to design MicroTOOLs (Microbiological Targets for Ocean Observing Laboratories), a high-density oligonucleotide microarray that targets functional genes of diverse taxa in pelagic and coastal marine microbial communities. MicroTOOLs integrates nucleotide sequence information from disparate data types: genomes, PCR-amplicons, metagenomes, and metatranscriptomes. It targets 19 400 unique sequences over 145 different genes that are relevant to stress responses and microbial metabolism across the three domains of life and viruses. MicroTOOLs was used in a proof-of-concept experiment that compared the functional responses of microbial communities following Fe and P enrichments of surface water samples from the North Pacific Subtropical Gyre. We detected transcription of 68% of the gene targets across major taxonomic groups, and the pattern of transcription indicated relief from Fe limitation and transition to N limitation in some taxa. *Prochlorococcus* (eHLI), *Synechococcus* (sub-cluster 5.3) and Alphaproteobacteria SAR11 clade (HIMB59) showed the strongest responses to the Fe enrichment. In addition, members of uncharacterized lineages also responded. The MicroTOOLs microarray provides a robust tool for comprehensive characterization of major functional groups of microbes in the open ocean, and the design can be easily amended for specific environments and research questions.
The ISME Journal (2014) **8,** 1476–1491; doi:10.1038/ismej.2014.1; published online 30 January 2014
Subject Category: Integrated genomics and post-genomics approaches in microbial ecology
Keywords: marine; microbial; microarray; transcription; molecular

Introduction

Marine microbial communities are complex, composed of diverse groups of Bacteria, Archaea, Eukaryotes and viruses. Molecular techniques frequently used in marine microbial ecology have shown strain-specific differences in genetic capabilities and transcriptional responses among the most abundant representatives of microbial communities (Fuhrman *et al.*, 2006; Giovannoni and Vergin, 2012). Clades of *Prochlorococcus* in the North Atlantic have more phosphorus-acquisition strategies than clades in the North Pacific, as an adaptation to chronic phosphate limitation (Coleman and Chisholm, 2010). Coastal clades of *Synechococcus* have higher number of regulatory systems and the use for metals than open ocean clades, the latter being adapted to relatively constant oligotrophic conditions (Palenik *et al.*, 2006). To link ocean processes to microbial metabolism and to build better models for predicting responses to future ocean states (Azam and Malfatti, 2007), in light of this strain-level heterogeneity, new research tools are needed that assess individual and microbial community responses.

Microarray technology can complement more commonly used molecular techniques, such as PCR and

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Received 29 May 2013; revised 16 December 2013; accepted 31 December 2013; published online 30 January 2014

next-generation sequencing, to provide cost-effective high-throughput gene and transcript detection from several organisms in a single sample. Microarrays have the advantages of sample replication, standardization and robust interpretations of strain-level variation in functional gene transcriptional patterns and lend themselves to better comparative quantification of specific genes and transcripts, especially in rare organisms.

Phylogenetic and functional microarrays have been developed and used for identification of microorganisms and their activity in diverse environments. The most comprehensive microbial functional microarray to date, the GeoChip 4.0, targets >10 000 sequences represented by 150 genes mainly from soil microbial communities and reduction–oxidation processes (He *et al.*, 2007, 2010; Bai *et al.*, 2013; Zhou *et al.*, 2013). In addition to contaminated soils, acid mine drainage sites and Antarctic soils (Yergeau *et al.*, 2007; Mason *et al.*, 2010; Xie *et al.*, 2011), the GeoChip has been applied to detect microbial DNA and RNA in the marine environment (Lu *et al.*, 2012; Wawrik *et al.*, 2012). Aside from the PhyloChip, which targets 16S rRNA genes (Brodie *et al.*, 2006), existing microarrays target a specific genus (Rinta-Kanto *et al.*, 2011), a particular process (Tiquia *et al.*, 2004; Moisander *et al.*, 2006, 2007; Ward *et al.*, 2007; Bulow *et al.*, 2008; Wu *et al.*, 2008; Bouskill *et al.*, 2011; Abell *et al.*, 2012) or ecosystem (Rich *et al.*, 2008; Smith *et al.*, 2010; Rich *et al.*, 2011). A comprehensive microarray that targets functional genes across diverse pelagic marine microbial communities has not yet been reported.

Various strategies have been used to overcome the lack of *a priori* knowledge of genomic sequences in target communities, which is a major limitation in the design of an environmental microarray. The most common strategy is to search public nucleotide (nt) sequence databases (such as National Center for Biotechnology Information (NCBI)) using key words (Rhee *et al.*, 2004; He *et al.*, 2007; Wu *et al.*, 2008; He *et al.*, 2010; Smith *et al.*, 2010). The resulting data sets, however, typically do not resemble the natural diversity of target genes, a problem that is exacerbated in undersampled environments. A second common strategy is to PCR-amplify a gene of interest and then either spot the products on a glass surface (Wu *et al.*, 2001) or use the derived sequence data in the design of oligonucleotide probes. Although this technique results in a fingerprint for an uncharacterized community and is especially valuable for rare targets (Taroncher-Oldenburg *et al.*, 2003; Moisander *et al.*, 2006; Ward *et al.*, 2007; Bulow *et al.*, 2008), the time-consuming cloning process makes this approach suitable for studying only a limited number of genes. The large nt databases obtained with next-generation sequencing (metagenomics) present an additional opportunity to access a cross-section of the diversity of a marker gene in natural populations.

Here, we report the design and application of a high-density oligonucleotide microarray, referred to as the MicroTOOLs (Microbiological Targets for Ocean Observing Laboratories) microarray, which targets 19 400 sequences across 145 genes associated with open ocean and coastal microbial communities. The microarray is based on existing data from genomes, metagenomes, metatranscriptomes and PCR-based assays and targets functional genes responsible for biogeochemical cycling and stress responses characteristic of the oceanic photic zone. As a proof-of-concept experiment, we analyzed microbial community responses to nutrient enrichments of inorganic phosphate (P) or ferric iron (Fe) from the oligotrophic Station (Stn.) ALOHA in the North Pacific Subtropical Gyre (NPSG). The physics, chemistry and biology of this region is well characterized (Karl and Lukas, 1996; White *et al.*, 2007), and its microbial metagenome is well represented in the nt sequence databases (DeLong *et al.*, 2006; Frias-Lopez *et al.*, 2008; Hewson *et al.*, 2010). In the North Pacific, where N_2 fixation is a major supply of nitrogen (Karl *et al.*, 1997), phosphorus and iron can be the limiting nutrients for N_2 fixation and primary production (Karl *et al.*, 2001; Moore *et al.*, 2006; Grabowski *et al.*, 2008; Karl and Letelier, 2008; Watkins-Brandt *et al.*, 2011). We hypothesized that if microbial taxa were Fe limited, Fe addition would result in decreased transcription of iron-stress genes and increased transcription of genes for energy, carbon and nitrogen metabolism. If microbial taxa were P limited, P addition would result in decreased transcription of P-stress genes and increased transcription of genes for energy metabolism, DNA replication and cell division. However, we expected to see heterogeneous transcriptional responses from individual taxa owing to differences in their genomic capabilities, nutrient requirements and life strategies (Tolonen *et al.*, 2006; Dupont *et al.*, 2008; Ilikchyan *et al.*, 2009; Stuart *et al.*, 2009; Tetu *et al.*, 2009; Kamennaya and Post, 2011; Thompson *et al.*, 2011).

Materials and methods

Design of the MicroTOOLs microarray

Target genes for oligonucleotide probe design were selected based on existing knowledge of gene markers that target microorganism interactions with their environment (for example, Lindell and Post, 2001; Webb *et al.*, 2001; Holtzendorff *et al.*, 2002; Chen *et al.*, 2004; Fuller *et al.*, 2005; Dyhrman and Haley, 2006; Zehr *et al.*, 2007; Orchard *et al.*, 2009; Sebastian and Ammerman, 2009; Kamennaya and Post, 2011; Mosier and Francis, 2011; Paerl *et al.*, 2011). Several genes for hypothetical proteins that were differentially expressed in response to specific stimuli in cultured marine microorganisms were also included (Scanlan *et al.*, 1996; Martiny *et al.*, 2009; Shi *et al.*, 2009; Tetu *et al.*, 2009; Thompson

Table 1 Marker genes targeted in the MicroTOOLs microarray

Process	*Gene*	*Annotation*	*Counts*	*Process*	*Gene*	*Annotation*	*Counts*
Carbon metabolism	*cdcA*	Cadmium containing carbonic anhydrase	79	Nitrogen metabolism	*glnB*	N regulatory protein P-II	53
	chpX	CO_2 hydration protein ChpX	5		*hao*	Hydroxylamine oxidoreductase	103
	dca1	Delta carbonic anhydrase	31		*metC*	Cystathionine beta-lyase family protein involved in Al resistance	113
	dxs	1-deoxy-D-xylulose-5-phosphate synthase	29		*narB*	Assimilatory nitrate reductase in bacteria	224
	fae	Formaldehyde-activating enzyme	118		*nifB*	Nitrogenase cofactor biosynthesis protein NifB	81
	fhcD	Formylmethanofuran-tetrahydromethanopterin formyltransferase	4		*nifD*	nitrogenase reductase	95
	gap	Glyceraldehyde-3-phosphate dehydrogenase	47		*nifE*	Nitrogenase MoFe cofactor biosynthesis protein NifE	241
	gidA	Glucose-inhibited division protein A	152		*nifH*	Nitrogenase iron protein NifH	92
	icd	Isocitrate dehydrogenase	313		*nifK*	Nitrogenase molybdenum-iron protein beta chain	265
	mch	Methenyltetrahydromethanopterin cyclohydrolase	3		*nifN*	Nitrogenase molybdenum-iron cofactor biosynthesis protein NifN	21
	mtdB	Methylenetetrahydromethanopterin dehydrogenase	55		*nifO*	Nitrogenase-associated protein NifO	236
	mxaF	Methanol dehydrogenase	71		*nifX*	Nitrogenase molybdenum–iron protein NifX	335
	pmoA	Methane monooxygenase	140		*nirA*	ferredoxin-nitrite reductase	81
	ppc	Phosphoenolpyruvate carboxylase	104		*nirS*	Dissimilatory nitrite reductase	159
	prsA	Ribose-phosphate pyrophosphokinase	275		*nirX*	Homeobox domain, in the nirA operon	73
	pyk	Pyruvate kinase	163		*NR*	Assimilatory nitrate reductase	227
	rbcL	RuBisCO	1		*nrtP*	Nitrate transporter	98
	sbtA	Sodium-dependent bicarbonate transporter	75		*ntcA*	N limitation transcriptional regulator	195
	zwf	Glucose-6-phosphate dehydrogenase	41		*slc17A*	Amino-acid transporter	540
Cell cycle and replication	*CwatDRAFT_4045*	Transposase CwatDRAFT_4045	41		*Tery_2117*	Hypothetical protein, expressed as nif	173
	dnaA	Replication-initiation protein	20		*Tery_2900*	Hypothetical protein, expressed as nif	476
	dnaE	DNA polymerase III, alpha subunit	110		*Tery_4333*	LysR family transcriptional regulator, expressed as nif	11
	elaC	Ribonuclease Z	97		*ure*	Urea transporter	229
	ftsZ	Cell division protein FtsZ	6		*ureA*	Urease alpha subunit	53
	kaiC	Circadian clock protein KaiC	609		*ureB*	Urease beta subunit	54
	pol	DNA polymerase	226		*ureC*	Urease	11
	recA	Recombinase A	222		*ureD*	Urease accessory protein UreD	755
	rpoD	RNA polymerase sigma factor	42		*ureE*	Urease accessory protein UreE	282
	sigA	RNA polymerase sigma factors	401		*ureF*	Urease accessory protein UreF	599
DMSP metabolism	*dddD*	DMSP CoA transferase	80		*ureG*	Urease accessory protein UreG	482
	dddL	DMSP lyase	238		*ureH*	Urease accessory protein UreH-like protein	854
	dddP	DMSP lyase	30		*ureX*	urease subunit	491
	dddQ	DMSP lyase	4		*urtA*	Urea ABC transporter, substrate-binding protein	33
	dmdA	Dimethyl sulfoniopropionate demethylase	35	Other metabolisms	*bop*	Proteorhodopsin	552
Energy metabolism	*coxA*	Cytochrome *c* oxidase subunit I	16		*chrA*	Chromate transporter	276
	coxB	Cytochrome *c* oxidase subunit II	4		*cobN*	Cobaltochelatase CobN	36
	cpcB	Phycocyanin, beta subunit	5		*mopA*	Heme-binding region from putitive Mn-oxidase	10
	hupS	Ni–Fe hydrogenase, small subunit HupS	51	Other stresses	*mfs*	Multidrug efflux transporter, proline/betaine transporter	374
	ndhI	NADH dehydrogenase subunit I	11		*NiSOD*	Putative nickel-containing superoxide dismutase precursor	468
	petB	Cytochrome b6f	154		*NUDIX*	nudix hydrolase	22
	psaA	Photosystem I P700 chlorophyll a apoprotein A1	149		*phrB*	DNA photolyase	172
	psaB	Photosystem I P700 chlorophyll *a* apoprotein A10	30		*pip*	Proline iminopeptidase	177
	psbA	Photosystem II PsbA protein (D1)	5		*pmm1148*	EF-1 guanine nucleotide exchange	279
	psbA1	Photosystem II PsbA protein (D1)	4		*pmm1462*	Conserved hypothetical protein PMM1462	1
	psbA2	Photosystem II PsbA protein (D1)	269		*ptox*	Plastoquinol terminal oxidase	9

Table 1 (Continued)

Process	Gene	Annotation	Counts	Process	Gene	Annotation	Counts
	psbB	Photosystem II PsbB protein (CP47)	262		*sigII*	Type II alternative sigma-70 family RNA polymerase sigma factor	5
Iron metabolism	*abc1*	ABC1 superfamily protein	55		*sodC*	Cu–Zn superoxide dismutase	1
	cirA	Ferric iron-catecholate outer membrane transporter	6	Phosphorus metabolism	*acr3*	Arsenite transport (efflux)	25
	dpsA	Ferritin-like diiron-binding domain	15		*arsC*	Arsenate reductase	4
	feoA	Ferrous iron transport protein A	9		*glpQ*	Glycerophosphoryl diester phosphodiesterase	40
	fepB	ABC-type Fe3 + − hydroxamate transport system	3		*phnA*	Phosphonoacetate hydrolase	252
	fepC	ABC-type cobalamin/Fe3 + − siderophores transport systems	209		*phnD*	Phosphonate transporter	1
	fepD	Fe3 + siderophore transport system	1035		*phnJ*	Phosphonate lyase	173
	fldA (isiB)	Flavodoxin eukaryotic	3		*phoA*	Alkaline phosphatase, Zn^{2+} binding	600
	fldB	Flavodoxin	217		*phoD*	Alkaline phosphatase	2
	fur	Ferric transcriptional regulator	2		*phoH*	P stress-inducible protein	2
	idiA	Iron (III) transporter	365		*phoU*	Transcriptional regulator, phosphate transport system protein	143
	isiA	Iron stress-induced chlorophyll-binding protein	12		*phoX*	Alkaline phosphatase, Ca^{2+} binding	2
	isiB	Flavodoxin	213		*polyP1*	Poly-phosphate accumulation	4
	isiP	Iron stress-induced protein	302		*psiP*	Highly expressed under low P	1
	pep_m20	Possible Peptidase family M20/M25/M56	11		*pstS*	Phosphate transporter	1
	petF	Ferredoxin	16		*ptrA*	Possible P transcriptional regulator	3
	piuC	Uncharacterized iron-regulated protein	9		*sqdB*	Sulfolipid biosynthesis protein	3
	pmm1359	Predicted membrane protein, iron-stress responsive	48	Silica transport	*sit*	Silicon transporter	5
	pvsB	Vibrioferrin biosynthesis protein PvsB	257		*sit1*	Silicon transporter	4
	sam	SAM-methyltransferase	98		*sit2*	Silicon transporter	167
Nitrogen metabolism	*aapJ*	Polar amino-acid ABC transporter	66		*sit3*	Silicon transporter	4
	amoA	Ammonia oxidation	5	Viral genes	*dnaPol*	Viral DNA polymerase	6
	amt	Ammonium transporter	5		*g20*	Viral capsid assembly protein g20	7
	arg	N-acetyl transferase	6		*gp23*	Viral major capsid proteins	1
	carA	Carbamoyl-phosphate synthase	5		*mcp*	Viral major capsid proteins	1
	cynA	Cyanate transporter	5		*RdRp*	RNA-dependent RNA pol	292
	glnA	Glutamine synthetase	1				

Abbreviations: DMSP, dimethylsulfoniopropionate; MicroTOOLs, Microbiological Targets for Ocean Observing Laboratories. Counts refer to gene ortholog counts on the array.

et al., 2011). A total of 145 genes provided molecular markers for metabolic and cellular processes (Table 1).

To obtain gene probes that adequately represent environmental nt diversity, we searched all marine metagenomic and metatranscriptomic databases along with sequences from clone libraries for known genes. For metagenomic and metatranscriptomic searches, a seed amino-acid sequence data set was built for selected taxa (Supplementary Material) for each targeted gene. This seed data set was used for a TBLASTN query against 'All Sanger reads' and 'All 454 reads' in the Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA, http://camera.calit2.net/, Sun *et al.*, 2011) with loose criteria: an E value cutoff of 1.0E-03 and up to 1000 hits per query. All TBLASTN hits with lengths >150 nt were used as queries in reciprocal BLASTX in CAMERA to confirm gene annotation. For this analysis, an E value cutoff of 1E-10 and 60% identity over a minimum 40 amino acid alignment were used. Subsequent BLASTN searches in CAMERA were used to retrieve corresponding taxonomic information. Sequences with >85% identity over 100 nt alignment length to targeted marine microorganisms were selected. Taxonomic affiliation means that the target sequence had the highest similarity to a specific organism by BLASTN search against the 'nr' database. Thus, the accuracy of affiliation to an organism depended on gene conservation and their representation in sequence databases. NimbleGen technology allows 5% nt mismatch in the whole probe region, thus sequences within a range of 95–100% nt identity to the target gene were detected. We use the phrase 'organism-like genotype' to refer to target gene affiliation if nt identity of the target gene to this organism was <95%. After reciprocal BLAST, sequences originating from metagenomic libraries that contained non-transcribed regions were trimmed at 5′- and 3′-ends

of the open reading frame region. Custom Java applications and R scripts were developed to filter all BLAST results and to trim the ends of sequences and are available upon request. Additionally, target sequences were added that derived from the clone libraries of genes (Supplementary Material) and from genomes of marine microorganisms contained in NCBI Genbank. Combined sequences were clustered using CD-HIT-EST (Li and Godzik, 2006; Huang *et al.*, 2010) at 95% nt similarity. The longest representative sequence from each cluster was selected as the target sequence for oligonucleotide probe design. Probe design was performed at Roche NimbleGen (Madison, WI, USA), and six probes of 60-nt length were designed for each target. Random oligonucleotide probes were included in addition to standard control and alignment NimbleGen probes. All oligonucleotide probes were tested *in silico* for possible cross-hybridization (Supplementary Material). Before the MicroTOOLs microarray design, we tested the specificity of oligonucleotide microarray technology in detecting transcripts from a mixed community and from environmental samples. The results showed cross-hybridization for highly conserved genes (such as the photosystem II *psbA* gene across cyanobacteria) when nt sequences were $<5\%$ dissimilar (Supplementary Material, Supplementary Figure S1). The final design of the MicroTOOLs microarray comprised of ca. 116 000 experimental and 19 000 control probes with one replication synthesized on a 12-plex 12×135 K NimbleGen array. The platform is available at NCBI Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo/) under accession number GPL16706.

Nutrient-enrichment incubation

An incubation experiment was performed at Stn. ALOHA (22°45′ N 158° W) in the NPSG during KM1016 cruise (R/V Kilo-Moana). Surface water was collected on 22 August 2010 from 10 m depth using Niskin bottles in a rosette mounted to a conductivity–temperature–depth instrument (cast S2c9), filtered through 10.0-μm pore-size mesh, and distributed into 12 4-l clear polycarbonate bottles. The bottles and tubing were cleaned with 10% HCl, but vigorous trace-metal cleaning precautions were not taken during experimental setup. Each treatment was done in triplicate: (1) control (no enrichment), (2) enrichment with 1.0 μM K_2PO_4, and (3) enrichment with 2.0 nM $FeCl_3$. The bottles were incubated in a deck incubator continuously flushed with surface seawater to maintain the proper temperature. Neutral density screening was used to attenuate sunlight to ca. 35% of surface sunlight. Four-liter samples were taken from the original seawater sample before nutrient additions (three replicates total) and from all treatments after 48 h of incubation in the morning. From each bottle, 3.9 l were then filtered onto Sterivex cartridges (0.22 μm, Millipore, Billerica, MA, USA) using gentle Masterflex (Cole Parmer, Vernon Hills, IL, USA) peristaltic pumping ensuring that filtration time did not exceed 25 min. Sterivex cartridges were immediately flash frozen in liquid nitrogen and stored at -80 °C until processing. The remaining 0.1 l from each bottle was used in Fast Repetition Rate Fluorometer (FRRF) analysis.

RNA extraction and processing for hybridization to the microarray

RNA was extracted using the Ambion RiboPure kit (Life Technologies, Grand Island, NY, USA) with modifications that included mechanical lysis using glass beads (Supplementary Material). Extracted RNA was treated with DNase to remove genomic DNA. RNA quantity and quality were determined with a NanoDrop 1000 (Thermo Scientific, Waltham, MA, USA) and a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) using the RNA 6000 Nano kit (Agilent Technologies). Only samples with RNA Integrity Number >7.0 and ratios of A260/A230 and A260/A280 $\geqslant 1.8$ were processed further. RNA yield from the incubations of the surface community at Stn. ALOHA ranged from 280 to 1130 ng l^{-1}, an amount insufficient for the NimbleGen microarray. cDNA was synthesized from 400 ng RNA from each sample, which was then amplified using the TransPlex Whole Transcriptome Amplification kit (WTA-1, Sigma-Aldrich, St Louis, MO, USA) and antibody-inactivated hot-start Taq DNA Polymerase (Sigma-Aldrich). The amplified cDNA was purified with the GenElute PCR cleanup kit (Sigma-Aldrich), and the quality and quantity of double-stranded (ds) cDNA was determined with NanoDrop 1000 and a 2100 Bioanalyzer using the Agilent DNA 7500 kit (Agilent Technologies). Four hundred nanograms of total RNA yielded on average 12 μg of ds-cDNA. The amplification efficiency was determined with a Taq-Man quantitative PCR (qPCR) assay targeting a spike-in transcript ERCC-00116 (Invitrogen, Life Technologies). One microliter of 1:100 dilution (corresponding to 4.7 attomoles of ERCC-0016) of the ERCC (External RNA Control Consortium, Lemire *et al.*, 2011) RNA spike-in mix 1 (Ambion) was added to RNA samples before amplification. Amplification of one replicate for Fe treatment was seven times less than the average amplification (150-fold), and the sample was excluded from microarray hybridization. The labeling and hybridization of cDNA samples (1.0 μg of ds-cDNA) to the microarray was done at the Sandler Center Functional Genomics Core Facility (University of California, San Francisco, CA, USA) according to the protocol in NimbleGen Arrays User's Guide: 'Gene Expression Arrays, version 6.0'.

DNA extraction and estimation of cell abundances for diazotrophs and Synechococcus *spp.*

DNA was extracted from the organic phase of the nucleic acid extract after RNA separation using the RiboPure kit (Ambion) according to the

manufacturer's instructions (Supplementary Material). DNA yield ranged from 9.2 to 26.6 $\mu g\,l^{-1}$. Diazotroph abundances were determined with Taqman qPCR assays targeting *nifH* as described in Moisander *et al.* (2010). *Synechococcus* spp. cell abundances were estimated using the qPCR assay targeting *narB* (group G) as described in Paerl *et al.* (2012). The Tukey's test was used to compare the qPCR data.

Microarray data analysis

All data analyses were performed with R (www.R-project.org) and the Bioconductor project (Gentleman *et al.*, 2004), specifically using the Linear Models for Microarray (LIMMA; Smyth, 2005), affy (Gautier *et al.*, 2004) and samr (Tusher *et al.*, 2001) packages; plots were made using gplots package. Transcription values were obtained using the robust multi-array average algorithm (Irizarry *et al.*, 2003) and using Li–Wong across-chip normalization (Li and Wong, 2001) (Supplementary Figure S2). The signal-to-noise ratio (SNR) of each chip was calculated as: $SNR = (S_i – BG)/BG$; where S_i is the hybridization signal for the gene and BG is the chip background signal determined as average of the lowest 5% of all signals. Transcription was considered detected if SNR of a transcript was $\geqslant 5$. The detection range was estimated based on the ERCC hybridization data (Supplementary Figure S3). The detection limit as percentage of total mRNA was calculated for 1000-nt long mRNA and considering that rRNA constitutes 95% of total RNA. The relative cell sensitivity limit was estimated based on the assumption of 1380 mRNA per cell (Neidhardt and Umbarger, 1996). For each group (Eukaryota, Bacteria non-picocyanobacteria, *Prochlorococcus*, *Synechococcus*, Viruses, Archaea), gene transcription was scaled to the median of the group in each sample. To identify differentially transcribed genes, the nonparametric method, Significance Analysis of Microarray (Tusher *et al.*, 2001) was used with the following parameter settings: delta = 0.3, 100 iterations, false discovery rate (FDR) = 0.05 (Supplementary Figure S4). In addition, LIMMA (Smyth, 2005) was used with the following parameters: fold change in $\log_2$ scale = 1; FDR = 0.05; $P < 0.1$ (Benjamini–Hochberg adjusted, Benjamini and Hochberg, 1995). The separation of samples by treatment was supported by a Wilcoxon's test (Bauer, 1972) performed with at least a 100 random resamplings of 1000 gene probes (Supplementary Figure S4). Transcription data was centered and scaled across genes, and a distance matrix was calculated by Pearson's correlation coefficient. The distance matrix was then used in hierarchical clustering by a complete agglomeration method. Raw and normalized microarray data were prepared in accordance to the MIAME standards (Brazma *et al.*, 2001) and submitted to NCBI GEO under accession number GSE44448.

FRRF measurements

Chlorophyll *a* variable fluorescence (Fv) and maximal fluorescence (Fm) were measured using FRRF as described in Kolber *et al.* (1998). FRRF measurements were taken for each sample in the beginning and after 48 h of incubation in six replications and using blue light (470 nm) for excitation. The Tukey's test was used to compare the FRRF data.

Results and discussion

Microarray design

The MicroTOOLs microarray targets marker genes for three domains of life in marine microbial communities along with known viruses. Using our approach, a total of 19 400 target sequences representing 145 genes (Table 1) were obtained from genomes (~8%) and from metagenomes/metatranscriptomes (~92%). The design was biased toward the picocyanobacteria *Prochlorococcus* and *Synechococcus* spp. and the Alphaproteobacteria clade SAR11 sequences (Figure 1, Supplementary Table S1) owing to their high abundances in surface oceanic waters, resulting in high abundances of their nt sequences in metagenomes and metatranscriptomes (for example, DeLong *et al.*, 2006; Hewson *et al.*, 2010) and the fact that gene function and diversity of these microorganisms have been relatively well studied (Scanlan and West, 2002; Scanlan *et al.*, 2009; Sun *et al.*, 2011; Brown *et al.*, 2012). Genes from less abundant prokaryotic microorganisms, such as from marine N_2-fixing cyanobacteria, were also included. Marine eukaryotic phytoplankton were primarily represented by the genes encoding the large subunit of RuBisCO (*rbcL*) and nitrate reductase (*NR*) (Figure 1). Additional eukaryotic genes were selected based on available genomes and/or expressed sequence tags for diatoms (Armbrust *et al.*, 2004; Bowler *et al.*, 2008). Genes for marine DNA and RNA viruses included genes for DNA polymerase, major capsid protein and RNA-dependent RNA polymerase. Probes for Archaea targeted genes encoding ammonia monooxygenase, RuBisCO and urease (Supplementary Table S1).

As a proof of concept, the transcriptional responses of the surface microbial community at Stn. ALOHA to phosphate (P) or iron (Fe) amendments were analyzed using the MicroTOOLs microarray. During the time of sampling, surface waters at Stn. ALOHA had 54–79 $nmol\,l^{-1}$ of P, 4–33 $nmol\,l^{-1}$ of nitrate plus nitrite, 0.79–1.07 $\mu mol\,l^{-1}$ of silicon and 67–78 $ng\,l^{-1}$ of chlorophyll *a* (data from 5–10 m depths). Fe concentrations were not measured during the time of study, but average near-surface Fe concentration at Stn. ALOHA is 0.44 $nmol\,l^{-1}$ (Boyle *et al.*, 2005). The long-term mean P concentrations at Stn. ALOHA are 58 ± 3 $nmol\,l^{-1}$ (Björkman *et al.*, 2012), and thus, out of P and Fe, Fe was believed to be the limiting nutrient at the time of incubation, at least for the diazotrophic community.

Microbial community transcription at Stn. ALOHA detected with the MicroTOOLs microarray

Hybridization signals above background were detected for a total of 15 507 genes (68% of the microarray set), with an average detection of 40% of target orthologs for each gene (Supplementary Table S3). The range of detection for the microarray was from 700 to 11E + 06 transcript copies based on the spike-in ERCC data (Supplementary Figure S3). Calculated for a 1000-nt long mRNA, the 700 transcript copies (absolute sensitivity) corresponded to 1.8E-06% of the total community mRNA and to 0.0025% as the lowest relative abundance of cells within the community that can be detected. Average transcription from pelagophytes (*Aureococcus*) and prymnesiophytes (*Chrysochromulina*, *Phaeocystis*, *Helicosphaera*) was up to sixfold higher than the median transcription in all eukaryotes across samples (Figure 2a, Supplementary Table S2). Average transcription among prokaryotes (*Prochlorococcus*, *Synechococcus*, Proteobacteria,) was distributed around the median transcription of the corresponding transcriptome in all samples (Figure 2a, insert). The exceptions were genes related to an uncultured *Prochlorococcus* species represented by a fosmid clone HOT0M, which had 16-fold higher transcription than the median (Figure 2a). The most highly transcribed genes across all treatments were *psaA* (photosystem I), *psbA* (photosystem II), *amt* (ammonium transport), *urtA* (urea transporter) and *rbcL* (Figure 2b). Among relatively low abundance taxa, diazotrophic cyanobacteria, *Trichodesmium erythraeum* IMS101, *Candidatus* Atelocyanobacterium thalassa (*Ca.* A. thalassa or unicellular cyanobacterial group A, UCYN-A) and an uncultured heterocystous cyanobacterium (NCBI 112280460, Moisander *et al.*, 2007) had high *nifH* (Fe-nitrogenase reductase) transcription (Supplementary Table S2). The maximum *nifH* transcription in these cyanobacteria occurs during the early morning hours (Church *et al.*, 2005), when samples from incubations were collected. Another N_2-fixing cyanobacterium *Crocosphaera watsonii* (*Crocosphaera*) had high transcription of *pstS* (high-affinity phosphate binding) and *ftsZ* (cell division) (Supplementary Table S2). *Ca.* A. thalassa and *Crocosphaera* cell abundances (Table 2) in the incubations ranged from 0.0002% to 0.04% of the total prokaryotic community (8.0E + 08 cells l^{-1}, Bjorkmann *et al.*, 2012), overlapping the estimated relative cell detection limit for the microarray (0.0025%). In addition, gene transcripts were detected for members of the *Phycodnavirus* family, which infects a number of eukaryotic phytoplankton, including *Micromonas* (Mayer and Taylor, 1979) and *Aureococcus* (Milligan and Cosper, 1994) and also for cyanophages from the *Myoviridae* family (DNA polymerase and viral capsid genes).

Overall, the genes with detected transcription reflected the composition and activity of the microbial community at Stn. ALOHA as previously described (DeLong *et al.*, 2006; Frias-Lopez *et al.*, 2008; Church *et al.*, 2009; Hewson *et al.*, 2010). Moreover, the high activity of the pelagophytes and prymnesiophytes was consistent with the detection of a eukaryotic phytoplankton bloom at that time (Björkman *et al.*, 2012).

Transcription by members of unknown lineages

A wide diversity of genotypes of *Prochlorococcus*, *Synechococcus* and Alphaproteobacteria were detected at the study site. *Prochlorococcus* probes that yielded detectable signals had a wide range of nt similarity to sequenced genomes (Figures 3a and d), indicating a broad representation of the known natural genetic diversity in *Prochlorococcus*, as well as transcriptional activity in genotypes with no currently sequenced genome. Gene probes with detectable transcription had a median of 91% nt identity to *Prochlorococcus* genome sequences, such as strain CCMP1986 (Figure 3a).

Detected *Synechococcus*-like transcripts had a median of 88.5% similarity at the nt level to their orthologs in known genomes (Figures 3b and d). Especially high transcriptional activity (normalized transcription > 2) was detected for genes similar to *Synechococcus* sp. RCC307, a strain from *Synechococcus* sub-cluster 5.3A. The low percentage of similarity to known genomes indicates the existence of an uncharacterized lineage, potentially within the sub-cluster 5.3, at Stn. ALOHA. The presence of this clade has recently been reported in the open ocean and in the Mediterranean Sea, with higher abundances in warm, low-nutrient waters (Mella-Flores *et al.*, 2011; Post *et al.*, 2011; Ahlgren and Rocap, 2012; Huang *et al.*, 2012).

With a median of 80.0% similarity, probe sequences targeting Alphaproteobacteria also displayed a degree of degeneracy relative to known genome sequences (Figure 3c), suggesting that new, active strains remain uncharacterized. This is consistent with another recent study, where a new group of the SAR11 clade was proposed based on 16S rRNA gene phylogeny (Allen *et al.*, 2012). Such uncharacterized genotypes with detectable transcription are candidates for further targeted genomic studies.

Response to nutrient amendments

Maximum chlorophyll *a* fluorescence (Fm) was not significantly different between each of the amendments and the control and between P and Fe amendments ($P > 0.2$, Table 2). The lower ratio of variable to maximum fluorescence (Fv/Fm) in the Fe treatment ($P < 0.05$) may be a result of either a shift in phytoplankton community composition or lower photosynthetic efficiency in Fe-enriched samples due to nutrient limitation (Vogel *et al.*, 2003; Sylvan *et al.*, 2007, 2011).

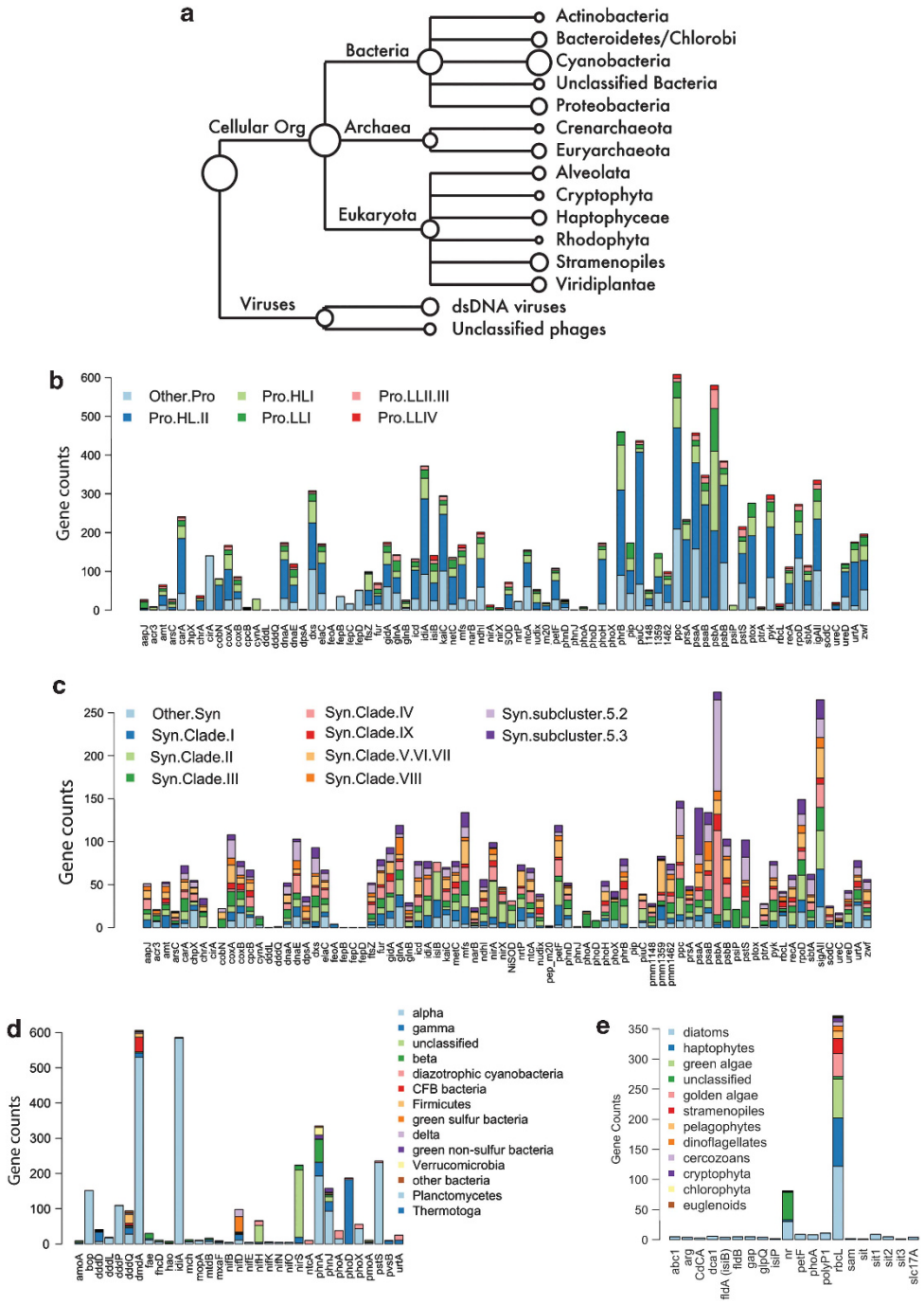

Figure 1 Microorganisms and viruses with genes targeted in the MicroTOOLs microarray. (**a**) Phylogenetic tree of all targeted genes with the number of genes reflected in the size of the circles. (**b–e**) Distribution of target genes in (**b**) *Prochlorococcus* spp. by clade, (**c**) *Synechococcus* spp. by clade, (**d**) bacteria other than *Prochlorococcus* and *Synechococcus* by phylogroups and (**e**) Eukaryota by phylogroup. The details for gene distribution are in Supplementary Table S1.

Cyanobacterial abundances increased in response to Fe amendments. Diazotrophic cyanobacteria *Crocosphaera* and *Ca.* A. thalassa and the non-diazotrophic cyanobacteria *Synechococcus* were 8, 19 and 23 times, respectively, more abundant in Fe amendments versus the control after incubation, as measured with qPCR (Table 2). *Ca.* A. thalassa and *Synechococcus* spp. were 8 and 11 times, respectively, more abundant in Fe-amended versus P-amended samples. This high increase in cell abundances in response to Fe and low response to P is consistent with previously reported variability in responses of diazotrophs to P and Fe availability in the NPSG (Zehr *et al.*, 2007; Grabowski *et al.*, 2008; Watkins-Brandt *et al.*, 2011).

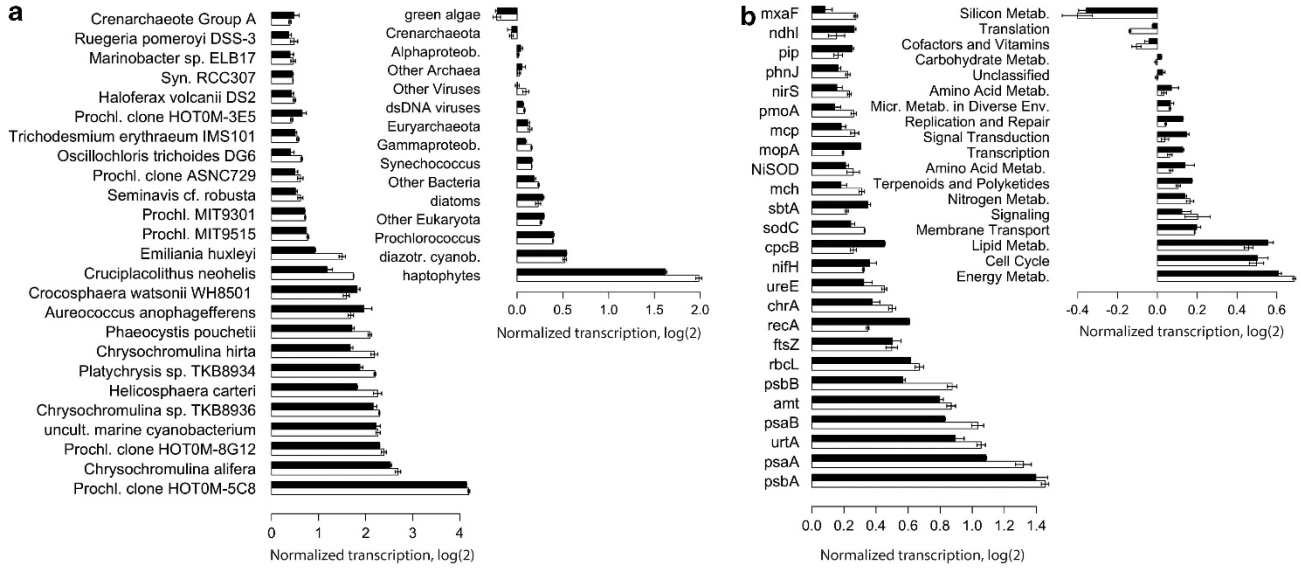

Figure 2 Average detected transcription by organisms (**a**) and by genes (**b**) normalized to the median transcription in each phylogroup in each sample. Only top 25 entries for each category are shown. Average transcription by phylogroups (**a**) and by gene pathways (**b**) are included as inserts. White and black represent P- and Fe-amended treatments, correspondingly.

Table 2 Cyanobacterial abundances measured as gene copy numbers with qPCR and chlorophyll *a* and Fv/Fm measured with FRRF

	Treatments			t-*value*		
	Cnt	*P*	*Fe*	*P-Cnt*	*Fe-Cnt*	*Fe-P*
Synechococcus, *narB* L-1	3.2 ± 0.9E + 03	6.7 ± 1.3E + 03	7.4 ± 2.0E + 04	0.22 ($n=6$)	4.53** ($n=6$)	4.31** ($n=6$)
Ca. A. thalassa, *nifH* L-1	1.3 ± 0.2E + 03	3.2 ± 0.6E + 03	2.4 ± 1.0E + 04	0.26 ($n=6$)	3.10* ($n=6$)	2.84* ($n=6$)
Crocosphaera, *nifH* L-1	4.3 ± 0.6E + 04	1.4 ± 0.4E + 05	3.2 ± 1.1E + 05	1.11 ($n=6$)	3.19* ($n=6$)	2.09 ($n=6$)
Fm	3.71 ± 0.28	4.39 ± 0.16	4.18 ± 0.44	1.85 ($n=9$)	1.13 ($n=9$)	− 0.61 ($n=9$)
Fv/Fm	0.50 ± 0.04	0.48 ± 0.01	0.43 ± 0.01	− 1.07 ($n=9$)	− 2.77* ($n=9$)	− 1.96 ($n=9$)

Abbreviations: Cnt, control; Fv/Fm, ratio of variable to maximum fluorescence; FRRF, Fast Repetition Rate Fluorometer; qPCR, quantitative PCR. Tukey's significance test: **0.01, *0.05.

Differential transcriptional responses to P and Fe amendments

After 48 h of incubation, Fe and P amendments yielded significant differences in transcript levels across the microbial community despite the high variability among biological replicates. When transcription of all genes was compared, biological replicates had a weak-positive-to-weak-negative correlation (Supplementary Table S4). Strong correlations between replicates were obtained at the level of specific phylogroups (eukaryotic, *Prochlorococcus* and *Synechococcus*) and metabolic functions (energy and N metabolism) (Figure 4). Factors that may have caused discrepancies in transcription profiles between replicates include differences in biological processes within individual bottles (such as protist grazing, viral lysis) and biases of sample collection and processing.

The Significance Analysis of Microarray analysis (see Materials and methods section) identified 3742 genes as significantly differentially transcribed between treatments (FDR = 0.05) from a total of 15 507 genes with detected transcription (Supplementary Figure S5, Supplementary Table S5). Overall, the addition of Fe resulted in the increased transcription of 1699 genes encoding for N metabolism, photosynthesis, oxidative phosphorylation and ABC-type transporters (Figure 4f). The category of genes not assigned to a KEGG pathway (NA) and upregulated in the Fe amendment included genes for ammonium transport, organic P assimilation, DNA replication and cell division. The 2043 genes upregulated in the P amendment were enriched in genes for carbon fixation (Figure 4g).

It is important to note that highly transcribed genes in all samples (for example, eukaryotic nitrate reductase *NR* NCBI GI: GU203403 (Supplementary Table S2) oversaturated the hybridization signal, and the difference in transcription could not be estimated.

Relief from Fe limitation in oligotrophic taxa

Transcriptional patterns showed that Fe amendment resulted in relief from Fe limitation in taxa common to oligotrophic waters.

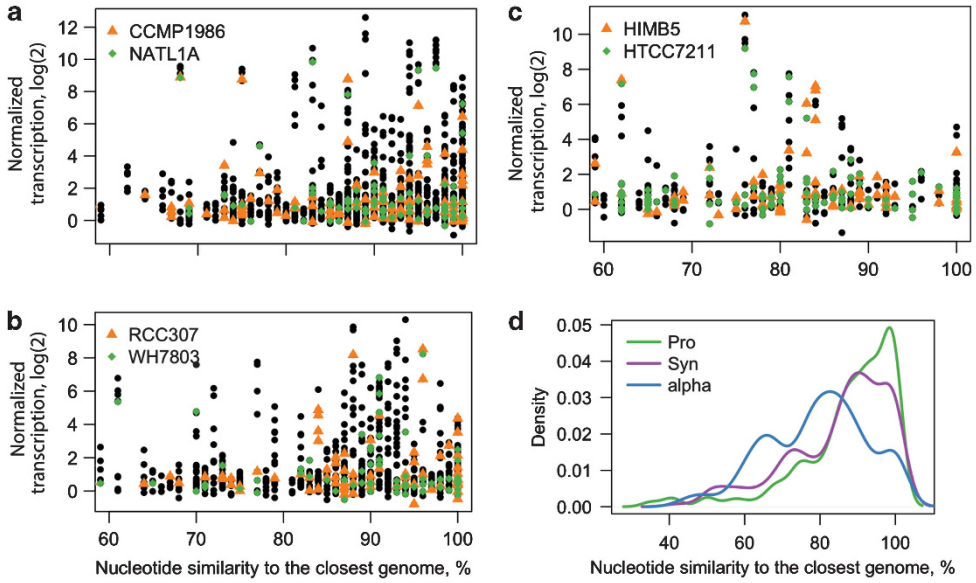

Figure 3 Transcription versus nucleotide similarity to the closest genome for *Prochlorococcus* spp. (**a**), with CCMP1986- and NATL1A-like genotypes highlighted; *Synechococcus* spp. (**b**), with RCC307- and WH7803-like genotypes highlighted; and Alphaproteobacteria (**c**) sequences, with HIMB5-and HTCC7211-like genotypes highlighted. Transcription was normalized to the median of each group in each sample, and only differentially transcribed genes are shown. (**d**) Distribution of differentially transcribed genes by nucleotide similarity to the closest genome for *Prochlorococcus*, *Synechococcus* and Alphaproteobacteria.

Transcription of genes for energy metabolism increased in picocyanobacteria upon Fe amendment (Figures 5a and b). Fe additions resulted in a twofold increased transcription of *psaA* (photosystem I) in both *Prochlorococcus* and *Synechococcus* spp, with an up to fivefold increase in transcript level for the *psaA* gene in the *Synechococcus* RCC307-like genotype. Transcription of the *coxA* gene (cytochrome *c* oxidase) in *Prochlorococcus* eHLI and eHLII genotypes was up to fivefold higher in the Fe-amended treatment. The role of Fe as a cofactor in electron transport is consistent with increases in transcripts for energy-metabolism genes in *Prochlorococcus* and *Synechococcus* in Fe amendments, a pattern also observed in cultures (for example, Singh *et al.*, 2003; Thompson *et al.*, 2011).

This increased transcription of energy-metabolism genes was accompanied by the increased transcription of genes for cellular activity (DNA replication *recA*, cell division *ftsZ*) in *Prochlorococcus* (high light ecotype I, eHLI) in Fe-amended treatments (Supplementary Figure S6). It is possible that addition of Fe resulted in the increased growth rate of *Prochlorococcus* as observed in the Eastern South Pacific (Mann and Chisholm, 2000). The higher sensitivity of the HLI ecotype in comparison to LL ecotypes to Fe availability (Thompson *et al.*, 2011) may explain the increased cellular activity of *Prochlorococcus* eHLI in response to the Fe amendment. The availability of fixed N from diazotrophs (Mulholland and Capone, 2001; Mulholland *et al.*, 2004) upon Fe amendment may have been an additional or alternative factor that enhanced the growth of *Prochlorococcus* eHLI in these treatments. This hypothesis is consistent with the similarity in responses of diazotrophs and *Prochlorococcus* HL spp. to Fe addition, both in this study and in a study in the Southwest Pacific Ocean (Moisander *et al.*, 2012).

The downregulation of *idiA* in oligotrophic taxa such as *Prochlorococcus* AS9601 (eHLII) and Alphaproteobacteria SAR11 clade strain HIMB59 (Supplementary Figure S6) in Fe amendments was consistent with the repression of the gene in Fe-replete cultures (Bagg and Neilands, 1987; Webb *et al.*, 2001; Smith *et al.*, 2010; Thompson *et al.*, 2011). In contrast, *idiA* transcription by more eutrophic *Synechococcus* RCC307-like (sub-cluster 5.3A, Mella-Flores *et al.*, 2011) and *Pelagibacter* HTCC7211-like genotypes was higher in the Fe-amendment. The differences between coastal and open ocean species have been reported before in cultures and include differences in Fe requirements and sensing (for example, Sunda *et al.*, 1991; Palenik *et al.*, 2006), uptake of siderophore- or porphyrin-bound Fe (Hutchins *et al.*, 1999) and post-translational regulation by antisense RNA (Hernández *et al.*, 2006).

This is the first report that validates differential Fe responses across a multitude of taxa in a mixed community. The relatively high ($0.44\,nmol\,l^{-1}$) average Fe concentrations in the surface waters at Stn. ALOHA are not usually considered limiting for microbial communities (Boyle *et al.*, 2005). The response to Fe amendments reported here indicates that either Fe concentrations at the time of study were lower than average or that much of this Fe was not bioavailable, at least for some taxa.

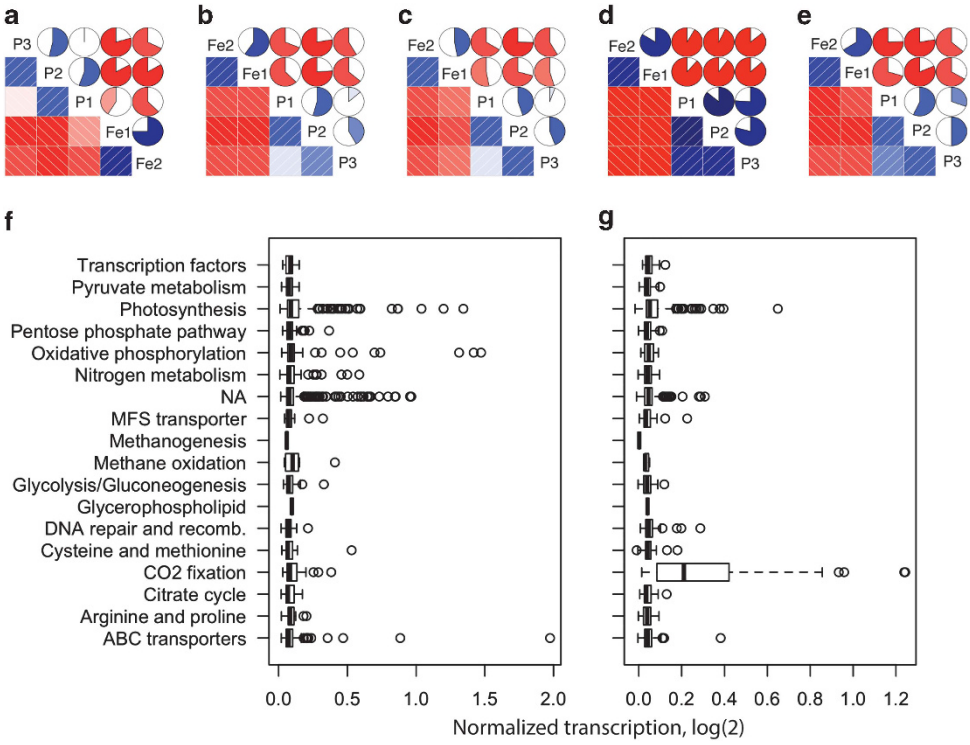

Figure 4 Correlograms for differentially transcribed genes from (**a**) Eukaryota (172 genes), (**b**) *Prochlorococcus* (2022 genes), (**c**) *Synechococcus* (1030 genes), (**d**) Energy metabolism (699 genes) and (**e**) Nitrogen metabolism (472 genes). The low triangle from the principal diagonal line in each panel contains correlation cells for each pair of samples, and the upper triangle contains pie charts showing the strength of correlation. Blue and red represent positive and negative correlations, respectively, and color intensity reflects magnitude of the correlation. Columns and rows were reordered based on principal component to reflect samples that group together. Correlation coefficients for all correlograms are in Supplementary Material (Supplementary Table S4). Transcription of genes upregulated in the Fe amendment (**f**) and in the P amendment (**g**) by KEGG pathway. Transcription was normalized to the mean across samples. 'NA' category comprises of genes not assigned to a KEGG pathway.

Increased N metabolism and N limitation in Fe amendments

The availability of Fe led to an increased transcription of N transport and metabolism genes (*amt*, *urtA*, *nrtP*, *glnA*, *nirA*, *sigAII*) in both *Synechococcus* and *Prochlorococcus* and possibly led to N limitation in *Prochlorococcus* eHL (Figure 5c, Supplementary Table S6). The observed fourfold downregulation of the *rbcL* transcripts in *Prochlorococcus* eHLI and eHLII in the Fe amendment (Figure 5d) might have resulted from N limitation (Tolonen *et al*., 2006). In contrast, *Synechococcus* spp. had twofold higher *rbcL* transcription in Fe amendments than in P amendments (Supplementary Table S6). We speculate that the differences in *rbcL* transcription were due to the ability of the majority of *Synechococcus* spp. to assimilate nitrate, while only few uncultured *Prochlorococcus* spp. have that capability (Moore *et al*., 2002; Martiny *et al*., 2009). Transcription of nitrite/nitrate-utilization genes (*nrtP*, *nirA* and *narB*) was detected in *Synechococcus* spp. and was upregulated in *Synechococcus* WH8109 (clade II) in Fe amendments (Supplementary Tables S2 and S6). Transcription of the *narB* gene was also detected for a few uncultured *Prochlorococcus* spp., but the precise affiliation of these genes is unknown. While not measured directly in the incubations, nitrate/nitrite concentrations at Stn. ALOHA during incubation were relatively high. It is possible that *Synechococcus* spp. were able to obtain sufficient nitrogen, and thus carbon fixation in *Synechococcus* was not downregulated.

The eukaryotic *NR* gene was upregulated in Fe amendments (Supplementary Table S6) possibly resulting from increased energy production, by sufficient Fe requirements for the nitrate reductase enzyme and/or by subsequent N limitation. Although transcription of *NR* can be induced by the presence of nitrate alone in eukaryotes (Song and Ward, 2004; Poulsen and Kröger, 2005), the lower Fv/Fm ratio was possibly due to insufficient N (Tolonen *et al*., 2006) for the dominant phytoplankton in chlorophyll *a* measurements, which at that time were eukaryotic phytoplankton. Similar to *Prochlorococcus*, eukaryotic phytoplankton downregulated *rbcL* genes in the Fe treatment, especially two Chrysophytes, *Epipyxis pulchra* and *Ochromonas aestuarti*, and two Prymnesiophytes, *Chrysochromulina alifera* and *Chrysochromulina flava* (Figure 5d). The downregulation of *rbcL* could be due to a reduced cellular N:C ratio in these organisms. Alternatively, if photosynthetic eukaryotic

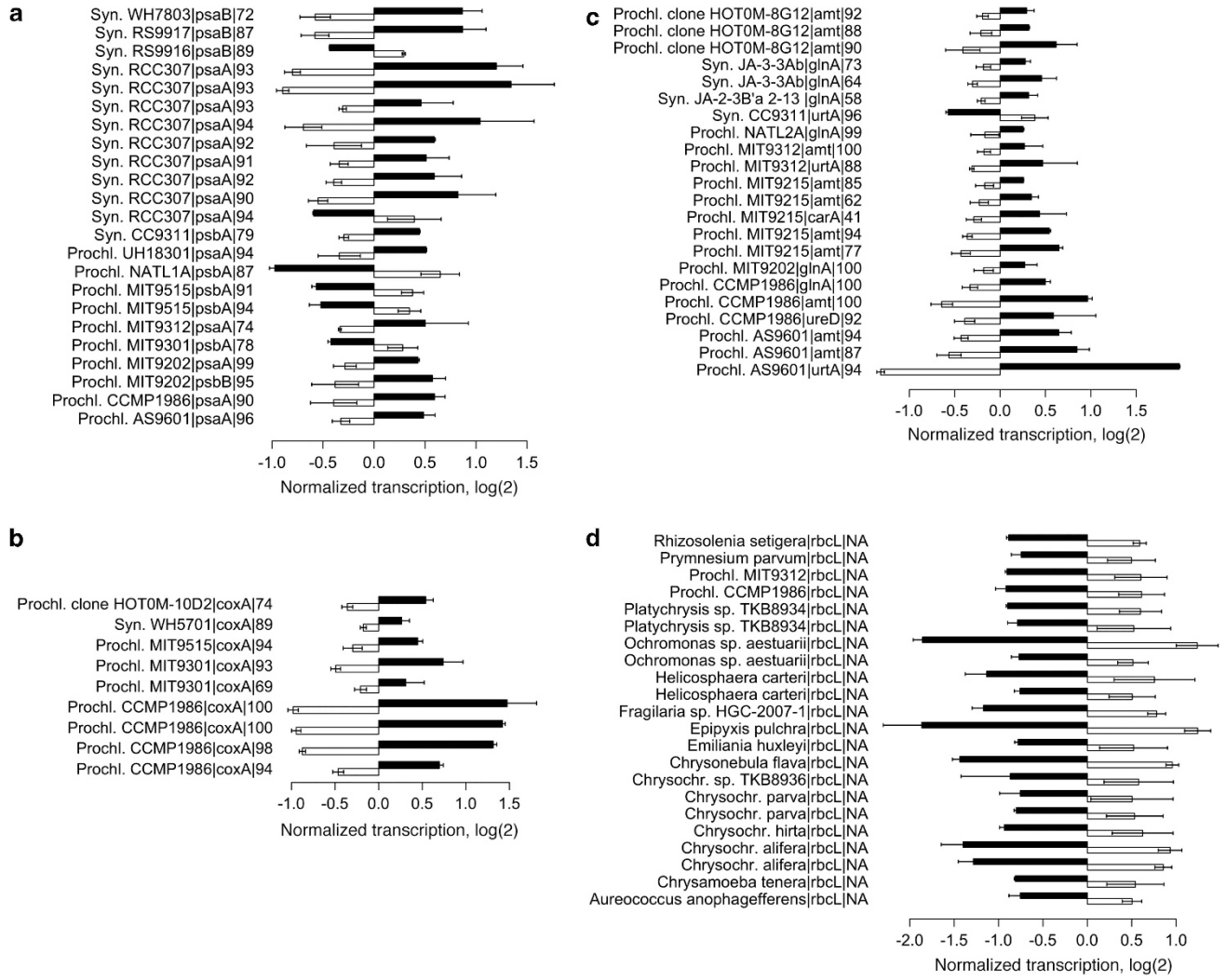

Figure 5 Normalized transcription for top differentially transcribed genes for (**a**) photosynthesis, (**b**) oxidative phosphorylation, (**c**) nitrogen metabolism and stress and (**d**) carbon fixation. Transcription was normalized to the mean transcription across samples.

phytoplankton were utilizing nitrate, they would have had to divert ATP and reductant away from carbon fixation, which would also result in decreased carbon fixation (Laws, 1991) by down-regulating *rbcL* transcription.

P limitation or increased P metabolism followed Fe addition

Individual taxa in the Fe treatment showed upregulated transcription of P stress-response genes, despite the generally high availability of phosphate. Transcription of *pstS* (the high-affinity phosphate-binding protein) in *Prochlorococcus* MIT9301-like genotypes (eHLII) was twofold higher in the Fe amendments in comparison to the P amendments (Supplementary Figure S6). The set of iron upregulated genes was enriched in *pstS* transcripts from Proteobacteria (especially, Alphaproteobacterium HIMB5) and cyanobacteria (*Synechococcus* WH8102) (Supplementary Table S6). The phosphonate utilization genes *phnJ* and *phnA* in Alphaproteobacteria and the alkaline phosphatase gene *phoD* in Gammaproteobacteria were upregulated in the Fe addition (Supplementary Figure S6, Supplementary Table S6). The 2-μm size fraction of phytoplankton was not P-depleted at the time of incubation (Björkman *et al.*, 2012), but it is possible that P limitation was induced by the fast removal of P in the Fe amendment by some members of the community, such as nitrate utilizers. Alternatively, increased energy generation in microorganisms in the Fe treatment might have provided energy for phosphate acquisition and membrane translocation systems for proteins involved in alternative P source assimilation (Cembella *et al.*, 1982; Jansson, 1988; Tetu *et al.*, 2009). Taxa that upregulated P transport and metabolism genes were largely different from taxa that demonstrated N limitation, but manifestations of both N and P limitations occurred after Fe was supplied, suggesting Fe as a primary limiting nutrient at that time.

Conclusions

This study reports the design of a high-density oligonucleotide microarray (MicroTOOLs) that targets marine microbial communities and was enabled by currently available environmental sequence data. We detected differential microbial community responses to nutrient amendments in the NPSG, ultimately demonstrating strain-specific community responses to relief of Fe stress that was followed by N or P limitation in some taxa. Such data provide a mechanistic understanding of changes in microbial communities in response to nutrient fluxes or other environmental factors.

Future technological improvements, such as automated probe design including probes for other marker genes and exploratory probes (Chung *et al.*, 2005; Dugat-Bony *et al.*, 2011), would improve the performance of the microarray. In addition to the utility of the MicroTOOLs array for incubation experiments, this microarray could be applied as a tool for pelagic marine microbiological studies for standardized information across study types and ocean basins. This would result in a high-resolution map of microbial genes and their transcriptional activities in the environment and provide the baseline for assessing the impacts of future perturbations of the global ocean.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work resulted from two workshops, and the design of the microarray was partially supported by the Gordon and Betty Moore Foundation (the MEGAMER facility), by a Gordon and Betty Moore Foundation Marine Investigator grant (JPZ) and by the NSF Center for Microbial Oceanography (C-MORE, NSF EF0424599). We thank all the participants of the October 2010 MicroTOOLs workshop sponsored by the Moore foundation. Particularly, we thank those who provided sequences for the design of microarray: Mahdi Belcaid, Dreux Chappell, Jackie Collier, Chris Francis, Scott Gifford, Jana Grote, Bethany Jenkins, Julie LaRoche, Pia Moisander, Annika Mosier, Micaela Parker, Holly Simon, Mariya Smit, Jody Wright, and Louie Wurch. We thank Shulei Sun and the team at CAMERA for support during microarray design. We also thank the Hawaii Ocean Time-series (HOT) program and C-MORE, specifically the captain and crew of R/V Kilo Moana and chief scientist of KM1016 cruise, Matt Church, for their expertise and for providing the opportunity and support for conducting experiments at sea. We thank Philip Heller and Jonathan Magasin for developing Java scripts for the microarray design and for computational support during the MicroTOOLs workshop in October 2010. Finally, we thank Nicole Pereira for help in running the incubation experiment, Sasha Tozzi for FRRF measurements, Rob Tibshirani for a consultation on microarray data normalization and analysis and the JPZ laboratory members for constructive discussions.

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