ORIGINAL ARTICLE

Phenotypic plasticity in heterotrophic marine microbial communities in continuous cultures

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Phenotypic plasticity (PP) is the development of alternate phenotypes of a given taxon as an adaptation to environmental conditions. Methodological limitations have restricted the quantification of PP to the measurement of a few traits in single organisms. We used metatranscriptomic libraries to overcome these challenges and estimate PP using the expressed genes of multiple heterotrophic organisms as a proxy for traits in a microbial community. The metatranscriptomes captured the expression response of natural marine bacterial communities grown on differing carbon resource regimes in continuous cultures. We found that taxa with different magnitudes of PP coexisted in the same cultures, and that members of the order _Rhodobacterales_ had the highest levels of PP. In agreement with previous studies, our results suggest that continuous culturing may have specifically selected for taxa featuring a rather high range of PP. On average, PP and abundance changes within a taxon contributed equally to the organism's change in functional gene abundance, implying that both PP and abundance mediated observed differences in community function. However, not all functional changes due to PP were directly reflected in the bulk community functional response: gene expression changes in individual taxa due to PP were partly masked by counterbalanced expression of the same gene in other taxa. This observation demonstrates that PP had a stabilizing effect on a community's functional response to environmental change.
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Introduction

Phenotypic plasticity (PP) has been defined as the ability of a given taxon to develop and express different phenotypes in response to distinct environmental conditions (Pigliucci, 2001). Along with evolutionary adaptation of genotype and physical dispersal to preferred environments, PP is a major mechanism by which species escape extinction because of changes in local conditions (Jump and Penuelas, 2005; Chevin _et al._, 2010). In the face of global change coupled to an increased risk of species loss, a better understanding of a mechanism that potentially stabilizes ecosystems is of increasing importance.

Because of methodological constraints, PP has usually been measured in individual taxa by targeting only one to a few traits expressed during changing conditions (Chevin _et al._, 2013). However, the recently developed molecular method of metatranscriptomics allows the detection of

expressed genes from all members of a community (Jansson _et al._, 2012) and can serve as a proxy for phenotype characterization. In essence, metatranscriptomic analyses distinguish expressed functions of individual taxa within complex communities. This is accomplished by binning transcripts based on identity to reference genomes and identifying functional gene classes nested within each bin. Functional patterns evident in the expressed genes within a bin allow the PP on the taxon bin level to be quantified (Figure 1). To the best of our knowledge, this is the first time that high-throughput metatranscriptomic sequencing has been used to estimate PP for individual members of a complex microbial community. This approach has the potential to provide novel insights into microbial structure–function relationships that so far have been only minimally addressed in metatranscriptomic studies.

In this study we were specifically interested in two major issues that are connected to the presence and magnitude of PP in single taxa within complex communities. First, how much does PP vary among taxa in a community and is phylogeny a significant predictor of PP? Second, what are the consequences of PP at the community level, in particular which proportion of the community functional change can be explained by PP rather than by changes in taxon

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Dataset	Treatment X	Treatment Y	Distance measure	Interpretation
a Genes			Bray-Curtis	Quantitative estimation of changes in community function
b Taxa			Bray-Curtis	Quantitative estimation of changes in the active community composition
c Genes nested within taxa (size normalized)			Bray-Curtis Bray-Curtis Bray-Curtis Bray-Curtis	Quantitative estimation of phenotypic plasticity in individual taxa
d Genes nested within taxa while considering transcript abundances changes of taxa			Bray-Curtis (corrected) Bray-Curtis (corrected) Bray-Curtis (corrected) Bray-Curtis (corrected) Bray-Curtis (corrected)	Quantitative estimation of total plasticity in individual taxa

Figure 1 Transcripts binned according to function (**a**), taxon (**b**) or both function and taxon (**c**, **d**) in different treatments (indicated as x and y) were used to quantitatively estimate the community functional change (**a**), changes in the actively transcribing community composition (**b**), PP of individual taxa in size-normalized taxon bins (**c**) and TP in taxon bins with gene counts multiplied by the taxon transcript abundance before calculating Bray–Curtis dissimilarities (**d**).

transcript abundance? PP may also increase the functional stability of a system if transcriptional changes in an individual taxon due to PP are counterbalanced by transcriptional changes in other taxa of the community. This effect would be similar to that of functional redundancy in a community that has usually been described in terms of shifts in the community composition rather than PP (Griffiths *et al.,* 2000; Langenheder *et al.,* 2006; Comte *et al.,* 2013) (Figure 2). The metric used to analyze PP responses in this study was based on high-throughput sequencing surveys of transcripts produced by members of a heterotrophic bacterioplankton community grown in continuous culture under varying dissolved organic matter (DOM) regimes. Transcripts that were retrieved from the continuous cultures were mapped to the closest bacterial reference genome and analyzed for differences between treatments in the composition of functional gene transcripts (Figure 1). To assess the effects of PP on the community functional change, functional differences introduced by PP of individual taxa alone and those introduced by taxon transcript abundance changes were compared with the community-wide functional change. Overall, our goal was to assess the structure–function relationships of marine microbes in the processing of marine DOM, with a special focus on the importance of PP and its effect on the community functional change.
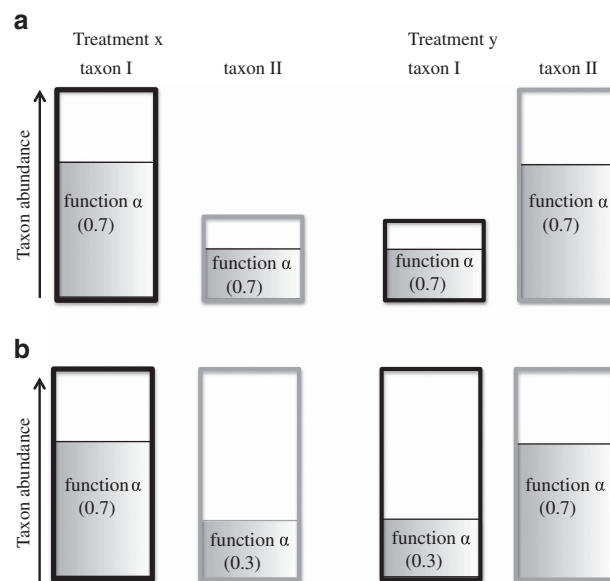
a

Treatment x — taxon I, taxon II   Treatment y — taxon I, taxon II

Taxon abundance

function α (0.7)   function α (0.7)   function α (0.7)   function α (0.7)

b

Taxon abundance

function α (0.7)   function α (0.3)   function α (0.3)   function α (0.7)

Figure 2 Functional stability can be caused by two scenarios: (**a**) abundance changes of taxa that express the same function at the same relative transcription level (that is, taxa that are functionally redundant) and (**b**) changes in relative transcription level of the same function within taxa that do not change their overall transcript abundance (that is, taxa that feature PP). Numbers in brackets indicate the fraction of bars occupied by function α in order to authenticate the schematically displayed functional stability.

Materials and methods

Continuous cultures and sample collection
Two independent continuous culture experiments were performed, referred to as Exp_SwDi (November 2009) and Exp_CyDi (March 2010), and each of the experiments consisted of two treatments that differed in DOM characteristics of the growth media. In Exp_SwDi, the two growth media were sea water aged for 6 weeks and the same aged sea water amended with DOM from a diatom culture (*Phaeodactylum tricornutum*). In Exp_CyDi, the two growth media were sea water aged for 6 weeks amended with DOM from *P. tricornutum* and the same aged sea water amended with DOM from the cyanobacterium *Synechococcus* sp. WH 7803. These strains were obtained from a culture collection at the Observatoire Océanologique de Banyuls.

The continuous culture system and the seawater sampling are described in detail in Landa *et al.* (2013a,b), where other analyses performed on the same experiment are reported. Surface sea water was collected in the northwest Mediterranean Sea (42°28.3' N—03°15.5' E, 95 m depth) and used to prepare the growth media and inoculate the continuous cultures. To make growth media, roughly 300 l was collected in September 2009 for Exp_SwDi and in January 2010 for Exp_CyDi and each stored in a polycarbonate carboy in the dark at room temperature for 6 weeks to allow degradation of most of the labile DOM. The phytoplankton cultures were

grown in low DOM (15 μM DOC) artificial sea water amended with nutrients according to the Guillard's F/2 medium (Guillard, 1975), and DOM was harvested in the early stationary phase by sequential filtration through a combusted GF/F filter (90 mm diameter, Whatman/GE Life Sciences, Pittsburgh, PA, USA) and an acid-washed, Milli-Q water rinsed 0.2 μm pore size polycarbonate membrane filter (90 mm diameter, Nucleopore/GE Life Sciences, Pittsburgh, PA, USA). In Exp_SwDi, *Phaeodactylum*-derived DOM was mixed with sea water (74 μM DOC) to obtain 13 μM carbon as phytoplankton-derived DOM in the diatom treatment. In Exp_CyDi, each of the DOM sources was mixed with sea water (63 μM DOC) to obtain 15 μM carbon as phytoplankton-derived DOM in each treatment. Nitrogen (NO_3^- in Exp_SwDi, and NO_3^- and NH_4^+ in Exp_CyDi) and phosphorus (PO_4^{3-}) were added in excess in the initial cultures as well as in the growth media to assure that organic carbon was the limiting factor for bacterial growth (Landa et al., 2013a, b).

The bacterial cultures were prepared by inoculating the growth media with a natural bacterial community (<0.8 μm filtrate) in a 10:1 ratio (final volume 6 l). Cultures from the same experiment were inoculated with the same bacterial community that was collected on the first day of each experiment in the NW Mediterranean Sea as described above on 14 November 2009 for Exp_SwDi and on 1 March 2010 for Exp_CyDi. Bacterial growth was maintained in batch mode until stationary phase was reached, and cultures were then switched to continuous mode for the duration of the incubation. For both experiments, duplicate cultures were established for each treatment. In Exp_SwDi, however, bacterial abundance decreased over time (Landa et al., 2013a) in one of the seawater cultures, and this replicate was eliminated from further analyses. The dilution rate in Exp_SwDi was 0.27 per day and in Exp_CyDi was 0.28 per day, and

cultures were maintained over 5 and 6 generations, respectively.

Sampling for metatranscriptomic analyses

Sampling and further processing of the metatranscriptomic analyses have been described previously (Beier et al., 2014). After 15 days, samples for metatranscriptomic analyses were siphoned from the incubation vessels into a combusted glass bottle. Roughly 1 liter (in duplicate) was immediately filtered through nitrocellulose membranes (GSWP, Millipore, Billerica, MA, USA, 0.2 μm pore size, 25 mm diameter) using a peristaltic pump. The flow rate was 100 ml min^{-1} and filtration time did not exceed 10 min. From each continuous culture (biological replicates), two filters for RNA extraction were prepared (serving as technical replicates; Table 1). These filters were stored in RNAlater at −80 °C. Further subsamples for DNA extraction (100 ml) were filtered onto polyethersulfone filters (SUPOR, Pall Cooperation, Port Washington, NY, USA) 0.2 μm pore size, 13 mm diameter) and stored in lysis buffer (Qiagen, Valencia, CA, USA) at −80 °C. DNA was extracted with the Qiagen kit following the manufacturer's instructions. The extracted DNA was later used to produce antisense ribosomal RNA (rRNA) to subtract rRNA from the metatranscriptome samples as described below.

RNA processing and sequencing

RNA was extracted as published previously (Gifford et al., 2011). Briefly, frozen filters for RNA extraction were vortexed with PowerSoil beads (MOBIO, Carlsbad, CA, USA) in 2 ml RTL buffer (Qiagen), and were then processed following the instructions of the RNAeasy Kit (Qiagen). DNA was removed from the extracted RNA using the turboDNAse (Applied Biosystems, Austin, TX, USA). To further

Table 1 Schematic overview displaying the experimental setup

Experiment	Treatment	Biological replicates	Technical replicates
Exp_SwDi	Control (aged sea water)	Control 1	Control 1a
			Control 1b*
		Control 2	Control 2a*
			Control 2b*
	Diatom (aged sea water + *Phaeodactylum* exudate)	Diatom1	Diatom1a
			Diatom1b
		Diatom2	Diatom2a
			Diatom2b
Exp_CyDi	Cyano (aged sea water + *Synechococcus* exudate)	Cyano1	Cyano1a
			Cyano1b
		Cyano2	Cyano2a
			Cyano2b*
	Diatom (aged sea water + *Phaeodactylum* exudate)	Diatom1	Diatom1a
			Diatom1b
		Diatom2	Diatom2a*
			Diatom2b

Samples marked with an asterisk were lost during the experimental procedure or excluded from data analyses for reasons given in the text (this figure has been published previously, Beier et al., 2014).

subtract rRNA, we used a protocol based on hybridization with antisense rRNA amplified from DNA of the same samples (Stewart *et al.,* 2010). Analyses of the DNA-free RNA extract (Bioanalyzer, Agilent Technologies, Santa Clara, CA, USA) indicated the presence of eukaryotic rRNA in Exp_CyDi, but not in Exp_SwDi; we therefore subtracted eukaryotic rRNA from the samples of Exp_CyDi in addition to bacterial rRNA using the same antisense rRNA method. The remaining RNA was amplified using the Message Amp II-Bacteria kit (Life Technologies, Grand Island, NY, USA) and finally reverse transcribed into complementary DNA using Super-Script III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) and NEBNext mRNA Second-Strand Synthesis Module (New England Biolabs, Ipswich, MA, USA). Transcripts were sheared to ∼220 bp and paired-end sequencing was performed at the Duke Institute for Genome Sciences and Policy (GAIIx platform, Illumina Inc., San Diego, CA, USA). Sequences are available via the CAMERA database (http://camera.calit2.net) under accession number CAM_P_0001134.

Bioinformatics analysis

The SHE-RA software (Rodrigue *et al.,* 2010) was used to join paired-end reads and sort out low-quality reads using 0.5 as the cutoff value. Further quality trimming was carried out with the SeqTrim pipeline (Falgueras *et al.,* 2010) with the parameters set as follows: minimal read length = 100; maximal frequency of Ns = 0.18; minimal nucleotide quality for a called base = 20; window width = 10. Non-mRNA reads (rRNAs, internal standards) were detected by a Usearch (using the ublast nucleotide algorithm with standard parameters, Edgar, 2010) of the quality trimmed reads against a sequence database containing reference rRNA gene sequences of taxa in major phylogenetic lineages (Gifford *et al.,* 2012). All reads with a bitscore ⩽50 were categorized as putative mRNA sequences and kept for further analyses. The average read length of these mRNA reads was 197 bp.

The potential mRNA reads were compared in a local Blastx search (Altschul *et al.,* 1997) against the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) RefSeq database (version 49) and top hits with a bitscore ⩾40 were kept for information on taxonomic affiliation, with the exception of hits (defined by GI numbers) that were only detected once in the total data set (Table 2). Transcripts were sorted into genome bins, referred to as 'taxa', based on the closest hit to a bacterial reference genome (Table 3).

Reads that were affiliated with genomes of prokaryotic origin (Bacteria, Archaea) were functionally assigned to orthologs of the Kyoto Encyclopedia of Genes and Genomes (KEGG, Kanehisa *et al.,* 2007). For this purpose, a local Blastx search against the full KO-database provided

Table 2 Sequencing results

Technical replicate	No. of mRNA-tax	No. of mRNA-fnc	%fnc	No. of taxa	No. of genes
Exp_SwDi: control 1a	4 602 232	3 091 085	67	1822	6095
Exp_SwDi: diatom1a	1 942 427	1 311 716	68	1783	5251
Exp_SwDi: diatom1b	2 560 303	1 601 515	63	1796	5234
Exp_SwDi: diatom2a	1 385 098	988 978	71	1740	4817
Exp_SwDi: diatom2b	1 383 864	1 058 036	76	1650	4241
Exp_CyDi: cyano1a	734 343	548 611	75	1699	4790
Exp_CyDi: cyano1b	809 356	615 961	76	1700	4806
Exp_CyDi: cyano2a	553 250	414 514	75	1626	4362
Exp_CyDi: diatom1a	740 665	531 561	72	1731	4804
Exp_CyDi: diatom1b	1 336 344	913 605	68	1774	5272
Exp_CyDi: diatom2b	1 176 335	841 247	72	1758	5170
Total	17 224 217	11 502 315	71	1868	7876

Abbreviations: %fnc, percent of prokaryotic reads that were assigned to any function; genes, number of detected genes; mRNA-fnc, number of prokaryotic reads with assigned function (genes); mRNA-tax, number of mRNA reads with prokaryotic taxonomic assignment; taxa, number of detected taxa.
Modified from Beier *et al.* (2014).

by KOBAS 2.0 (http://kobas.cbi.pku.edu.cn/home.do; download: February 2012) was performed using the Usearch ublast protein algorithm while adjusting the settings to keep the 10 first hits and reject up to 1000 sequences. The best hit that could be assigned to a KEGG ortholog was kept for further statistical analyses. Hits were selected according to cutoff criteria tested previously (Mou *et al.,* 2008): *E*-value ⩽0.1, identity ⩾40% and alignment coverage ⩾23 amino acids. In the remainder of the article, KEGG orthologs are referred to as 'genes' (Table 3).

Sample similarity

Count data derived from the functional (KEGG ortholog) and taxonomic (genome bin) assignments were randomly subsampled to obtain an equal number of reads for each technical replicate (that is, size normalized; Figures 1a and b) in order to construct dendrograms (Bray–Curtis dissimilarity, Ward's linkage). In the dendrogram based on functional binning (Figure 3a), one technical replicate in each of the two treatments of Exp_CyDi clustered separately from the remaining replicates of the treatment. We found evidence for possible contamination of these samples with genomic DNA (Beier *et al.*, 2014) and reads from these two replicates were therefore excluded from further analyses (Table 1). In order to quantify the correlation between the functional and taxonomic response, a Mantel test (Mantel, 1967) using Pearson's correlation and 10 000 permutations was performed.

Plasticity of taxonomic bins

PP was estimated using the Bray–Curtis dissimilarity index. The Bray–Curtis distance is a dissimilarity measurement that assesses the degree of compositional dissimilarity between two samples by estimating the

Table 3 Technical definition of terms used in the manuscript

Term	Methodological definition
Taxon	All transcripts binning to an individual genome of the Refseq database (NCBI, http://www.ncbi.nlm.nih.gov/).
Gene	All transcripts binning to an individual entry of the KEGG database (Kanehisa *et al.,* 2007).
Taxon transcript abundance	Relative abundance of transcripts assigned to an individual taxon that are due either to abundance changes of the taxon or to changes in per-cell transcription levels.
Phenotypic plasticity (PP)	Quantitative changes in gene expression within taxa because of changes in gene composition between treatments. Taxa were normalized to a transcriptome size of 2000 transcripts and changes in gene composition were quantified using the Bray–Curtis dissimilarity index (Figure 1c).
Weighted average PP	Average PP for all taxa. A weighting factor was introduced to account for differences in taxon abundance. This value estimates the average degree of PP within the community (Equation (2)).
Total plasticity (TP)	Quantitative changes in gene expression within taxa that considers both changes in gene composition (PP) and taxon abundance change between treatments. Gene counts of size-normalized taxa (2000 transcripts) were multiplied with the fold difference of each taxon before calculating Bray–Curtis dissimilarity (Figure 1d). The resulting Bray–Curtis values were subsequently corrected for the changed sample size (Equation (1)).
Weighted average TP	Average TP for all taxa. A weighting factor was introduced to account for differences in taxon abundance. This value estimates the average degree of TP within the community (Equation (3)).
Community functional change	Quantitative changes in gene composition between treatments using the Bray–Curtis dissimilarity index (Figure 1).
Theoretical community functional change	This value equals the community functional change if changes of transcribed genes in individual taxa are never counterbalanced by transcription of the same function gene in other taxa (Equation (4)).
Functional stability	Difference between the theoretical community functional change and the community functional change (Equation (5)). This difference indicates the amount of change in gene transcription of individual taxa that were counterbalanced by other taxa.
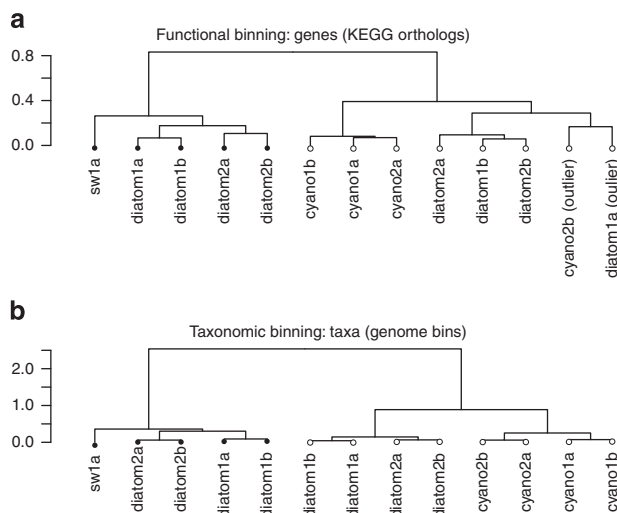
Figure 3 Dendrograms (Bray–Curtis dissimilarity, Ward's linkage) based on normalized count data after functional (**a**) and taxonomic (**b**) binning of the sequence data. Technical replicates from Exp_SwDi are labeled with filled circles and technical replicates from Exp_CyDi are labeled with open circles (modified from Beier *et al.*, 2014).

number of differences between samples in relation to the total number of elements in both samples: values from 0 (identical expressed genes in a taxon in two compared samples) to 1 (no overlap of expressed genes in a taxon in two compared samples) were assigned. The data used to estimate PP by the Bray–Curtis dissimilarity were normalized by both taxonomic bin and experimental treatment (Figure 1c and Table 3). In order to avoid biases of undersampling, only genome bins that had

assignments of at least 2000 reads in each treatment were considered, and each bin was randomly subsampled to 2000 reads. Genome bins were not compared across the two experiments (Exp_SwDi and Exp_CyDi), as they had different community inocula. To examine relationships between PP of individual taxa and phylogeny of these taxa, values for PP were grouped by phylogenetic order and subjected to a randomized analysis of variance (10 000 randomizations, RundomPro, vs 3.14, Jadwiszczak, 2009). Orders that contained < 5 taxa were removed from this analysis and unequal variances were assumed to account for the unbalanced distribution of taxa.

Functional changes in a microbial community are not only caused by changes in PP for the member taxa, but also by the changes in the number of transcripts each taxon is contributing that is related to cell abundance in the community. In order to estimate the magnitude of all gene transcription changes that a taxon contributes, we developed a parameter that we call total plasticity (TP, Table 3). TP was calculated similarly to PP but also included the effect of differences in transcript contributions to the community transcriptome. This was accomplished by multiplying the abundance of genes within a taxon that was previously normalized to a transcriptome size of 2000 (Figure 1c) by its fold difference in taxon transcript abundance (Supplementary Tables S1 and S2) between treatments. Subsequently Bray–Curtis dissimilarities were computed. This scaling allowed the values of PP and TP to be compared by keeping gene richness and evenness constant; extracting the corresponding number of taxon transcripts directly from the total

data set would have changed the richness. However, scaling increases the total number of transcripts, meaning that less weight is given to the number of introduced changes when compared with PP. To be able to directly relate PP to TP, we introduced the following correction that increases the Bray–Curtis dissimilarity after multiplication of taxon-specific gene abundance data with the fold difference (BC_{fd}) for the same factor as the number of gene counts was increased:

$$TP = \frac{BC_{fd} + fd \times BC_{fd}}{2} \tag{1}$$

where fd is the fold difference (Supplementary Table S2).

This measurement for TP can reach values >1 and typically provides higher dissimilarities than those calculated for PP because including changes in taxon transcript abundance further accentuates the expression differences of taxa between treatments. The ratio of PP to TP gives the frequency of changes within a taxon that were introduced by PP and not by transcript abundance changes.

To estimate the importance of PP in the community we calculated the contribution of average PP to average TP. The average contained values for PP and TP of all taxa with at least 2000 transcripts and was weighted according to the taxon transcript abundances in each experiment in order to give a higher weight to taxa with high transcript abundance:

$$\overline{PP} = \sum_{i=1}^{n} (PP_i \times ab_i) \tag{2}$$

$$\overline{TP} = \sum_{i=1}^{n} (TP_i \times ab_i) \tag{3}$$

where ab is the average relative abundance of a taxon in both compared samples (Supplementary Table S2) and n is the number of taxa exceeding 2000 transcripts. As values for the weighted average TP and PP rely on subsampling, calculation of these values was repeated 100 times to retrieve a bootstrapped mean value and estimate the variation introduced by subsampling.

The influence of PP on community function
To obtain estimates of the community functional change, Bray–Curtis dissimilarities were calculated for the pooled transcripts of all taxa included in the weighted average measure (Figure 1a). To be able to compare the data sets of individual taxa and the pooled community, we normalized the community data by pooling the 2000 transcripts extracted for individual taxa after accounting for fold difference of taxon transcript abundance. This procedure allows us to relate values for weighted average PP/TP and the community functional change to each other. Values retrieved for community functional change are only representative of the subset of the

community with sufficient transcript coverage. However, because different numbers of taxa were considered in each experiment (46 versus 99 taxa in Exp_SwDi and Exp_CyDi) the values for the community functional change are not directly comparable across experiments. This pooling of community data was repeated 100 times to retrieve a bootstrapped mean value for the community's functional change.

Changes in community function can be caused either by changes in taxon transcript abundance or by PP that together make up the metric of TP. However, the sum of gene transcription changes in individual taxa is not fully reflected in the community functional change if counteracting changes in other taxa outweigh changes introduced by an individual taxon. We refer to the replacement of transcribed genes by another taxon as 'functional stability' (Figure 2 and Table 3). The community functional change equals the average weighted Bray–Curtis dissimilarities multiplied by the fold difference (BC_{fd}) if transcription by other members in a community are not considered to counteract transcription differences in individual taxa (see Supplementary Table S1). This value was therefore designated as the theoretical community functional change and the presence and magnitude of functional stability (FS) in a community was quantified as the theoretical community functional change minus the community functional change (Figure 2 and Supplementary Table S1):

$$TFC = \sum_{i=1}^{n} BC_{fdi} \times ab_i \tag{4}$$

$$FS = TFC - FC \tag{5}$$

where TFC is the theoretical community functional change and FC is the community functional change.

The minimal contribution of PP (min_{fc}) to the community functional change or the (min_{fs}) functional stability was then estimated by first scaling values of average weighted PP to the TFC using the ratio of average weighted PP to TP:

$$min_{fc} = \frac{\left(TFC \times \frac{\overline{PP}}{\overline{TP}}\right) - FS}{FC} \tag{6}$$

$$min_{fs} = \frac{\left(TFC \times \frac{\overline{PP}}{\overline{TP}}\right) - FC}{FS} \tag{7}$$

If Equation (6) or (7) results in a value $\leqslant 0$, the minimum contribution was not detectable.

To assess diversity in the assembled communities, we calculated the Shannon index as well as Pielou's evenness for both treatments in Exp_SwDi and Exp_CyDi.

Results

Microbial dynamics in the continuous cultures

Nutrient conditions remained stable in the continuous cultures during the 15-day experimental period (Landa et al., 2013a, b). Bacterial abundances stabilized after the initial 24 h in 3 out of 4 cultures in Exp_SwDi, but substantially decreased over time in one of the seawater cultures; this replicate was discarded in all further analyses. Bacterial abundances ranged from 2.6 to 4.6×10^6 cells per ml in the remaining Exp_SwDi cultures (Landa et al., 2013a). In Exp_CyDi, bacterial abundances remained stable at $4.2–5.1 \times 10^6$ cells per ml until day 8, after which a decrease in bacterial abundances was detectable until day 11, followed by a second stable phase at $5.2–7.5 \times 10^5$ cells per ml. This decrease in bacterial abundance was accompanied by an increase in heterotrophic nanoflagellates (Landa et al., 2013b). Metatranscriptomic analyses were performed on samples collected for both experiments at day 15.

Sample similarity

In total, we obtained > 17 million prokaryotic reads of suitable quality for downstream analysis, of which 71% could be assigned to a function (Table 2). Cluster analyses revealed congruent grouping of the sequence data when binned according to either taxonomic or functional categories, with the exception that the reads derived from two samples in Exp_CyDi (cyano2b/diatom1a) clustered separately from the replicates of the same treatment in functional-based clustering (Figure 3a) but not taxonomic-based clustering (Figure 3b). Read patterns from these technical replicates suggested that genomic DNA may have remained in the RNA preparations (Beier et al., 2014) and the replicates were therefore removed from all downstream analyses. The reads of all the remaining technical replicates grouped according to the treatments as defined in Table 1. Cluster analyses based on gene bins (Figure 3a) therefore revealed a functional response of the microbial community at the gene level to the treatments in both experiments, a prerequisite for analyzing PP.

The Mantel test performed after removing cyano2b and diatom1a (Exp_CyDi) confirmed analog grouping of technical replicates within the dendrograms for both functional and taxonomic binning, returning a highly significant correlation between both data sets ($R^2 = 0.93$, $P = 10^{-4}$).

Plasticity of taxonomic bins

PP and TP estimates were made for 46 and 99 genome bins for Exp_SwDi and Exp_CyDi, respectively, restricting the analysis to bins with a minimum of 2000 transcripts per treatment. None of the excluded taxa accounted for > 0.8% of the community transcriptome, and therefore the individual influence of an excluded taxon was minimal. In experiment Exp_SwDi, the majority of genome bins had PP values ranging from 0.18 to 0.75 (mean: 0.45 ± 1.34; Figure 4a and Supplementary Table S2). PP was less variable in Exp_CyDi, with values ranging from 0.02 to 0.62 (mean: 0.43 ± 0.10; Figure 4b and Supplementary Table S2).

A randomized analysis of variance suggested a significant relationship between the PP of a taxon and its phylogenetic affiliation at the order level ($P = 0.001$, F = 5.1). Some orders were excluded from the analysis of variance because they were represented by < 5 individual taxa and only taxa within four orders were subjected to the analyses. Rhodobacterales had a significantly higher PP than other taxa, with other taxa also having more spread in PP values (Figure 5).

In both experiments, the weighted average PP contributed substantially to the weighted average TP, with 62% and 39% in Exp_SwDi and Exp_CyDi, respectively (Table 4). This means that an average taxon changed its gene expression by 62% or 39% because of PP, and by 48% or 61% owing to abundance changes in Exp_SwDi and Exp_CyDi, respectively. Consequently, in the theoretical case of functional stability being absent from our systems, 62% and 39% of the community functional change was based only on PP.

The influence of PP on community function

By applying Equation (5), we could demonstrate that in both experiments the theoretical community functional change was higher than the community functional change (Table 4). This demonstrates that
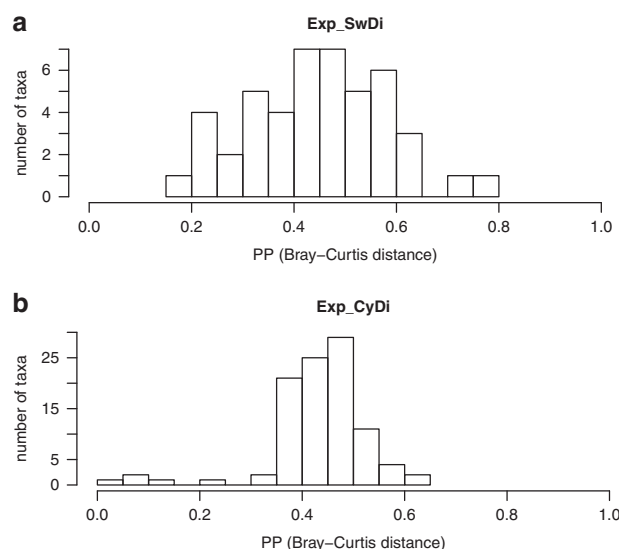

Figure 4 Histogram displaying the variability of PP in 46 (**a**) and 99 (**b**) taxa (> 2000 transcripts each) in Exp_SwDi and Exp_CyDi, respectively. The magnitude of PP was estimated by Bray–Curtis dissimilarities of functional bins for the same taxon in different treatments within an experiment. Increasing Bray–Curtis values indicate increasing PP.

counteracting transcriptional changes in some taxa balanced transcriptional changes in others and suggests a degree of functional stability in the communities (Supplementary Table S1). Our results further point to higher functional stability in Exp_CyDi than in Exp_SwDi.

Both the community functional change and functional stability must be because of changes in transcript abundance of individual taxa and/or PP: if the weighted average PP alone introduces more change than reflected in the community functional change, this 'excess PP' must contribute to functional stability of a system and indicates a minimum
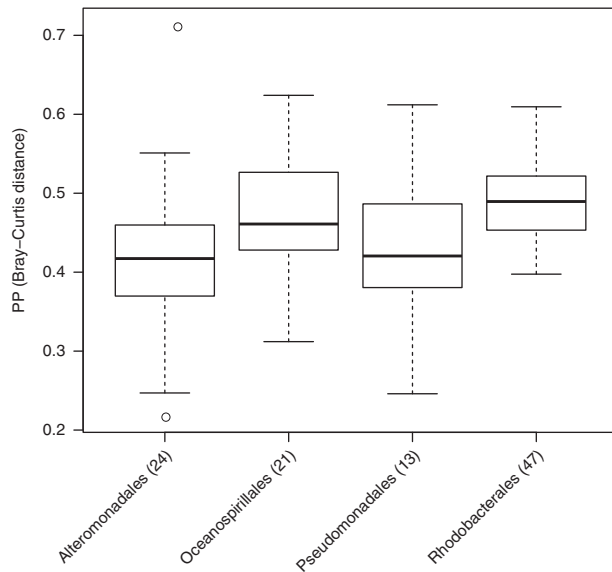

Figure 5 Boxplot displaying PP of taxa from Exp_SwDi and Exp_CyDi after grouping taxa according to their phylogenetic order. Numbers in brackets following the order names indicate the number of taxa affiliated with the order. Orders with <5 taxa were excluded from analysis. Open circles indicate outliers with values exceeding by 1.5-fold the distance of the interquartile range.

estimate of the fraction of weighted average PP that contributes to functional stability. On the other hand, if changes due to weighted average PP are lower than the community functional change, we cannot delineate a minimum fraction of PP that contributed to functional stability nor can we exclude that PP contributed to it. These considerations can be applied similarly to obtain minimum contributions of the weighted average PP to the community functional change. Accordingly, the results of Equations (6) and (7) demonstrate that the minimum contribution of average weighted PP to the community functional change in Exp_SwDi was 38% and the minimum contribution of PP to functional stability in Exp_CyDi was 6% (Table 4). However, we could not evaluate whether and to what extent the weighted average PP contributed to the functional stability in Exp_SwDi and the community functional change in Exp_CyDi, because for these parameters the minimum estimates (from Equations (6) and (7)) did not exceed 0 (Table 4). The taxon diversity of the assembled community was lower in Exp_SwDi than in Exp_CyDi, and accordingly evenness in Exp_SwDi was reduced compared with Exp_CyDi (Table 4).

Discussion

PP has usually been measured based on the response of a few traits in single taxa (Chevin *et al.*, 2013). Using metatranscriptomic data to quantify PP provides a unique opportunity to follow differences across all expressed traits of multiple taxa within a complex community. We believe that we can neglect possible evolutionary adaptation of individual taxa to different treatments in our case because the cultures were sampled after only 5 or 6 generation times. High PP may enhance the success of species in a changing environment (Wennersten and Forsman, 2012; Chevin *et al.*, 2013), but may

Table 4 Summary of PP and its contribution to community functioning and diversity indices

	Exp_SwDi	Exp_CyDi
Number of taxa considered	46	99
Weighted average TP	0.618 ± 0.007	1.084 ± 0.003
Weighted average PP (% of TP)	0.380 ± 0.007 (62%)	0.420 ± 0.002 (39%)
Community functional change	0.258 ± 0.005	0.185 ± 0.004
Theoretical community functional change	0.413 ± 0.005	0.521 ± 0.002
Functional stability	0.155	0.336
Minimum contribution of PP to community functional change	40%	0%
Minimum contribution of PP to functional stability	0%	5%
Shannon diversity in the control/cyano treatment	2.02	3.75
Shannon diversity in the diatom treatment	2.00	3.69
Pielou's evenness in the control/cyano treatment	0.52	0.81
Pielou's evenness in the diatom treatment	0.53	0.82

Abbreviations: PP, phenotypic plasticity; TP, total plasticity.
Values for weighted average TP, weighted average PP, community functional change and theoretical community functional change are derived from Bray–Curtis dissimilarities and numbers given behind '±' are s.d. values from bootstrapping after subsampling the total data set with $n = 100$.

also introduce costs (Auld et al., 2010). Thus, life history strategies based on both high and low PP may be ecologically advantageous in the same community. Indeed, we found a wide range of PP across the microbial taxa surveyed in the continuous culture communities, even though only a subset of the natural diversity was analyzed (46 taxa in Exp_SwDi and 99 taxa in Exp_CyDi).

The significant relationship between the PP of a taxon and its phylogenetic affiliation at the order level (Figure 5) indicates that a trend for high plasticity may be a consistent trait of some lineages, with *Rhodobacterales* featuring a genetic propensity for high PP. This is in agreement with earlier findings that species within the *Rhodobacterales,* including the *Roseobacter* clade, are versatile organisms that can adapt rapidly to changing conditions (Moran et al., 2004; Polz et al., 2006). The higher contribution of members of the *Rhodobacterales* to the community in Exp_CyDi (Supplementary Table S2) may explain the lower variance in PP in this experiment (Figure 4).

In both experiments we found that ∼50% of the weighted average TP was contributed by the weighted average of PP (Table 4). Although comparable data from other studies are not available because the metric for PP is new, our results point to the importance of PP in explaining functional attributes of prokaryotic communities in the continuous cultures. High PP allows taxa to react to changing environmental conditions. Consequently, our results may in part explain why the composition of the bacterial communities was not different between treatments in Exp_CyDi (Landa et al. 2013b). The continuous cultures possibly selected for versatile taxa that are adapted to a variety of available carbon sources, and indeed the microbial taxa dominating the continuous culture communities, have been identified in previous studies as opportunists that grow well in laboratory situations (Supplementary Table S2). Conversely, organisms with an oligotrophic lifestyle like *Pelagibacter* were not present or were found in low abundance and therefore excluded from our analyses (Supplementary Table S2). Opportunists are thought to exploit spatially and temporally variable resources (Polz et al., 2006) and may therefore feature higher PP. If so, marine prokaryotic communities in the ocean might have a distribution of PP that is shifted to lower values as compared with those measured in our continuous cultures (Figure 4), and a future research objective is to determine whether faster growing taxa are in general more versatile than slower growing taxa.

The magnitude of PP may also depend on characteristics of habitat structure other than those related to growth rate, as fine-grained habitats (featuring little or no physical structure) are hypothesized to foster an increased plastic response in taxa (Wennersten and Forsman, 2012). The present study had a well-mixed (fine-grained) environment where niches for specialized metabolic strategies associated with low PP may be scarce. Systems with high particle loads, on the other hand, may provide more physical niches in close proximity to one another and favor taxa that move to new environments rather than switch metabolism. To the best of our knowledge, this is the first time that PP has been measured by considering all expressed traits simultaneously in multiple taxa of a complex community, and it will be interesting to compare our results with similar studies in environments with different physical and chemical substructure.

In communities with a highly skewed distribution of taxa expressed in low values for evenness (Table 4), the highly abundant members of the community (for example, gamma proteobacterium IMCC 1989 in Exp_SwDi with 47% transcript abundance; Supplementary Table S2) have a considerable impact on the weighted average PP, and that may lead to more extreme values for the parameter in communities with low evenness.

A caveat in our use of metatranscriptomic data to estimate PP is that taxa are defined based on binning to reference genomes. This may lead to the possibility that transcripts of individual organisms in our cultures are split between genome bins (because of equidistance of transcripts to different reference genomes), or aggregated into genome bins that do not represent single species. In future studies, ever increasing sequencing depths will allow reference genomes to be assembled routinely from metagenomes, including from less abundant taxa, allowing further decreases in any biases due to binning errors.

In our current data, results of several tests indicated that the genes transcribed by the same taxon in different treatments were largely derived from the same gene pool (Supplementary Figures S1 and S2 and Supplementary Table S3) and PP of individual taxa was independent of transcript abundance (Supplementary Figure S3). High similarity of 16S rRNA sequences to the 16S rRNA sequences from the reference genomes (Supplementary Table S2) demonstrate that close relatives of the organisms were present in the RefSeq database, increasing the quality of binning. The presence of transcripts coding for ribosomal proteins, oxidative phosphorylation and rpoB in the great majority of taxon bins (Supplementary Table S2 and Supplementary Figure S4) further indicates suitable binning of the transcripts into biologically relevant taxon bins. Finally, use of weighted average values for estimating the impact on PP in our experiments balances any biases in the individual taxon bins, as biases introduced by binning errors would not be expected to cause a directional shift to either consistently higher or lower values for PP. Binning of transcripts to related genomes is therefore not likely to result in biases that are sufficiently strong or systematic to influence our overall conclusions.

The importance of PP from an autecological perspective has been well recognized (Wennersten and Forsman, 2012). However, less is known about how PP of individual taxa influences the function of a total community, and whether it weakens the link between community composition and functional response. The significant correlation found here between functional and taxonomic data at the community level (Figure 3) points to a coupling between taxon composition and gene transcription despite evidence for pronounced PP.

Because the sum of functional changes within individual taxa (measured as theoretical community functional change) was higher than the community functional change, our data demonstrate that the coexistence of several taxa in microbial communities induced functional stability in our continuous culture system. Our results further indicate a higher degree of functional stability in Exp_CyDi, where more taxa were considered (99 vs 46 taxa in Exp_CyDi vs Exp_SwDi) and may point to a stabilizing effect in systems with increased taxonomic diversity. Minimum estimations (Equations (6) and (7)) predict that PP can potentially contribute to both community functional change and functional stability (Table 4).

The present study demonstrates that considering only shifts in species composition may miss a substantial aspect of community adaptation to changing environments, particularly for microbial systems. An improved appreciation of the role of PP will help to better understand characteristics of a community, such as taxonomic diversity and its connection to functional stability (Yachi and Loreau, 1999), in response to environmental change.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

References

Altschul SF, Madden TL, Schäffer AA, Zhang JH, Zhang Z, Miller W et al. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Auld JR, Agrawal AA, Relyea RA. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proc R Soc B Biol Sci* **277**: 503–511.

Beier S, Rivers AR, Moran MA, Obernosterer I. (2014). The transcriptional response of prokaryotes to phytoplankton-derived DOM in seawater. *Environ Microbiol;* e-pub ahead of print 25 February 2014; doi:10.1111/1462-2920.12434.

Chevin L-M, Gallet R, Gomulkiewicz R, Holt RD, Fellous S. (2013). Phenotypic plasticity in evolutionary rescue experiments. *Philos Trans R Soc Lond B Biol Sci* **368**: 20120089.

Chevin L-M, Lande R, Mace GM. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol* **8**: e1000357.

Comte J, Fauteux L, Del Giorgio PA. (2013). Links between metabolic plasticity and functional redundancy in freshwater bacterioplankton communities. *Front Microbiol* **4**: 112.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.

Falgueras J, Lara A, Fernández-Pozo N, Cantón F, Pérez-Trabado G, Claros MG. (2010). SeqTrim: a high-throughput pipeline for pre-processing any type of sequence read. *BMC Bioinformatics* **11**: 38.

Gifford SM, Sharma S, Booth M, Moran MA. (2012). Expression patterns reveal niche diversification in a marine microbial assemblage. *ISME J* **7**: 281–298.

Gifford SM, Sharma S, Rinta-Kanto JM, Moran MA. (2011). Quantitative analysis of a deeply sequenced marine microbial metatranscriptome. *ISME J* **5**: 461–472.

Griffiths BS, Ritz K, Bardgett RD, Cook R, Christensen S, Ekelund F et al. (2000). Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. *Oikos* **90**: 279–294.

Guillard RR. (1975). Culture of phytoplankton for feeding marine invertebrates. In Smith CL, Chanley MH (eds) *Culture of Marine Invertebrate Animals*. Plenum Press: New York, pp 26–60.

Jadwiszczak P. (2009). Rundom Pro 3.14. Software for classical and computer-intensive statistics. http://pjadw.tripod.com.

Jansson JK, Neufeld JD, Moran MA, Gilbert JA. (2012). Omics for understanding microbial functional dynamics. *Environ Microbiol* **14**: 1–3.

Jump AS, Penuelas J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecol Lett* **8**: 1010–1020.

Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M et al. (2007). KEGG for linking genomes to life and the environment. *Nucleic Acids Res* **36**: D480–D484.

Landa M, Cottrell MT, Kirchman DL, Blain S, Obernosterer I. (2013a). Changes in bacterial diversity in response to dissolved organic matter supply in a continuous culture experiment. *Aquat Microb Ecol* **69**: 157–168.

Landa M, Cottrell MT, Kirchman DL, Kaiser K, Medeiros PM, Tremblay L et al. (2013b). Phylogenetic and structural response of heterotrophic bacteria to dissolved organic matter of different chemical composition in a continuous culture study. *Environ Microbiol* **16**: 1668–1681.

Langenheder S, Lindström ES, Tranvik LJ. (2006). Structure and function of bacterial communities emerging from different sources under identical conditions. *Appl Environ Microbiol* **72**: 212–220.

Mantel N. (1967). Detection of disease clustering and a generalized regression approach. *Cancer Res* **27**: 209–230.

Moran MA, Buchan A, Gonzalez JM, Heidelberg JF, Whitman WB, Kiene RP *et al.* (2004). Genome sequence of Silicibacter pomeroyi reveals adaptations to the marine environment. *Nature* **432**: 910–913.

Mou XZ, Sun SL, Edwards RA, Hodson RE, Moran MA. (2008). Bacterial carbon processing by generalist species in the coastal ocean. *Nature* **451**: 708–U4.

Pigliucci M. (2001). *Phenotypic Plasticity Beyond Nature and Nurture*. The John Hopkins University Press: Baltimore.

Polz MF, Hunt DE, Preheim SP, Weinreich DM. (2006). Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. *Philos Trans R Soc B Biol Sci* **361**: 2009–2021.

Rodrigue S, Materna AC, Timberlake SC, Blackburn MC, Malmstrom RR, Alm EJ *et al.* (2010). Unlocking short read sequencing for metagenomics. *PLoS One* **5**: e11840.

Stewart FJ, Ottesen EA, DeLong EF. (2010). Development and quantitative analyses of a universal rRNA-subtraction protocol for microbial metatranscriptomics. *ISME J* **4**: 896–907.

Wennersten L, Forsman A. (2012). Population-level consequences of polymorphism, plasticity and randomized phenotype switching: a review of predictions. *Biol Rev Camb Philos Soc* **87**: 756–767.

Yachi S, Loreau M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc Natl Acad Sci USA* **96**: 1463–1468.

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