Dynamics of the bacterial community associated with *Phaeodactylum tricornutum* cultures

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Bacterial community profile of diatom cultures

Abstract

The pennate diatom *Phaeodactylum tricornutum* is a model organism able to synthesise industrially-relevant molecules. Large-scale monocultures are prone to bio-contamination, however, little is known about the identity of the invading organisms. To gain insight into the bacterial community associated with diatoms, we translated the complexity of a natural system into reproducible experiments where we investigated the microbiome of *P. tricornutum* cultures. The results revealed a dynamic bacterial community that changed over time and in differing media conditions. We propose a network of putative interactions between *P. tricornutum* and the main bacterial factions, which is translated into a set of ordinary differential equations constituting a computational dynamic model. The proposed mathematical model is able to capture the population dynamics, further supporting the hypothesised interactions. The interdisciplinary approach implemented provides a framework for understanding the dynamics of diatom-associated microbial communities, and provides a foundation for further systematic investigations of host-microbe interactions.

Introduction

Phaeodactylum tricornutum is a diatom first described by Bohlin in 1897 when he found it in samples collected off the coast of Plymouth, United Kingdom. Diatoms belong to the Phylum *Heterokontophyta* and the Class *Bacillariophyceae* (Dangeard, 1933). They are the result of a secondary endosymbiotic event that took place around one billion years ago between a red alga (Rhodophyta) and a heterotrophic eukaryote (Bhattacharya *et al.*, 2007). Unlike most diatoms, which have the distinct ability to precipitate soluble silicic acid to form a silica cell wall, *P. tricornutum* has a poorly silicified cell wall and therefore does not have an obligate requirement for silicic acid (Montsant *et al.*, 2005; Martino *et al.*, 2007). *P. tricornutum* is found in coastal regions such as rock pools and estuaries where aquatic environmental parameters (salinity, temperature) vary greatly as a consequence of tidal changes and solar irradiation (Martino *et al.*, 2011). Its habitual characteristics, peculiar ability to form

45 oval, fusiform, and triradiate cells, as well as its poorly silicified cell wall, have triggered a tremendous
46 increase in scientific research on *P. tricornutum*. The genome sequencing of *P. tricornutum* was
47 completed in 2008, and the subsequent generation of expressed sequence tag (ESTs) databases make
48 *P. tricornutum* an excellent model organism (Montsant *et al.*, 2005; Martino *et al.*, 2007; Bowler *et al.*,
49 2008).

50 Driven by photosynthesis, *P. tricornutum* is able to synthesise a number of commercially relevant
51 molecules, applicable to various industries. In aquaculture, *P. tricornutum* is used as feed for bivalve,
52 echinoderm, crustacean and fish hatcheries (Ryther and Goldman, 1975; Tredici *et al.*, 2009). On
53 average, 18% of the *P. tricornutum* biomass are lipids, making it a potential candidate for biofuel
54 production (Kates and Volcani, 1966; Rebolloso-Fuentes *et al.*, 2001). Furthermore, *P. tricornutum* has
55 the ability to produce the poly-unsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA; 20:5n-3)
56 and docosahexaenoic acid (DHA; 22:6n-3) in high proportions of the total fatty acid content (Siron *et*
57 *al.*, 1989; Rebolloso-Fuentes *et al.*, 2001; Fajardo *et al.*, 2007). Marine-derived EPA and DHA,
58 colloquially known as omega-3 PUFAs, are important in human nutrition with a vast number of health
59 benefits (Yashodhara *et al.*, 2009). *P. tricornutum* is therefore an ideal source of omega-3 PUFAs for
60 the pharma- and nutraceutical industries.

61 To fully exploit the industrial potential of *P. tricornutum* derived products, substantial amounts of
62 microalgal biomass are required, preferably with low production costs. This is achieved by
63 implementation of large-scale cultivation methods such as open raceway ponds and various types of
64 photobioreactors. Microalgal cultivation methods rely on keeping monocultures of the desired
65 species, especially if the final product is a bioactive molecule for human consumption (Mata *et al.*,
66 2010). Photobioreactors (PBRs) are closed systems that allow for the production of monoseptic
67 cultures, fully isolated from potential contamination if cultivation protocols are followed correctly
68 (Grima and Fernández, 1999). However, high operational costs of PBRs would increase production
69 costs. The other option is open raceway ponds, which are simple open-air cultivation systems that
70 have been in use since the 1950s (Chisti, 2007). They are highly susceptible to contamination, and

unless the desired species is a halophile or thermophile (Parmar *et al.*, 2011), it is hard to maintain monocultures. Irrespective of the cultivation method, the establishment of unwanted organisms such as amoeba, ciliates, rotifers, bacteria, viruses, and other photosynthetic organisms in microalgal cultures, is a serious obstacle for large-scale microalgae cultivation (Day *et al.*, 2012; Wang *et al.*, 2013). Although much research is carried out in the field of microalgal culture upscaling, very little is known about the identity and characteristics of these invading organisms, responsible for microalgal culture 'crashes' which lead to loss of biomass, and therefore, loss of revenue.

The establishment of non-target organisms in microalgal cultures should not come as a surprise. Microalgae are not found in monoculture in nature and imposing such an environment is counterintuitive leading to unstable cultures. Rather than looking at these organisms as contaminants, understanding them could allow for the exploration of 'synthetic ecology' as a novel scaling up technique, a concept proposed by Kazamia *et al.*, 2012. The cornerstone of synthetic ecology is the Competitive Exclusion Principle, or Gause's Law, which states 'as a result of competition two species scarcely ever occupy similar niches, but displace each other in such a manner that each takes possession of certain peculiar kinds of food and modes of life in which it has an advantage over its competitor' (Gause, 1934; Hardin, 1960). By 'synthesising' a community of organisms that fills every niche in the ecosystem (i.e. the microalgal culture) supporting the growth of the desired microalgae, we prevent the establishment of other, potentially harmful organisms in the culture, and optimise the utilisation of nutrients (Kazamia *et al.*, 2012).

In order for synthetic ecology to be a legitimate contender as a novel scaling up technique, greater understanding of species-specific interactions is required. Bacteria are present in all of the Earths' biomes (Dykhuizen, 1998), and insight into the microorganisms (plankton) inhabiting our oceans was greatly improved by the three-year study abroad the schooner *Tara*. In May 2015, Sunagawa *et al.* published the metagenomics data from 243 samples collected from 68 unique locations during the *Tara* expedition. The data showed that 58.8% of the sequences belonged to bacteria, even though bacterial densities (10^5 to 10^6 per gram of seawater) in our oceans are orders of magnitudes less than

97 those found in sediments (10^8 cells per gram), humans (10^{14} cells per gram), or soil (10^9 cells per gram)
98 (Whitman *et al.*, 1998; Amin *et al.*, 2012). The data generated by the *Tara* project shows the sheer
99 amplitude of genetic material belonging to bacteria, coupled with their co-existence with diatoms for
100 more than 200 million years (Amin *et al.*, 2012), fuelled our interest in the microbiome of diatom
101 cultures. Furthermore, in 1958, Provasoli suggested that bacteria can enhance the growth of algae
102 (Provasoli, 1958). In the subsequent decades, species-specific studies have further corroborated his
103 initial idea (Delucca and Mccracken, 1977; Suminto and Hirayama, 1997). Furthermore, Bruckner *et*
104 *al.* showed an increase in growth of *P. tricornutum* when co-cultured with an Alphaproteobacterium
105 strain as well as when cultured in the spent media of the bacteria (Bruckner *et al.*, 2011). A recent
106 study conducted by Amin *et al.* shows a species-specific interaction between a coastal diatom, *Pseudo-*
107 *nitzschia multiseries*, and a bacterial *Sulfitobacter* species (SA11), where the bacteria was shown to
108 promote diatom cell division via secretion indole-3-acetic acid IAA, synthesised by the bacterium using
109 diatom secreted and endogenous tryptophan. The IAA and tryptophan act as signalling molecules in
110 this intricate diatom-bacteria relationship (Amin *et al.*, 2015).

111 With respect to the application in industry, the bacteria act as probiotics for the microalgae culture,
112 just as bacterial probiotics have been successfully implemented in human diet by the pharma- and
113 nutraceutical industries (Parvez and Malik, 2006), poultry industries (Kabir, 2009), and aquaculture
114 industries (Qi *et al.*, 2009), to name a few. By identifying the bacterial community in non-axenic *P.*
115 *tricornutum* cultures we can start to identify and characterise those that may have a beneficial role in
116 the cultures. Subsequently, a suitable candidate to fill a certain niche in the hypothetical synthetic
117 ecosystem could be chosen.

118 ## Results

119 In order to translate the complexity of a natural system into a reproducible, systematic experimental
120 approach, batch cultures of *Phaeodactylum tricornutum* (CCAP 1052/1B) were cultivated in two media
121 conditions; (1) complete F/2 medium with the addition of sodium metasilicate as the source of silicon,

and (2) minimal media with a source of nitrogen ($NaNO_3$) and phosphorus ($NaH_2PO_4.2H_2O$) at the same concentration as in the F/2 medium recipe. Samples were taken at different stages of growth and subsequent barcoded 16S-V6-Next Generation Sequencing carried out. After the implementation of a stringent bioinformatics approach, the identity and abundance of the bacteria present in *P. tricornutum* cultures was revealed. The in the temporal evolution of the relative abundances of bacteria were used to infer a network of interactions between the diatom and the four dominant bacteria families, which was then translated into a mathematical model reproducing the community dynamics.

Characteristics of *Phaeodactylum tricornutum* culture growth

The media composition was shown to have a significant effect on the growth characteristics of *P. tricornutum*. A significant difference (p=0.042, unpaired Wilcoxon signed rank) in the maximal cell density when *P. tricornutum* is cultivated in complete (9.3 x 10^6 cells/mL) or minimal media (11.2 x 10^6 cells/mL). The growth rates during the exponential phase in both cultures were $\mu_{complete} = 0.43 \pm 0.07$ d^{-1} and $\mu_{minimal} = 0.51 \pm 0.04$ d^{-1} respectively. In contrast, the death rates when the cultures 'crash' are $\delta_{complete} = 0.09 \pm 0.02$ d^{-1} and $\delta_{minimal} = 0.08 \pm 0.04$ d^{-1} respectively.

Bacterial community profile of *Phaeodactylum tricornutum* cultures

In order to identify the bacteria present in the *P. tricornutum* cultures, the Ion Torrent™ barcoded Next Generation Sequencing protocol was used to sequence the bacterial gDNA (Quail *et al.*, 2012; Grada and Weinbrecht, 2013). The subsequent 16S rRNA gene sequences were clustered to defined Operational Taxonomic Units (OTUs) using a threshold of ≥97% sequence identity, most of which could be assigned to the genera level (Supplementary Figure S2). Of the 9727 OTUs identified, 8109 corresponded to known sequences in the SILVA database (v.118) (Quast *et al.*, 2013). The OTU abundance at the phylum level showed that 99.97% of all OTUs belonged to Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes (Figure 1). A comparison of the number of individual

146 reads to the number of unique OTUs showed that the high number of reads per phyla is not the result
147 of a single OTU (Supplementary Figure S3). OTUs with hits to known 16S *P. tricornutum* chloroplast
148 sequences were discarded.

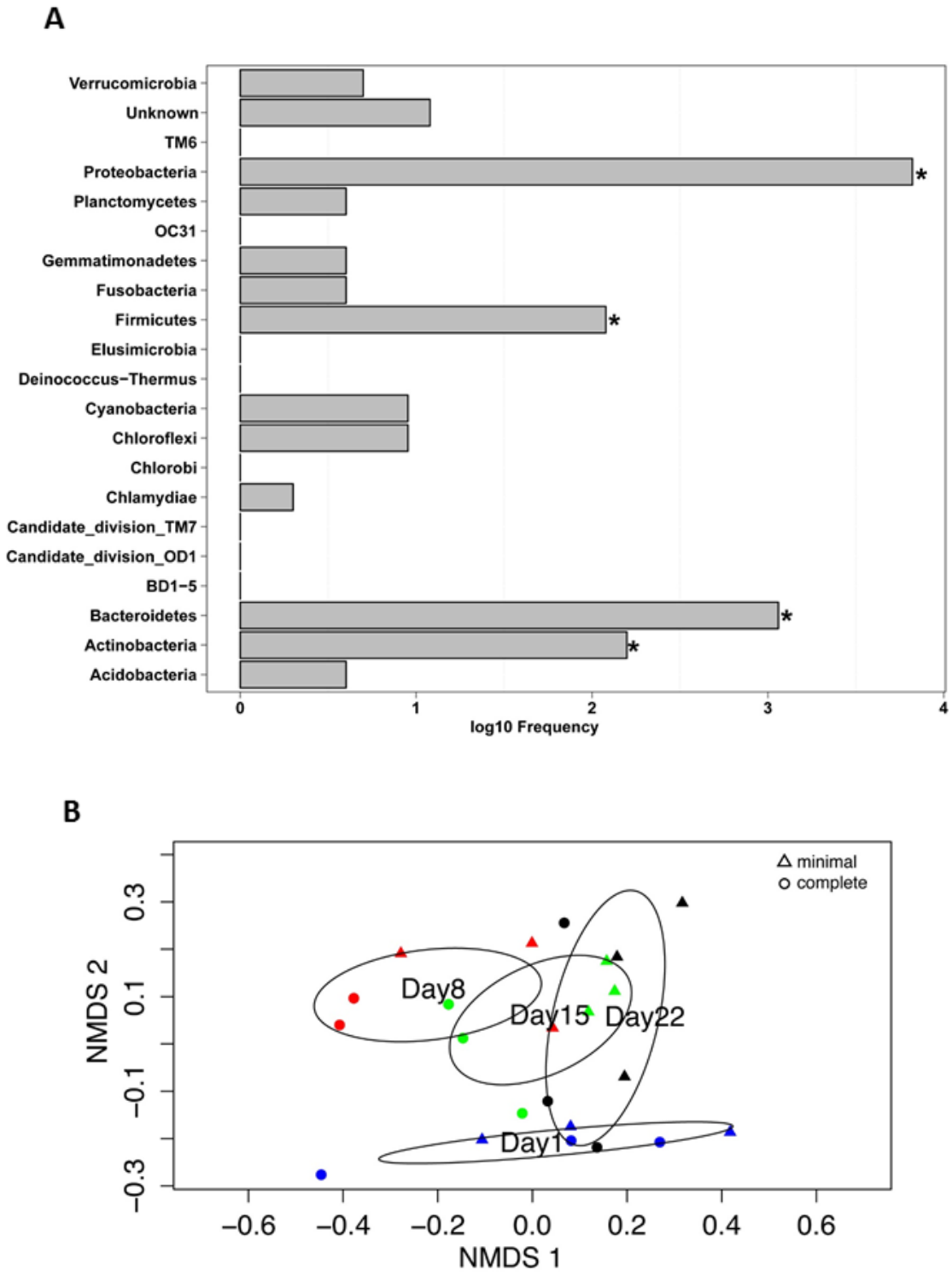

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Figure 1. (A) Distribution of Operational Taxonomic Unit (OTU) abundance (LOG scaled) within phyla from complete data set. The bins marked with asterisks correspond to 99.97% of all which belong to Proteobacteria, Bacteriodetes, Actinobacteria and Firmicutes. **(B) Ordination plot of bacterial community in the two media conditions for all sampling points.** To compare the species composition between the different samples (days / media) we used a non-metric multidimensional scaling (NMDS) function based on generalised UniFrac distances (Chen *et al.*, 2012). Triangles and circles correspond to minimal media and complete media conditions, respectively. Blue represents Day 1. Red represents Day 8. Green represents Day 15. Black represents Day 22. The ellipses correspond to the 99% confidence interval to each group centroid.

Rarefaction curves were used to evaluate the alpha diversity in the different media conditions as well as at the different time points (Supplementary Figure S4). Species richness in both minimal and complete media was ~3000. Species richness over time remained between ~2400 and ~2600, with reduced species richness (~1300) on Day 8 (both minimal and complete media) possibly due to elevated levels of 16S *P. tricornutum* chloroplast reads which had to be omitted. Greatest species richness (~3000) was shown on Day 22. Overall, all datasets showed less increase in the number of unique species as the sample size increased, confirming adequate species richness in all culture conditions.

To compare the species composition between the different samples (days/media) we used a non-metric multidimensional scaling (NMDS) function based on generalised UniFrac distances (Chen *et al.*, 2012). We observed a clear divergence in the bacterial community in the two media conditions. Ordination based on the sampling day indicated that the bacterial community was dynamic with a clear divergence visible between Day 1 and the other three sampling days. Day 15 and 22 showed a slight overlap (Figure 2). An adapted version of PermanovaG was used to carry out permutational multivariate analysis of variance using multiple distance matrices which were previously calculated based on the generalised UniFrac distance (Chen *et al.*, 2012). The significance for the test was assessed by 5000 permutations. The results of the PermanovaG tests support the NMDS ordination,

confirming a statistically significant effect in the bacterial community profile at the different sampling points and in the two media conditions whereas no significant effect was found in the experimental replicates (Supplementary Figure S5).

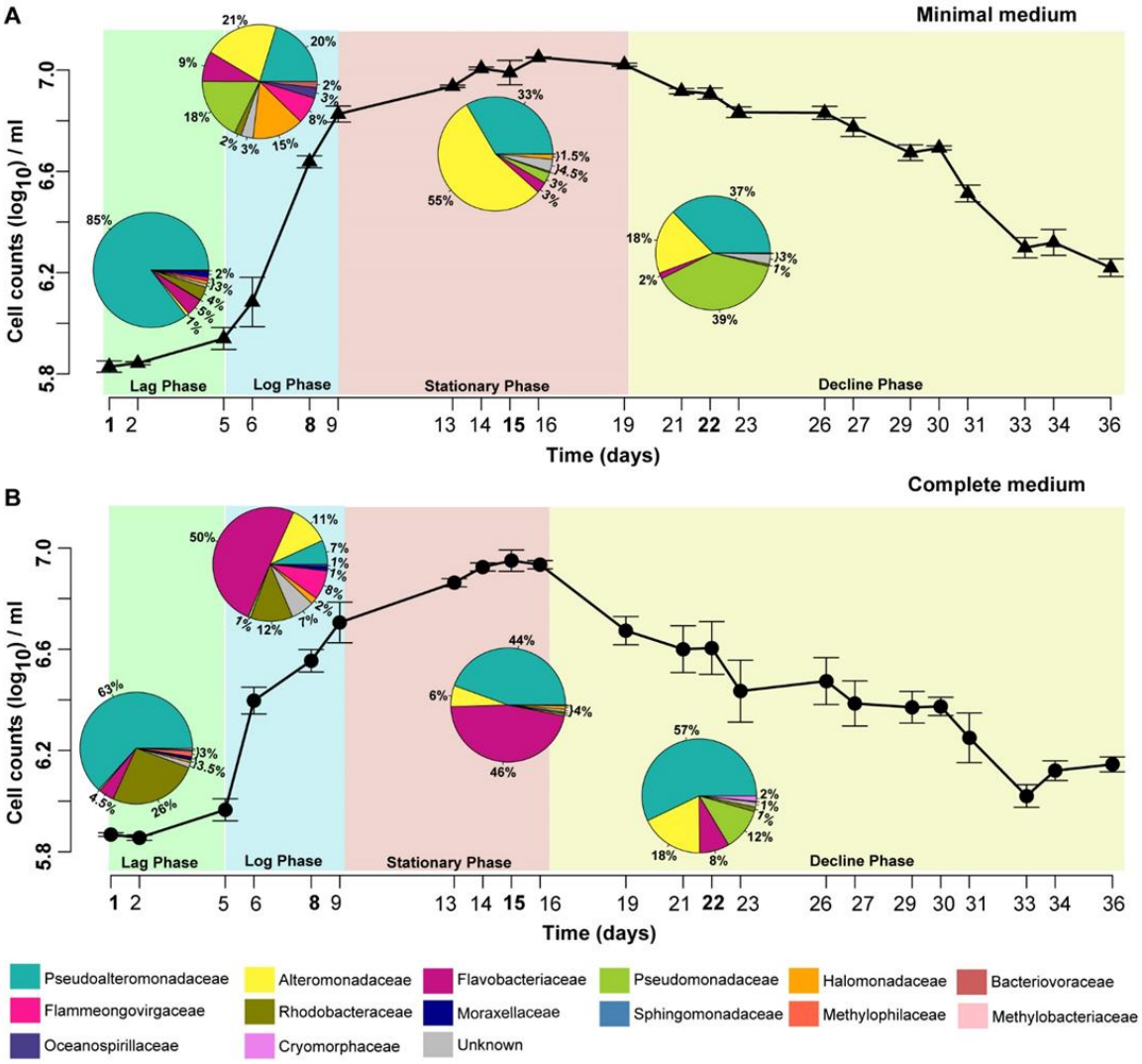


Figure 2. Bacterial community profile of *Phaeodactylum tricornutum* cultures over time and in differing media conditions. Both panels illustrate the growth of *P. tricornutum* (CCAP 1052/1B) over a 36 day period. The growth curves have been partitioned into lag (green), log (blue), stationary (red), and decline (yellow) phases. The abundance (%) of the 'Top Ten' bacterial families (corresponding colours described in the key) is depicted in pie charts on Days 1, 8, 15 and 22 in both media conditions. The existence of one dominant family at each investigated time point is a peculiar characteristic. In **minimal media (A)**, the lag phase of *P. tricornutum* growth is dominated

by Pseudoalteromonadaceae (85%). However, during the log phase, a wide diversity of bacterial families is observed, with members of the Alteromonadaceae family (21%) beginning to dominate. During the stationary phase, a clear dominance of Alteromonadaceae species (55%) in the community can be observed. The decline phase, however, shows the Pseudomonadaceae (39%) as a dominant family, with Pseudoalteromonadaceae species (37%) increasing in abundance again. In **complete media (B)**, the lag phase is also dominated by Pseudoalteromonadaceae (63%). During the log phase, 50% of the community is composed of members of the Flavobacteriaceae family, with the other 50% distributed amongst a number of different families. Flavobacteriaceae (46%) remain high in abundance during the stationary phase, with Pseudoalteromonadaceae species (44%) beginning to increase in abundance again. As for minimal media (A), Pseudoalteromonadaceae (57%) show clear dominance of the community during the decline phase.

Effect of temporal evolution and media composition on the bacterial community profile

We compared the bacterial community profiles over time and in the different media conditions at the family level so as to avoid diluting the signal of the less abundant genera. Supplementary Figure S6 shows no dynamical difference within the genera that cannot be observed at the family level. By investigating the bacterial community dynamics at the family level, we also include taxonomical information that is unavailable at the genus level.

Overall, the families over-represented in all samples are Pseudoalteromonadaceae, Alteromonadaceae, Flavobacteriaceae and Pseudomonadaceae. Figure 2 illustrates the temporal evolution of the bacterial community in both minimal and complete media with a unique composition at each time point. A remarkable feature is that at all investigated time points there exist one or two dominant families.

Bacterial community in complete media

Members of the Pseudoalteromonadaceae family were highly abundant when *P. tricornutum* cell densities are low (63% and 57% on Day 1 and Day 22, respectively). Flavobacteriaceae species

dominated (50%) when the *P. tricornutum* culture is growing exponentially (Day 8). Day 15, when *P. tricornutum* cell densities are at their highest, shows co-dominance of both Flavobacteriaceae (46%) and Pseudoalteromonadaceae (44%).

Bacterial community in minimal media

Similarly, in the minimal media, members of the Pseudoalteromonadaceae family were highly abundant when *P. tricornutum* cell densities are low. However, on Day 22 Pseudomonadaceae (39%) and Pseudoalteromonadaceae (37%) are both overrepresented. When the *P. tricornutum* culture is growing exponentially (Day 8) a cluster of Families dominate; namely Alteromonadaceae (21%), Pseudoalteromonadaceae (20%), Pseudomonadaceae (18%), Halomonadaceae (15%) and Flavobacteriaceae (9%). When the cell density of *P. tricornutum* peaks (Day 15), the Alteromonadaceae species take over (55%).

The bacterial communities within the two media conditions on Day 1 are more closely related than the communities on days 8 and 15 (see Table S2 for generalised UniFrac distances). As the cultures begin to 'crash' (Day 22), the bacterial communities in the two media conditions increase in similarity again.

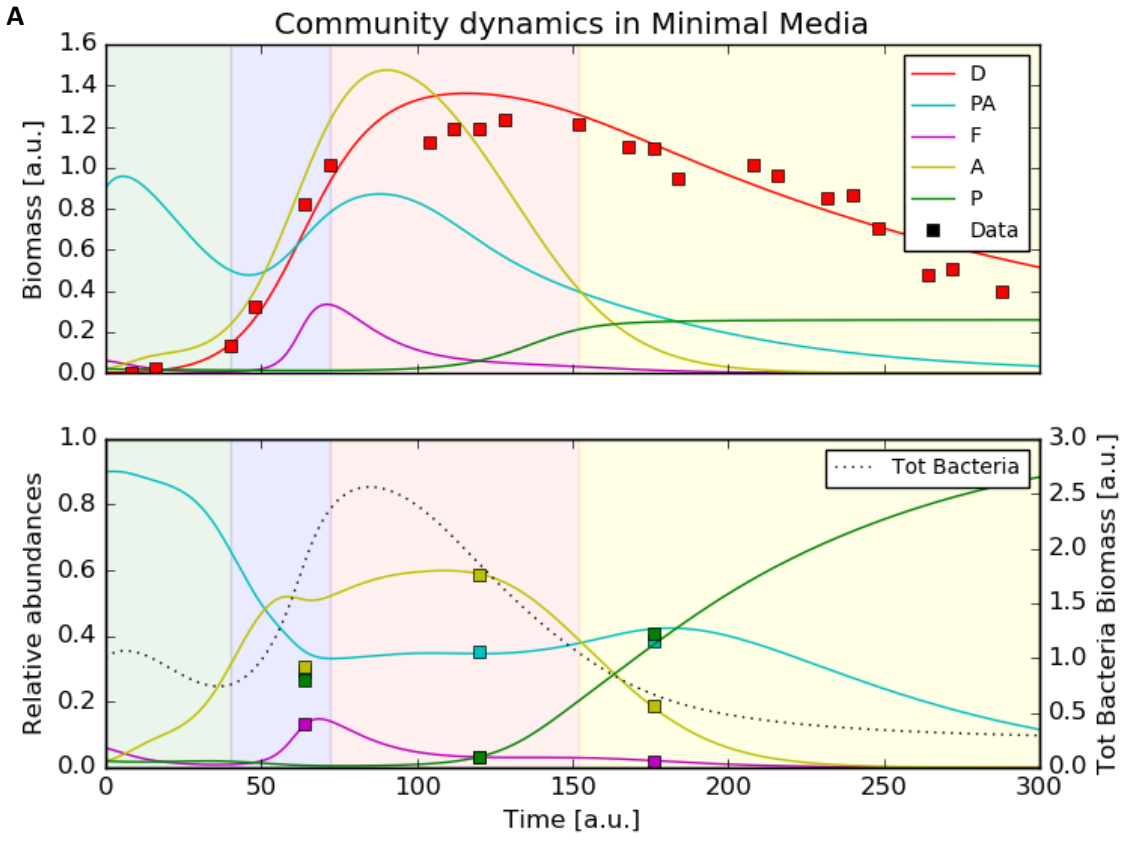
In general, the main families identified show a distinct pattern of disappearance and regeneration within the bacterial community. In the complete media, Pseudoalteromonadaceae species start at 63% (Day 1), drops in abundance to 7% (Day 8) then recovers to 57% (Day 22). Flavobacteriaceae species, in complete media, start at 4.5% (Day 1), increases in abundance to 50% (Day 8), and then falls back to 8% (Day 22). In the minimal media, Alteromonadaceae species have an abundance of only 1% (Day 1), peaks at 55% (Day 15), and decreases down to 18% (Day 22).

Mathematical model

The dynamic changes of the bacterial communities associated with *P. tricornutum* at different growth stages led us to the formulation of a network of bacteria - diatom interactions. In order to test its

plausibility, we developed a qualitative mathematical model starting from few key assumptions about nutrients availability and metabolite exchange between the organisms involved, i.e. *P. tricornutum* and general representatives of the four most abundant bacteria families Pseudoalteromonadaceae, Alteromonadaceae, Flavobacteriaceae and Pseudomonadaceae.

In Figure 3A and B we show the simulation results from the model performed in complete media and minimal media conditions, respectively, with experimental data superimposed. The top panel shows biomasses of the five organisms (data available only for the diatom), the bottom panel shows relative bacteria abundance versus time (biomass divided by total bacteria biomass). The figures show that the model is able to reproduce the main features of the bacterial community dynamics, like the disappearance and return of Pseudoalteromonadaceae in complete media and the peak of Alteromonadaceae at the end of the diatom's exponential growth phase in minimal media. Because of the qualitative nature of the model, units are arbitrary and the parameters used for simulation do not claim any biological significance (see Supplementary Model Information for more details).



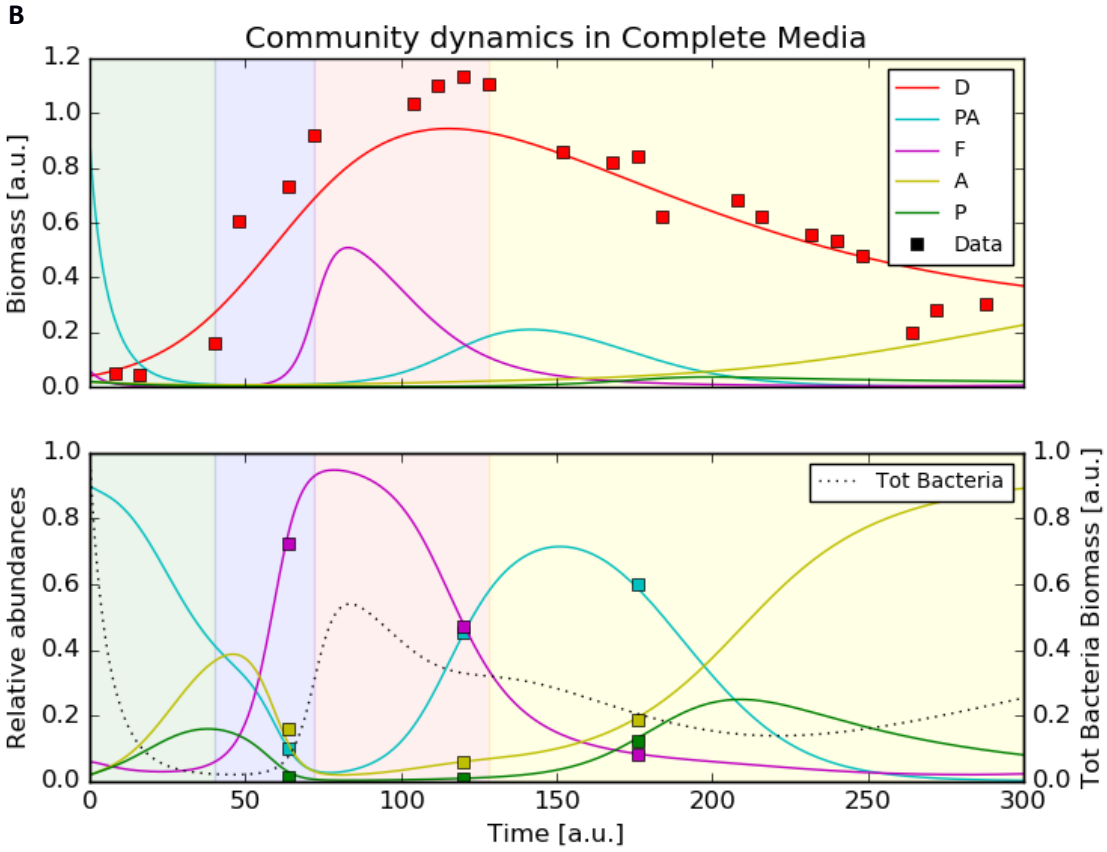


Figure 3A and B. Simulation results (lines) and experimental data (squares) for communities of *P. tricornutum* (D), Pseudoalteromonadaceae (PA), Flavobacteriaceae (F), Alteromonadaceae (A) and Pseudomonadaceae (P) in minimal (A) and complete (B) media conditions. The top panel shows the biomass time course (arbitrary units) for the five organisms and the rescaled data points (squares) for the *P. tricornutum*. The bottom panel shows the variations in relative abundances of the four bacteria (single bacteria biomass/total bacteria biomass) over time and the three sets of data points from the sequencing analysis (the first data point is used as starting condition at time 0). Also shown in the bottom plot (dotted line, right y-axis) is the total bacterial biomass in arbitrary units.

Discussion

In nature, *Phaeodactylum tricornutum* is not an isolated sovereign entity impassive to its environment including other organisms. In fact, it is part of a complex ecosystem, which is poorly understood. To

reduce the complexity of a natural system, but nevertheless to gain valuable insights into the dynamics of the bacterial communities associated with diatoms, we investigated here non-axenic cultures of laboratory strains of *P. tricornutum*. Our results showed the trends of the bacterial community dynamics during the batch growth of the *P. tricornutum*.

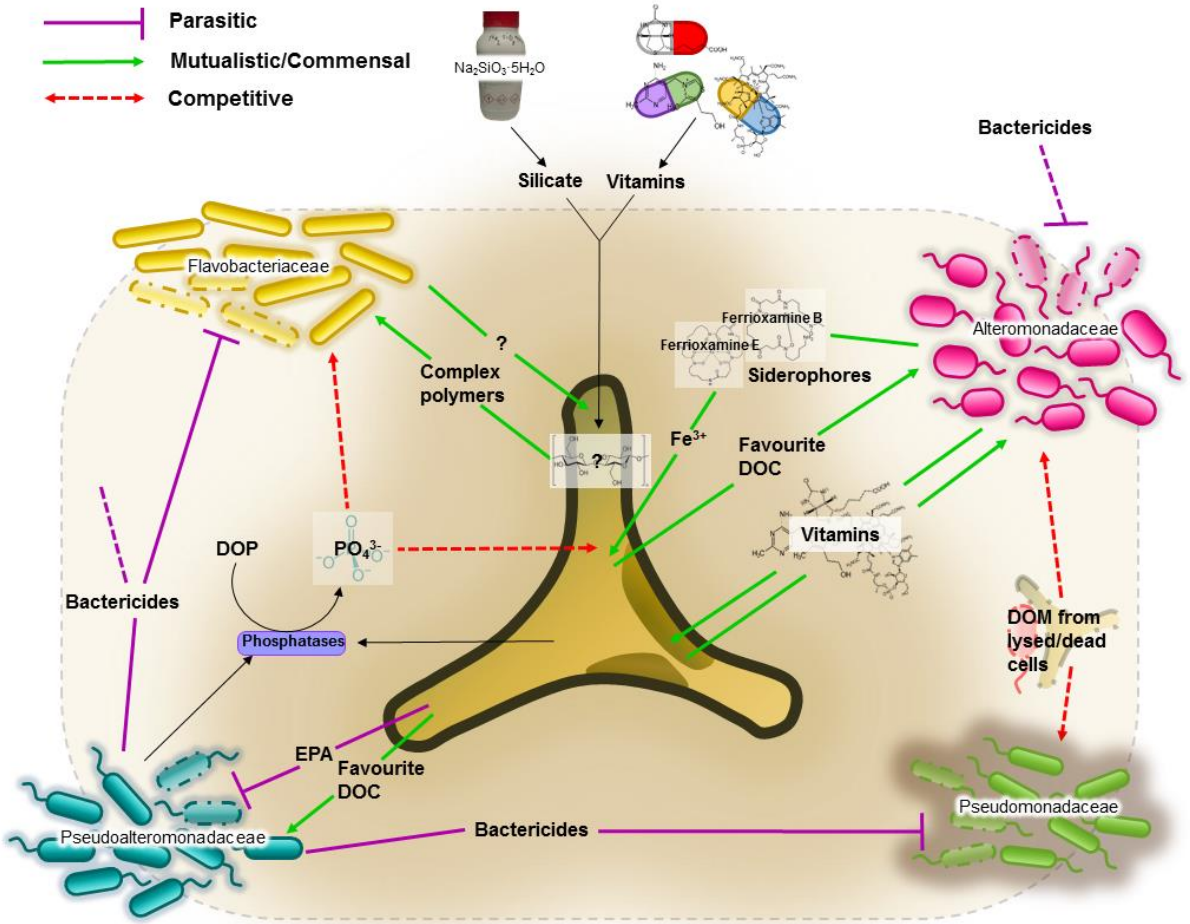
To progress towards the goal of creating a synthetic community, an in-depth understanding of the naturally occurring diatom-bacterial interactions, which are predominantly based on a 'biological barter trade system' between diatoms and bacteria – where substances such as trace metals, vitamins, and nutrients (nitrate, phosphate, silicate, carbon) are traded – is necessary. Based on our findings and additional insights from previous studies on diatom-bacterial interactions as well as on existing characterisation of known species from each family, we will postulate the role of the particular bacterial families in the *P. tricornutum* cultures. From this we will derive a mathematical model with the aim of reproducing the dynamical evolution of the community composition over time.

The growth dynamics of *P. tricornutum* in the two media conditions showed an accelerated 'culture crash' in the complete media compared to the minimal media, which suggests a more stable culture in the minimal media (Figure 2). Simultaneously, the dynamics of the bacterial community reveals that the community in the minimal media increases in complexity over time. The link between ecosystem complexity and stability based on theoretical and experimental data has been debated by ecologists for over half a century (MacArthur, 1955; Elton, 1958; Gardner and Ashby, 1970; Pimm, 1984). Our observations are in agreement with more recent hypotheses indicating that diversity generally increases the stability of an ecosystem (McCann, 2000).

Prospective role of central bacterial families

The putative roles of each of the dominant families are illustrated in Figure 4. The presence of **Pseudoalteromonadaceae** species is not unexpected as members of this family have been isolated from coastal, open and deep-sea waters, sediments, marine invertebrates, as well as marine fish and algae (Ivanova *et al.*, 2004). The Pseudoalteromonadaceae family has three genera, namely

288 *Pseudoalteromonas*, *Algicola* and *Psychrosphaera* (Rosenberg *et al.*, 2014, 28). Several species of
289 Pseudoalteromonadaceae are reported to possess antibiotic properties with bactericidal effects
290 (Bowman, 2007). For example, concentrated supernatant of a marine bacterium *Pseudoalteromonas*
291 *sp.* strain A28 contained various enzymes including proteases, DNases, cellulases, and amylases,
292 capable of causing the lysis of the diatom *Skeletonema costatum* (Lee *et al.*, 2000). Species of
293 Pseudoalteromonadaceae are also capable of producing cold-adapted enzymes (Venkateswaran and
294 Dohmoto, 2000; Chen *et al.*, 2007; Khudary *et al.*, 2010; Lu *et al.*, 2010; Albino *et al.*, 2012; He *et al.*,
295 2012). Pseudoalteromonadaceae species can produce extracellular polymeric substances allowing
296 them to colonise surfaces, enhancing nutrient uptake whilst limiting diffusion of particular substances
297 across the cell membrane (Holmström and Kjelleberg, 1999). The ability of Pseudoalteromonadaceae
298 species to suppress the growth of competing bacteria could explain the dominance of
299 Pseudoalteromonadaceae in almost all cultures irrespective of media composition, particularly when
300 *P. tricornutum* abundance is limited (Figure 2, Days 1 and 22). *P. tricornutum* on the other hand, may
301 protect other bacterial community members from the bacteriolytic ability of
302 Pseudoalteromonadaceae by producing specific antibacterial compounds themselves. Desbois *et al.*
303 showed that *P. tricornutum* excreted bacteriolytic fatty acids such as eicosapentaenoic acid (EPA;
304 20:5n-3), nucleotides, peptides, and pigment derivatives that can eliminate unwanted competition for
305 nutrients such as organic phosphates from certain bacteria (Desbois *et al.*, 2009).



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307 **Figure 4. Network of putative interactions between *Phaeodactylum tricornutum* and identified**
308 **bacterial families.** The dotted grey line depicts the **'phycosphere'**; a term coined by Bell and
309 Mitchell in 1972 as an aquatic equivalent of the 'rhizosphere', denoting the region extending
310 outwards from the algal cell in which bacterial growth is stimulated by extracellular products of
311 the alga (Bell and Mitchell, 1972). **Bactericidal Effects.** Several species of the
312 Pseudoalteromonadaceae family have been reported to possess bactericidal effects (Bowman,
313 2007). *P. tricornutum*, however, can excrete fatty acids (such as eicosapentaenoic acid or EPA),
314 nucleotides, peptides, and pigment derivatives to protect themselves against opportunistic attack
315 or pathogenic damage (Desbois *et al.*, 2009). **Iron.** Siderophores are a group of iron scavengers
316 that act by chelating iron (III). Siderophores are produced and excreted by bacteria, and some
317 cyanobacteria, which then reuptake the siderophores with bound iron (III) via outer-membrane
318 transporters that are siderophore-specific (Vraspir and Butler, 2009). Diatoms are not known to
319 produce siderophores (Soria-Dengg and Horstmann, 1995; Amin *et al.*, 2009). However, based on

genome sequence analyses, the presence of a gene orthologue of a bacterial ferrichrome binding protein suggests the possibility of iron (III)-siderophore utilisation by *P. tricornutum*. Furthermore, it was shown that *P. tricornutum* was able to uptake siderophores ferrioxamines B and E (Soria-Dengg and Horstmann, 1995). **Vitamins.** Prokaryotes are thought to be the main producers of B vitamins (Provasoli, 1963; Provasoli and Carlucci, 1974). Although *P. tricornutum* does not require cobalamin, thiamine and biotin (Croft *et al.*, 2006), production of organic compounds such as EPA can by considerably enhanced by the bioavailability of co-factors such as cobalamin (Yongmanitchai and Ward, 1991). This provides the basis for potential mutualistic interactions. For example, Alteromonadales, dominant in our cultures, are thought to be capable of producing B vitamins (Sañudo-Wilhelmy *et al.*, 2014). **Dissolved Organic Carbon (DOC).** It is estimated that up to 50% of carbon fixed via phytoplankton-mediated photosynthesis is utilised by marine bacteria (Azam *et al.*, 1983), mainly as DOC compounds, defined as the organic material <0.7µm in size (Stocker, 2012). DOC from diatoms originates either from live cells or recently lysed or grazed cells, which determines the type of DOCs available, and therefore likely determining the bacterial consortia associated with the diatom (Amin *et al.*, 2012). **Dissolved Organic Phosphate (DOP).** Both diatoms and bacteria primarily utilise orthophosphate as a source of phosphorus. However, to access phosphate from DOP compounds, both diatoms and bacteria developed mechanisms such as the excretion of enzymes, including phosphatases, to release orthophosphate (PO_4^{3-}) from DOP. The mechanism is not species-specific, which consequently means the 'free' orthophosphates can be acquired by any organism (Persson *et al.*, 1988).

The **Alteromonadaceae** family consists of 16 (yet annotated) named genera (LPSN, 2016a) found predominantly in marine environments (Rosenberg *et al.*, 2014, 5). Members of this family were isolated from nutrient-rich environments such as coastal, open, and deep-sea waters, sediments, marine invertebrates and vertebrates, algae, and temperate and Antarctic marine environments (Ivanova and Mikhaïlov, 2001). They are able to utilise a vast array of compounds as carbon sources; from glucose to glycerol (Rosenberg *et al.*, 2014, 5). Members of this family are known siderophore producers (Reid and Butler, 1991; Holt *et al.*, 2005; Amin *et al.*, 2009). Greek for 'iron carrier',

siderophores are a group of iron scavengers that act by chelating iron (III) that are produced and excreted by bacteria, and some cyanobacteria, which then reuptake the siderophores with bound iron (III) via outer-membrane transporters that are siderophore-specific (Vraspir and Butler, 2009). Iron acquisition is essential for biological processes such as photosynthesis, respiration and nitrogen fixation. Most bioactive trace metals, including iron, exist at nanomolar (10^{-9} M) to picomolar (10^{-12} M) concentrations in our oceans, approximately one-millionth of the intracellular concentration in diatoms (Bruland *et al.*, 1991; Morel and Price, 2003). Diatoms are not known to produce siderophores (Soria-Dengg and Horstmann, 1995; Amin *et al.*, 2009) but previous studies have shown that diatoms can use siderophores as an iron source (Soria-Dengg *et al.*, 2001). However, based on genome sequence analyses, the presence of a gene orthologue of a bacterial ferrichrome-binding protein suggests the possibility of iron (III)-siderophore utilisation by *P. tricornutum* (Soria-Dengg and Horstmann, 1995). No trace metals, including iron (III), were provided to minimal media cultures. However, natural seawater may contain minute traces of bioactive trace metals. The high abundance of Alteromonadaceae in the minimal media suggests a potential supportive role in sequestering traces of iron (III) that may be present in the sterile natural seawater to the *P. tricornutum* (Figure 2). This is further supported by the very low level of Alteromonadaceae in the complete media (11% in complete media compared to 55% in minimal media, both on Day 15) where the culture has been supplied with 11.7 µM of iron (III) chloride hexahydrate.

Flavobacteriaceae are members of the Bacteroidetes phylum and include over 120 genera (LPSN, 2016b) found in soil, sediments and seawater (see (Yoon *et al.*, 2015) for further references). Flavobacteriaceae belong within the Cytophaga-Flavobacterium cluster which has been shown to account for more than 10% of the total bacterial community in coastal and offshore waters (Glöckner *et al.*, 1999; Abell and Bowman, 2005; DeLong *et al.*, 2006). Members of Flavobacteriaceae are proficient degraders of various biopolymers such as cellulose, chitin and pectin (Manz *et al.*, 1996; Kirchman, 2002). They were shown to be omnipresent during phytoplankton blooms, and their

preference for consuming more complex polymers rather than monomers suggests an active role in the processing of organic matter during these blooms (Cottrell and Kirchman, 2000; Pinhassi *et al.*, 2004). Although the exact mechanisms behind them are not perfectly understood, algal blooms are a consequence of exponential growth of phytoplankton (Smayda, 1997). In this respect, the phase of exponential growth of *P. tricornutum* in complete media, when our results showed highest abundance of Flavobacteriaceae, is the artificial equivalent of an algal bloom of *P. tricornutum* (Figure 2). In the minimal media, the abundance of Flavobacteriaceae remains very low; at its maximum on Day 8 it only accounts for 9% of the total bacterial community. Members of the Flavobacteriaceae family could be more demanding than other bacteria that require lower nutrient levels to thrive. It is estimated that up to 50% of carbon fixed via phytoplankton-mediated photosynthesis is utilised by marine bacteria (Azam *et al.*, 1983), mainly as Dissolved Organic Carbon (DOC) compounds, defined as the organic material <0.7µm in size (Stocker, 2012). DOC from diatoms originates either from live cells or recently lysed or grazed cells, which determine the type of DOCs available, and therefore are likely to influence the bacterial consortia associated with the diatom (Amin *et al.*, 2012). This suggests a dynamic complexity within the bacterial consortia based solely on the type of DOC available. Members of the Flavobacteriaceae family might possess the genetic ability to utilise specific DOC produced by *P. tricornutum* grown in complete media.

Pseudomonadaceae are an extraordinarily diverse family of bacteria found in almost all habitats on Earth; in soils, freshwater as well as marine environments, as well as plant and animal-associated pathogens (Starr *et al.*, 1981, 58). Species from the *Pseudomonas* genus are the best studied of the Pseudomonadaceae family, whose sheer genetic diversity explains the ability to thrive in such a wide range of environments (Anzai *et al.*, 2000). Marine isolates from the *Pseudomonas* genus have been shown to produce a wide range of bioactive compounds, many of which exhibit antibacterial as well as antiviral properties (see (Isnansetyo and Kamei, 2009) for further references). Our results, indeed show an elevated level of Pseudomonadaceae OTUs evident on Day 22 of the complete media cultures, and on Days 8 and 22 of the minimal media cultures. The increased presence of

Pseudomonadaceae when *the P. tricornutum* culture has 'crashed' could be attributed to its ability to produce antibacterial compounds allowing members of this family to begin to thrive in the community through inhibition of its competitors. Given its exceptional genetic diversity, and thus, its metabolic versatility, allows for members of Pseudomonadaceae to be truly saprophytic; providing a hypothetical explanation of its abundance we could measure when the *P. tricornutum* cultures crash (Figure 2, Day 22 in both media conditions).

Mathematical Model

We observed that the bacterial community associated with *Phaeodactylum tricornutum* cultures changed over time, correlating with the growth and subsequent crashing of the diatom cultures. The bioavailability or absence of vitamins, trace metals and silicon, as well as nutrients or bactericidal substances can alter the bacterial community. We built a mathematical model based on simple assumptions extracted from the putative roles we assigned to the dominant bacterial families (see Figure 4) and applied them to standard methods for modelling population dynamics. In particular, we introduced growth limitation from nutrients/micronutrients, as well as from bactericidal-induced death. An ordinary differential equation model cannot, of course, capture mechanisms such as metabolic shifts caused by changes in the environment such as the supplementation of minimal or complete media. Therefore, we did not implement a unique set of parameters for the model in the two conditions. The current qualitative model provides an important proof-of-concept to emphasise the validity of our assumptions, and serves as the motivation for further research bringing the model to a quantitative, predictive level. Indeed, mathematical models are powerful tools towards the goal of synthetic community establishment and control, and the model parameters can be experimentally measured to bring predictive power to the simulations.

Concluding remarks

We postulate that a role within the community can be filled, not by one specific species of bacteria, but rather a number of bacterial species capable of carrying out said role. Which bacteria fill the role

423 is dependent upon the environmental characteristics and the prevailing needs of the community as a
424 whole at any given time. If a niche is unfilled, bacteria with the ideal metabolic functionality will seize
425 the opportunity and thrive within that niche. The absence of certain micronutrients creates a new
426 niche that can be filled by a certain unique bacterial faction.

427 Further work is necessary to explore the hypotheses postulated in the Discussion section. This can be
428 achieved by carrying out systematic co-culture experiments with culturable members of the bacterial
429 families of interest. The role of each representative of the bacterial families can be identified by
430 carrying out subsequent –omics studies, which provide a holistic view of the genes (genomics), mRNA
431 (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific biological
432 sample in a non-targeted and non-biased manner (Horgan and Kenny, 2011). The resulting
433 experimental measurements will allow the dynamic model presented here to develop from qualitative
434 to quantitative, providing a powerful predictive tool for culture monitoring such as predicting
435 harvesting point based on the bacterial community.

436 # Materials and methods

437 **Strains and culture conditions**

438 All *Phaeodactylum tricornutum* cultures were obtained from the Culture Collection of Algae and
439 Protozoa (CCAP) based in Oban, Scotland (http://www.ccap.ac.uk/our-cultures.htm). All cultures are
440 obtained non-axenic. Based on previous experimental evidence (unpublished data), the *P. tricornutum*
441 strain CCAP1052/1B displayed optimal growth in 5L cultures. *P. tricornutum* was cultured in Guillard's
442 medium for diatoms (F/2 + Si) in filtered natural seawater chemically sterilised using sodium
443 hypochlorite and sodium thiosulphate pentahydrate. *P. tricornutum* was grown in two media
444 conditions; (1) complete F/2 medium with the addition of sodium metasilicate as the source of silicon,
445 as per Guillard and Ryther, 1962; Guillard, 1975, and (2) minimal media with a source of nitrogen
446 ($NaNO_3$) and phosphorus ($NaH_2PO_4.2H_2O$) at the same concentration as in the F/2 medium recipe.

Recipe was obtained from the Culture Collection of Algae and Protozoa website (see http://www.ccap.ac.uk/pdfrecipes.htm). All cultures were grown in hanging 5L polyethylene bags with a 'V' shaped bottom prepared using a heat sealer (Supplementary Figure S1). All cultures had a modified aeration system provided by a 10ml pipette attached to the main pressurised air supply via 0.2 µm sterile air filters. A modified access port was created to allow for sampling and measurement of environmental parameters. Cultures were kept at 18-20°C and 24hr light at an average of 132.3 µmol m^{-2} s^{-1} using Phillips TL-D 58W 33-640 M9 fluorescent tube lights. All cultures, irrespective of media condition, were inoculated with 250ml from the same 5L stock culture of actively growing non-axenic *P. tricornutum*.

Growth measurements

Growth was monitored every 24 to 48 h using a light microscope and carrying out cell counts of each culture in quadruplicates for each culture. During the cell counts the ratios of the four different morphotypes (oval, fusiform, triradiate and cruciform) were recorded, and descriptions of each culture noted. Samples of each culture were subsequently taken using a sterile 10ml syringe and placed in 50ml Falcon centrifuge tubes and placed in -20°C freezer.

Genomic DNA extraction

All samples from Day 1, 8, 15, and 22 were thawed in a water bath set at 25°C. As per de Gouvion Saint Cyr *et al.*, 2014, samples were centrifuged for 5mins at 2000g to gather the *P. tricornutum* in the pellet while particles such as debris, other organisms, bacteria, and soluble substances remain in the supernatant. Because the bacteria might be attached to the *P. tricornutum* cells in the pellet, the pellet was washed with deionised water and then centrifuged for 5mins at 2000g. This was repeated twice. Genomic DNA extraction was carried out in the Aquaculture and Fisheries Development Centre and University College Cork. The Mo Bio's PowerWater® DNA Isolation Kit (catalogue no. 14900-100-NF) was utilised to carry out the genomic DNA extraction. The protocol provided with the kit was followed.

Presence of gDNA was detected by running a 1% agarose-ethidium bromide gel with 72 wells. The samples were sent on dry ice to Heinrich Heine University, Düsseldorf, for the V6 16S sequencing.

Barcoded 16S-V6-Next Generation Sequencing

Ion Torrent™ barcoded Next Generation Sequencing protocol was used to sequence the bacterial gDNA (Quail *et al.*, 2012; Grada and Weinbrecht, 2013). Amplification of the V6 hyper variable region of 16S rRNA with forward and reverse primers (Supplementary Table S2) was carried out. Ion Reporter™ software assembles all the raw sequencing data and sorts all the reads using the unique sample-specific barcode sequences and removes them from the reads. The outcome is raw FASTQ files which are ready for analysis using bioinformatics tools.

Bioinformatics

A total of 87,077,374 reads were identified. The smallest sample was just over 1 million reads; the largest sample was just under 10 million reads. The sequencing data was subjected to a pipeline adapted and modified from Pylro *et al.*, 2014. Primers were trimmed with fastq-mcf (version 1.04.807) (Aronesty, 2011), the resulting sequences were quality filterted and clustered into OTUs (operational taxonomic units) with usearch (version 8.0.1517; 32Bit – opensource) (Edgar, 2010, 2013). Taxonomy assignment was done by QIIME (version 1.9.0) (Caporaso *et al.*, 2010) with the implemented uclust classifier based on 97% sequence identity to the reference 16S sequences from SILVA 111 database (Quast *et al.*, 2013). Statistical analyses were performed in R (R Development Core Team, 2015).

The complete protocol containing all processing steps is available on https://github.com/QTB-HHU.

Modelling approach

Population dynamics models have been developed since quite some time (Verhulst, 1838; Lotka, 1925; Volterra, 1926) spanning the broad fields of ecology, epidemiology and economics. Starting from our understanding of the organism-to-organism interactions, we developed a dynamic model consisting

494 of 13 ordinary differential equations and including 56 (55 free) parameters. The parameters are fitted
495 using a genetic algorithm (Mitchell, 1996) which is run in different steps to optimise the fit of *P.*
496 *tricornutum* growth and/or the bacteria relative abundances to the experimental data in evolving
497 system conditions (see Supplementary Model Information). The model is written in Python (Python
498 Software Foundation, https://www.python.org/) and is available on GitHub (https://github.com/QTB-
499 HHU/communityODE) with instructions and scripts for running.

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509 Competing interests

510 To the best of our knowledge, we do not have competing interest to declare.

511 References

512 Abell, G. and Bowman, J. (2005) Ecological and biogeographic relationships of class Flavobacteria in the Southern
513 Ocean. *FEMS Microbiol. Ecol.* **51**: 265–277.

514 Albino, A., Marco, S., and Maro, A. Di (2012) Characterization of a cold-adapted glutathione synthetase from the
515 psychrophile Pseudoalteromonas haloplanktis. *Mol. Biosyst.* **8**: 2405–2414.

516 Amin, S.A, Green, D.H., Hart, M.C., Küpper, F.C., Sunda, W.G., and Carrano, C.J. (2009) Photolysis of iron,
517 siderophore chelates promotes bacterial, algal mutualism. *Proc. Natl. Acad. Sci.* **106**: 17071–17076.

518 Amin, S.A, Parker, M.S., and Armbrust, E.V. (2012) Interactions between diatoms and bacteria. *Microbiol. Mol.*

519 *Biol. Rev.* **76**: 667–84.

520 Amin, S.A., Hmelo, L.R., van Tol, H.M., Durham, B.P., Carlson, L.T., Heal, K.R., et al. (2015) Interaction and
521 signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* **522**: 98–101.

522 Anzai, Y., Kim, H., Park, J., Wakabayashi, H., Oyaizu, H., and The, P. (2000) Phylogenetic affiliation of the
523 pseudomonads based on 16S rRNA sequence become a dumping ground for incompletely characterized
524 polarly flagellated , of 128 valid and invalid Pseudomonas species , which included almost valid species of
525 the genus Pseudomona. *Int. J. Syst. Evol. Microbiol.* **50**: 1563–1589.

526 Aronesty, E. (2011) Command-line tools for processing biological sequencing data. *Expr. Anal. Durham NC.*

527 Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reil, L., and Thingstad, F. (1983) The Ecological Role of Water-
528 Column Microbes in the Sea. *Mar. Ecol. Prog. Ser.* **10**: 257–263.

529 Bell, W. and Mitchell, R. (1972) Chemotactic and growth responses of marine bacteria to algal extracellular
530 products. *Biol. Bull.* **143**: 265–277.

531 Bhattacharya, D., Archibald, J.M., Weber, A.P.M., and Reyes-Prieto, A. (2007) How do endosymbionts become
532 organelles? Understanding early events in plastid evolution. *BioEssays* **29**: 1239–1246.

533 Bowler, C., Allen, A.E., Badger, J.H., Grimwood, J., Jabbari, K., Kuo, A., et al. (2008) The Phaeodactylum genome
534 reveals the evolutionary history of diatom genomes. *Nature* **456**: 239–244.

535 Bowman, J. (2007) Bioactive compound synthetic capacity and ecological significance of marine bacterial genus
536 Pseudoalteromonas. *Mar. Drugs* **5**: 220–241.

537 Bruckner, C.G., Rehm, C., Grossart, H.P., and Kroth, P.G. (2011) Growth and release of extracellular organic
538 compounds by benthic diatoms depend on interactions with bacteria. *Environ. Microbiol.* **13**: 1052–1063.

539 Bruland, K.W., Donat, J.R., and Hutchins, D.A. (1991) Interactive influences of bioactive trace metals on biological
540 production in oceanic waters. *Limnol. Oceanogr.* **36**: 1555–1577.

541 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. (2010) QIIME allows
542 analysis of high-throughput community sequencing data. *Nat. Methods* **7**: 335–336.

543 Chen, J., Bittinger, K., Charlson, E.S., Hoffmann, C., Lewis, J., Wu, G.D., et al. (2012) Associating microbiome
544 composition with environmental covariates using generalized UniFrac distances. *Bioinformatics* **28**: 2106–
545 2113.

546 Chen, X., Xie, B., Lu, J., He, H., and Zhang, Y. (2007) A novel type of subtilase from the psychrotolerant bacterium
547 Pseudoalteromonas sp. SM9913: catalytic and structural properties of deseasin MCP-01. *Microbiology*
548 **153**: 2116–2125.

549 Chisti, Y. (2007) Biodiesel from microalgae. *Biotechnol. Adv.* **25**: 294–306.

550 Cottrell, M.T. and Kirchman, D.L. (2000) Natural assemblages of marine proteobacteria and members of the
551 Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl.*
552 *Environ. Microbiol.* **66**: 1692–7.

553 Croft, M.T., Warren, M.J., and Smith, A.G. (2006) Algae need their vitamins. *Eukaryot. Cell* **5**: 1175–1183.

554 Dangeard, P.-A. (1933) Traité d'algologie: introduction à la biologie et à la systématique des algues. Paul
555 Lechevalier & Fils.

556 Day, J.G., Thomas, N.J., Achilles-Day, U.E.M., and Leakey, R.J.G. (2012) Early detection of protozoan grazers in
557 algal biofuel cultures. *Bioresour. Technol.* **114**: 715–719.

558 DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.-U., et al. (2006) Community genomics
559 among stratified microbial assemblages in the ocean's interior. *Science* **311**: 496–503.

560 Delucca, R. and Mccracken, M.D. (1977) Observations on interactions between naturally-collected bacteria and
561 several species of algae. *Hydrobiologia* **55**: 71–75.

562 Desbois, A.P., Mearns-Spragg, A., and Smith, V.J. (2009) A Fatty Acid from the Diatom Phaeodactylum
563 tricornutum is Antibacterial Against Diverse Bacteria Including Multi-resistant Staphylococcus aureus
564 (MRSA). *Mar. Biotechnol.* **11**: 45–52.

565 Dykhuizen, D. (1998) Santa Rosalia revisited: why are there so many species of bacteria? *Antonie Van*
566 *Leeuwenhoek* **73**: 25–33.

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

568 Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **10**:
569 996–8.

570 Elton, C. (1958) The Ecology of Invasions by Animals and Plants. Methuen (ed) London.

571 Fajardo, A.R., Cerdán, L.E., Medina, A.R., Fernández, F.G.A., Moreno, P. a. G., and Grima, E.M. (2007) Lipid
572 extraction from the microalga Phaeodactylum tricornutum. *Eur. J. Lipid Sci. Technol.* **109**: 120–126.

573 Gardner, M. and Ashby, W. (1970) Connectance of large dynamic (cybernetic) systems: critical values for
574 stability. *Nature* **228**: 784.

575 Gause, G. (1934) The Struggle for Existence. Williams and Wilkins (ed) Baltimore (MD).

576 Glöckner, F.O., Fuchs, B.M., and Amann, R. (1999) Bacterioplankton compositions of lakes and oceans: a first
577 comparison based on fluorescence in situ hybridization. *Appl. Environ. Microbiol.* **65**: 3721–6.

578 de Gouvion Saint Cyr, D., Wisniewski, C., Schrive, L., Farhi, E., and Rivasseau, C. (2014) Feasibility study of
579 microfiltration for algae separation in an innovative nuclear effluents decontamination process. *Sep. Purif.*
580 *Technol.* **125**: 126–135.

581 Grada, A. and Weinbrecht, K. (2013) Next-Generation Sequencing: Methodology and Application. *J. Invest.*
582 *Dermatol.* **133**: e11–4.

583 Grima, E. and Fernández, F. (1999) Photobioreactors: light regime, mass transfer, and scaleup. *J. Biotechnol.* **70**:
584 231–247.

585 Guillard, R. (1975) Culture of phytoplankton for feeding marine invertebrates. In, W. L. Smith and M. H. Chanley
586 (ed), *Culture of marine invertebrate animals.* Plenum Press, New York, pp. 29–60.

587 Guillard, R. and Ryther, J. (1962) Studies of marine planktonic diatoms: i. *Cyclotella nana* Hustedt, and *Detonula*
588 *confervacea* (Cleve) gran. *Can. J. Microbiol.* **8**: 229-239.

589 Hardin, G. (1960) The competitive exclusion principle. *Science* **131**: 1292–7.

590 He, H., Guo, J., Chen, X., Xie, B., and Zhang, X. (2012) Structural and functional characterization of mature forms
591 of metalloprotease E495 from Arctic sea-ice bacterium Pseudoalteromonas sp. SM495. *PLoS One* **7**:
592 e35442.

593 Holmström, C. and Kjelleberg, S. (1999) Marine Speudoalteromonas species are associated with higter organisms
594 and produce biologically active extracellular agents. *FEMS Microb. Ecol.* **30**: 285–293.

595 Holt, P.D., Reid, R.R., Lewis, B.L., Luther, G.W., and Butler, A. (2005) Iron(III) coordination chemistry of

alterobactin A: A siderophore from the marine bacterium Alteromonas luteoviolacea. *Inorg. Chem.* **44**: 7671–7677.

Horgan, R.P. and Kenny, L.C. (2011) "Omic" technologies: genomics, transcriptomics, proteomics and metabolomics. *Obstet. Gynaecol.* **13**: 189–195.

Isnansetyo, A. and Kamei, Y. (2009) Bioactive substances produced by marine isolates of Pseudomonas. *J. Ind. Microbiol. Biotechnol.* **36**: 1239–1248.

Ivanova, E.P., Flavier, S., and Christen, R. (2004) Phylogenetic relationships among marine Alteromonas-like proteobacteria: Emended description of the family Alteromonadaceae and proposal of Pseudoalteromonadaceae fam. nov., Colwelliaceae fam. nov., Shewanellaceae fam. nov., Moritellaceae fam. nov., Ferri. *Int. J. Syst. Evol. Microbiol.* **54**: 1773–1788.

Ivanova, E.P. and Mikhaĭlov, V. V (2001) A new family of Alteromonadaceae fam. nov., including the marine proteobacteria species Alteromonas, Pseudoalteromonas, Idiomarina i Colwellia. *Mikrobiologiia* **70**: 15–23.

Kabir, S. (2009) The role of probiotics in the poultry industry. *Int. J. Mol. Sci.* **10**: 3531–3546.

Kates, M. and Volcani, B.E. (1966) Lipid components of diatoms. *Biochim. Biophys. Acta* **116**: 264–278.

Kazamia, E., Aldridge, D.C., and Smith, A.G. (2012) Synthetic ecology – A way forward for sustainable algal biofuel production? *J. Biotechnol.* **162**: 163–169.

Khudary, R. Al, Venkatachalam, R., and Katzer, M. (2010) A cold-adapted esterase of a novel marine isolate, Pseudoalteromonas arctica: gene cloning, enzyme purification and characterization. *Extremophiles* **14**: 273–285.

Kim, S.M., Jung, Y., and Kwon, O. (2012) A Potential Commercial Source of Fucoxanthin Extracted from the Microalga Phaeodactylum tricornutum. *Appl. Biochem. Biotechnol.* **166**: 1843–1855.

Kirchman, D.L. (2002) The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiol. Ecol.* **39**: 91–100.

Lee, S., Kato, J., Takiguchi, N., Kuroda, A., and Ikeda, T. (2000) Involvement of an Extracellular Protease in Algicidal Activity of the Marine Bacterium Pseudoalteromonas sp. Strain A28. *Appl. Environ. Microbiol.* **66**: 4334–4339.

Lotka, A. (1925) Elements of Physical Biology Williams and Wilkins (ed).

LPSN (2016a) List of Prokaryotic names with Standing in Nomenclature - Alteromonadaceae.

LPSN (2016b) List of Prokaryotic names with Standing in Nomenclature - Flavobacteriaceae.

Lu, M., Wang, S., Fang, Y., Li, H., Liu, S., and Liu, H. (2010) Cloning, expression, purification, and characterization of cold-adapted α-amylase from Pseudoalteromonas arctica GS230. *Protein J.* **29**: 591–597.

MacArthur, R. (1955) Fluctuations of animal populations and a measure of community stability. *Ecologycology* **36**: 533–536.

Manz, W., Amann, R., Ludwig, W., Vancanneyt, M., and Schleifer, K.H. (1996) Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology* **142**: 1097–1106.

Martino, A. De, Bartual, A., Willis, A., Meichenin, A., Villazán, B., Maheswari, U., and Bowler, C. (2011) Physiological and Molecular Evidence that Environmental Changes Elicit Morphological Interconversion in

the Model Diatom Phaeodactylum tricornutum. *Ann. Anat.* **162**: 462–481.

Martino, A. De, Meichenin, A., Shi, J., Pan, K., and Bowler, C. (2007) Genetic and phenotypic characterization of Phaeodactylum tricornutum (Bacillariophyceae) accessions 1. *J. Phycol.* **43**: 992–1009.

Mata, T., Martins, A., and Caetano, N. (2010) Microalgae for biodiesel production and other applications: a review. *Renew. Sustain. Energy Rev.* **14**: 217–232.

McCann, K.S. (2000) The diversity-stability debate. *Nature* **405**: 228–33.

Mitchell, M. (1996) An introduction to genetic algorithms MIT Press, Cambridge, MA.

Montsant, A., Jabbari, K., Maheswari, U., and Bowler, C. (2005) Comparative genomics of the pennate diatom Phaeodactylum tricornutum. *Plant Physiol.* **137**: 500–513.

Morel, F.M.M. and Price, N.M. (2003) The biogeochemical cycles of trace metals in the oceans. *Science* **300**: 944–7.

Owens, T.G. and Wold, E.R. (1986) Light-harvesting function in the diatom Phaeodactylum tricornutum: II. Distribution of excitation energy between the photosystems. *Plant Physiol.* **80**: 732–738.

Parmar, A., Singh, N., and Pandey, A. (2011) Cyanobacteria and microalgae: a positive prospect for biofuels. *Bioresour. Technol.* **102**: 10163–10172.

Parvez, S. and Malik, K. (2006) Probiotics and their fermented food products are beneficial for health. *J. Appl. Microbiol.* **100**: 1171–1185.

Peng, J., Yuan, J.P., Wu, C.F., and Wang, J.H. (2011) Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health. *Mar. Drugs* **9**: 1806–1828.

Persson, G., Jansson, M., and Kluwer, C. (1988) Phosphate uptake and utilizaton by bacteria and algae. *Hydrobiologia* **170**: 177–189.

Pimm, S. (1984) The complexity and stability of ecosystems. *Nature* **307**: 321–326.

Pinhassi, J., Sala, M.M., Havskum, H., Peters, F., Guadayol, O., Malits, A., and Marrasé, C. (2004) Changes in bacterioplankton composition under different phytoplankton regimens. *Appl. Environ. Microbiol.* **70**: 6753–66.

Provasoli, L. (1958) Nutrition and ecology of Protozoa and Algae. *Annu. Rev. Microbiol.* **12**: 279–308.

Provasoli, L. (1963) Organic regulation of phytoplankton fertility. In, Hill,M. (ed), *The sea: ideas and observations on progress in the study of the seas*. Wiley-Interscience, New York, pp. 165–219.

Provasoli, L. and Carlucci, A. (1974) Vitamins and growth regulators. In, Stewart,W. (ed), *Algal Physiology and Biochemistry, Botanical monographs, 10*. Blackwell Scientific Publications, California, pp. 741–787.

Pylro, V.S., Roesch, L.F.W., Morais, D.K., Clark, I.M., Hirsch, P.R., and Tótola, M.R. (2014) Data analysis for 16S microbial profiling from different benchtop sequencing platforms. *J. Microbiol. Methods* **107**: 30–37.

Qi, Z., Zhang, X.-H., Boon, N., and Bossier, P. (2009) Probiotics in aquaculture of China — Current state, problems and prospect. *Aquaculture* **290**: 15–21.

Quail, M., Smith, M.E., Coupland, P., Otto, T.D., Harris, S.R., Connor, T.R., et al. (2012) A tale of three next generation sequencing platforms: comparison of Ion torrent, pacific biosciences and illumina MiSeq sequencers. *BMC Genomics* **13**: 341.

672 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA ribosomal RNA gene
673 database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**: D590–D596.

674 R Development Core Team (2015) R: A language and environment for statistical computing. *R Found. Stat.*
675 *Comput. Vienna, Austria.* http://www.R–project.org/.

676 Rebolloso-Fuentes, M.M., Navarro-Pérez, A., Ramos-Miras, J.J., and Guil-Guerrero, J.L. (2001) Biomass nutrient
677 profiles of the microalga Phaeodactylum tricornutum. *J. Food Biochem.* **25**: 57–76.

678 Reid, R. and Butler, A. (1991) Investigation of the mechanism of iron acquisition by the marine bacterium
679 Alteromonas luteoviolaceus: Characterization of siderophore production. *Limnol. Oceanogr.* **36**: 1783–
680 1792.

681 Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F. eds. (2014) The Prokaryotes Springer
682 Berlin Heidelberg, Berlin, Heidelberg.

683 Ryther, J. and Goldman, J. (1975) Microbes as food in mariculture. *Annu. Rev. Microbiol.* **29**: 429–443.

684 Sañudo-Wilhelmy, S. a., Gómez-Consarnau, L., Suffridge, C., and Webb, E. A. (2014) The Role of B Vitamins in
685 Marine Biogeochemistry. *Ann. Rev. Mar. Sci.* **6**: 339–367.

686 Siron, R., Giusti, G., and Berland, B. (1989) Changes in the fatty acid composition of Phaeodactylum tricornutum
687 and Dunaliella tertiolecta during growth and under phosphorus deficiency. *Mar. Ecol. Prog. Ser.* **55**: 95–
688 100.

689 Smayda, T. (1997) Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in
690 the sea. *Limnol. Oceanogr.* **42**: 1137–1153.

691 Soria-Dengg, S. and Horstmann, U. (1995) Ferrioxamines B and E as iron sources for the marine diatom
692 Phaeodactylum tricornutum. *Mar. Ecol. Prog. Ser.* **127**: 269–277.

693 Soria-Dengg, S., Reissbrodt, R., and Horstmann, U. (2001) Siderophores in marine coastal waters and their
694 relevance for iron uptake by phytoplankton:experiments with the diatom Phaeodactylum tricornutum.
695 *Mar. Ecol. Prog. Ser.* **220**: 73–82.

696 Starr, M.P., Stolp, H., Trüper, H.G., Balows, A., and Schlegel, H.G. eds. (1981) The Prokaryotes Springer Berlin
697 Heidelberg, Berlin, Heidelberg.

698 Stocker, R. (2012) Marine Microbes See a Sea of Gradients. *Science* **338**: 628–633.

699 Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., et al. (2015) Structure and function
700 of the global ocean microbiome. *Science* **348**: 1–10.

701 Suminto and Hirayama, K. (1997) Application of a growth-promoting bacteria for stable mass culture of three
702 marine microalgae. *Hydrobiologia* **358**: 223–230.

703 Tredici, M.R., Biondi, N., Ponis, E., Rodolfi, L., Zittelli, G.C., Burnell, G., and Allan, G. (2009) Advances in microalgal
704 culture for aquaculture feed and other uses. In, Burnell,G. and Allan,G. (eds), *New Technologies in*
705 *Aquaculture: Improving Production Efficiency, Quality and Environmental Management.* Woodhead
706 Publishing Ltd, Cambridge, pp. 610–676.

707 Venkateswaran, K. and Dohmoto, N. (2000) Pseudoalteromonas peptidolytica sp. nov., a novel marine mussel-
708 thread-degrading bacterium isolated from the Sea of Japan. *Int. J. Syst. Evol. Microbiol.* **50**: 565–574.

709 Verhulst, P. (1838) Notice sur la loi que la population suit dans son accroissement. correspondance
710 mathématique et physique publiée par a. *Quetelet* **10**: 113–121.

711 Volterra, V. (1926) Fluctuations in the abundance of a species considered mathematically. *Nature* **118**: 558–560.

712 Vraspir, J.M. and Butler, A. (2009) Chemistry of marine ligands and siderophores. *Ann. Rev. Mar. Sci.* **1**: 43–63.

713 Wang, H., Zhang, W., Chen, L., Wang, J., and Liu, T. (2013) The contamination and control of biological pollutants
714 in mass cultivation of microalgae. *Bioresour. Technol.* **128**: 745–750.

715 Whitman, W.B., Coleman, D.C., and Wiebe, W.J. (1998) Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci.*
716 *U. S. A.* **95**: 6578–6583.

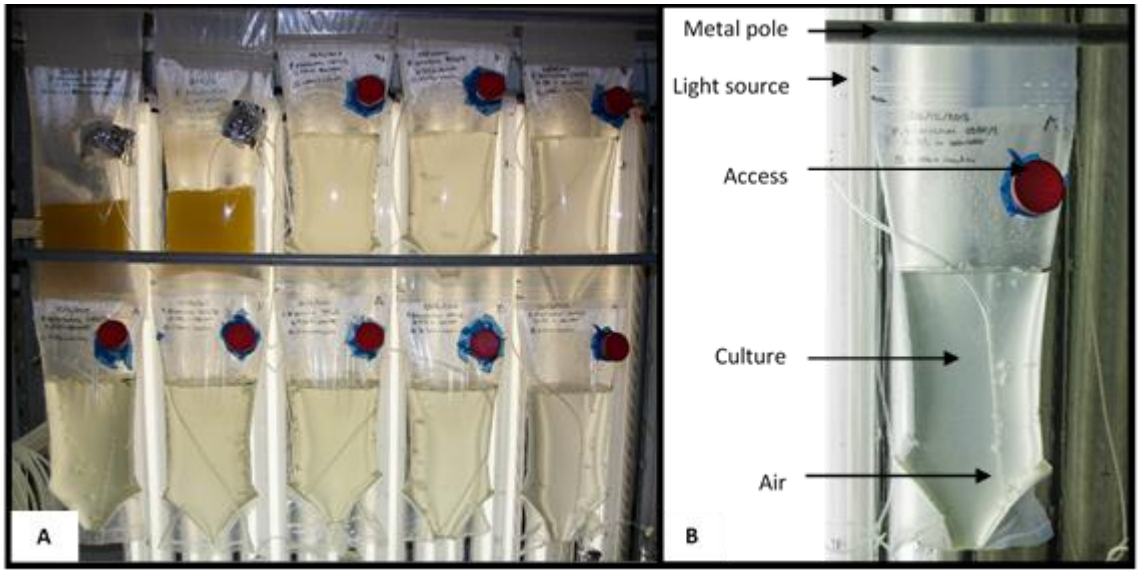
717 Yashodhara, B.M., Umakanth, S., Pappachan, J.M., Bhat, S.K., Kamath, R., and Choo, B.H. (2009) Omega-3 fatty
718 acids: a comprehensive review of their role in health and disease. *Postgrad. Med. J.* **85**: 84–90.

719 Yongmanitchai, W. and Ward, O.P. (1991) Growth of and omega-3 fatty acid production by Phaeodactylum
720 tricornutum under different culture conditions. *Appl. Environ. Microbiol.* **57**: 419–425.

721 Yoon, J., Jo, Y., Kim, G.J., and Choi, H. (2015) Gramella lutea sp. nov., a Novel Species of the Family
722 Flavobacteriaceae Isolated from Marine Sediment. *Curr. Microbiol.* **71**: 252–8.

723

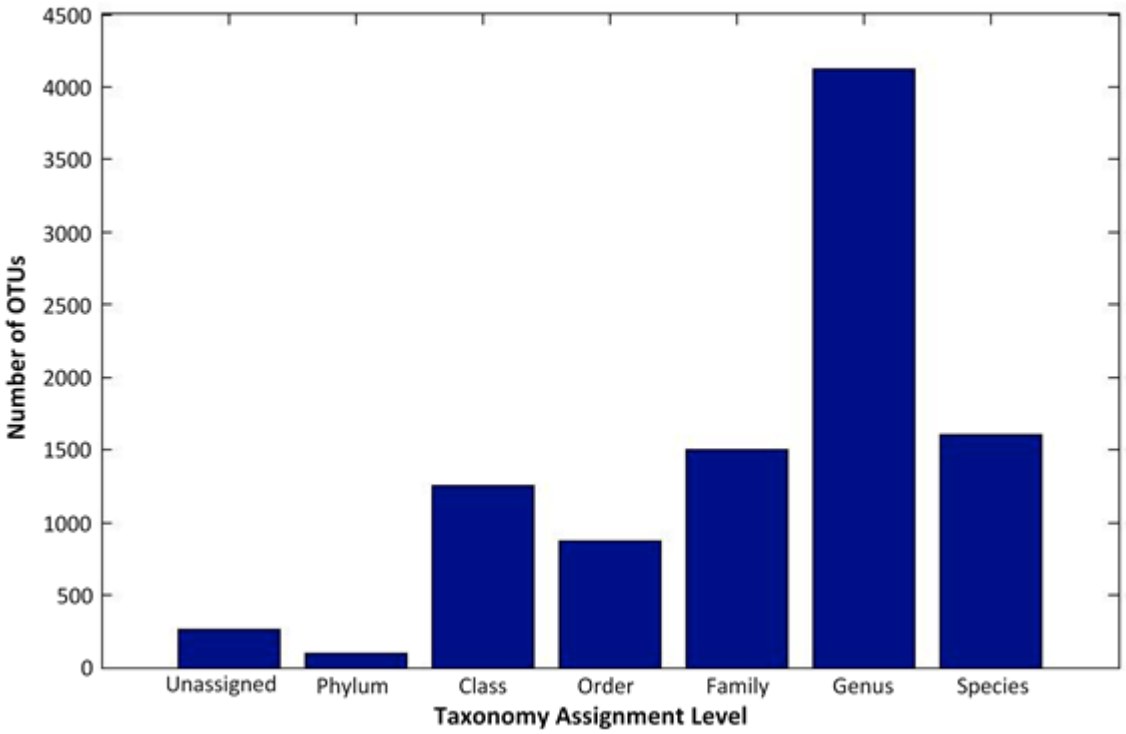
724 # Supplementary Material



725

726 **Figure S1. Non-axenic *Phaeodactylum tricornutum* culture set up.** 5L polyethylene bags with a 'V'
727 shaped bottom were created using the heat sealer machine. The bags were then rinsed and filled with
728 5L of filtered seawater. Afterwards each bag was sealed and hung approximately 30 cm from the light
729 source. A small incision was made to insert the aeration tubing. This consists of a 10ml pipette
730 attached to silicon tubing which is attached to a sterile air filter connecting it to the main air supply. A
731 modified access port was created to take samples and measure the environmental parameters
732 (Photographs courtesy of Maria Rubio Bernal)

733



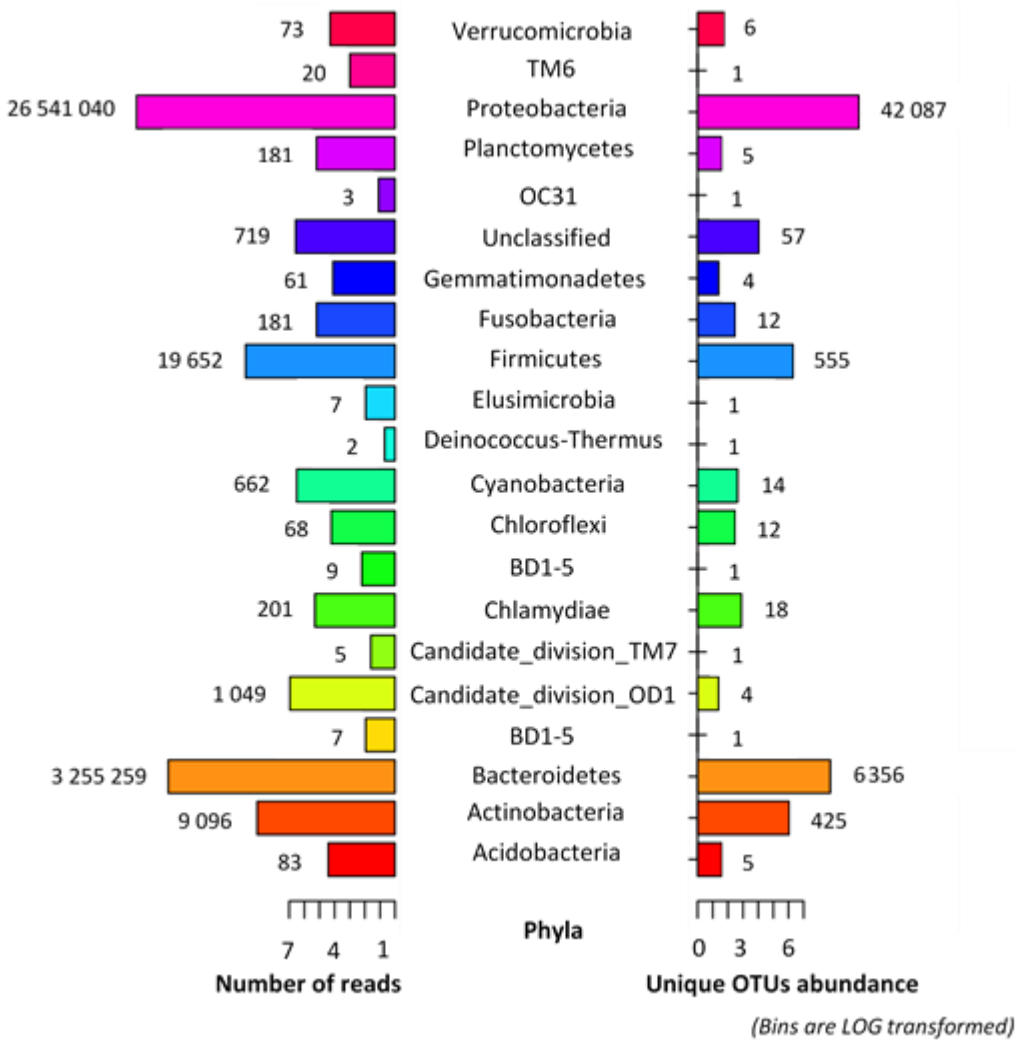
734

735 **Figure S2. Operational Taxonomic Unit (OTU) Taxonomy Assignment Level.** The 16S rRNA gene

736 sequences were clustered to defined Operational Taxonomic Units (OTUs) at ≥97% sequence identity.

737 Most OTUs could be assigned to the genera level, using the SILVA database (v.118) (Quast *et al.*, 2013).

738



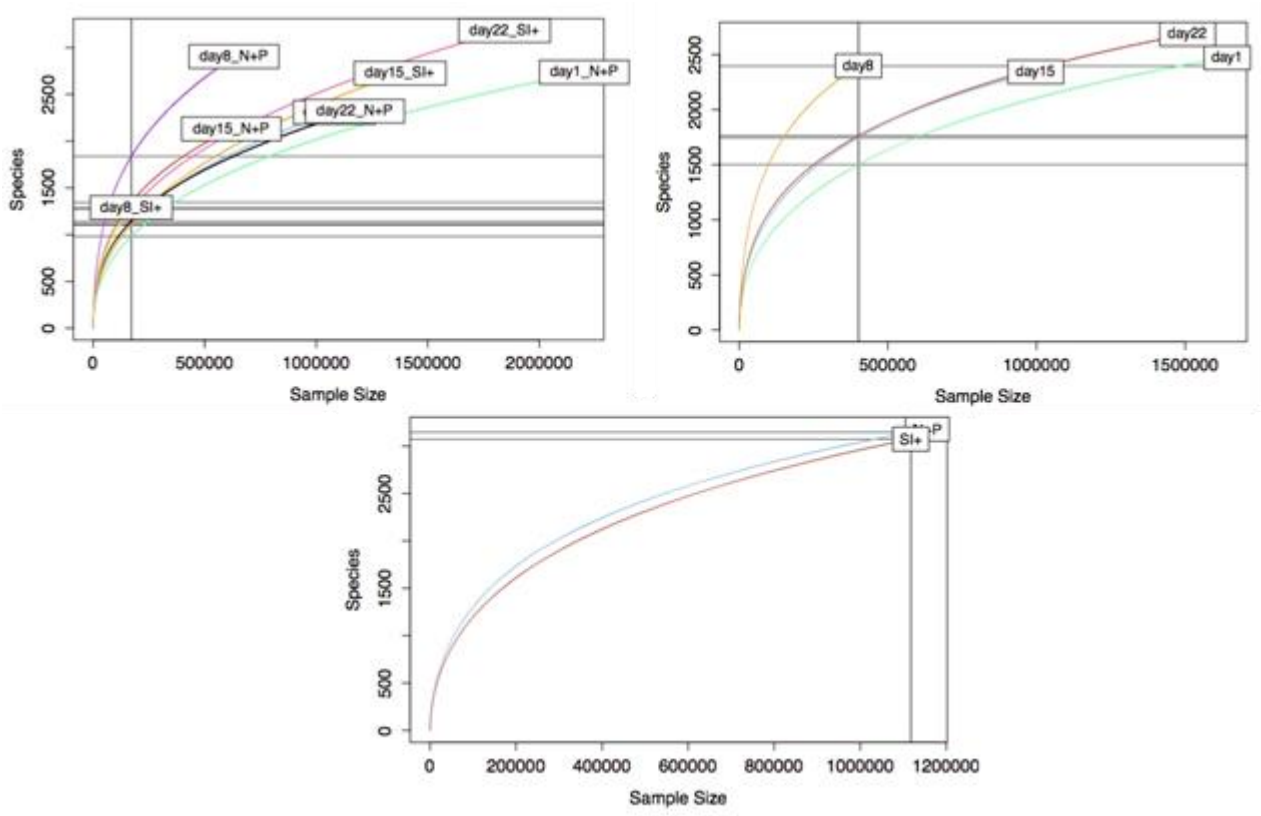
739

740 **Figure S3. Number of reads per unique OTU abundance (at the phylum level).** A comparison of the

741 number of individual reads to the number of unique OTUs shows that phyla with high number of reads

742 do not result in single OTUs.

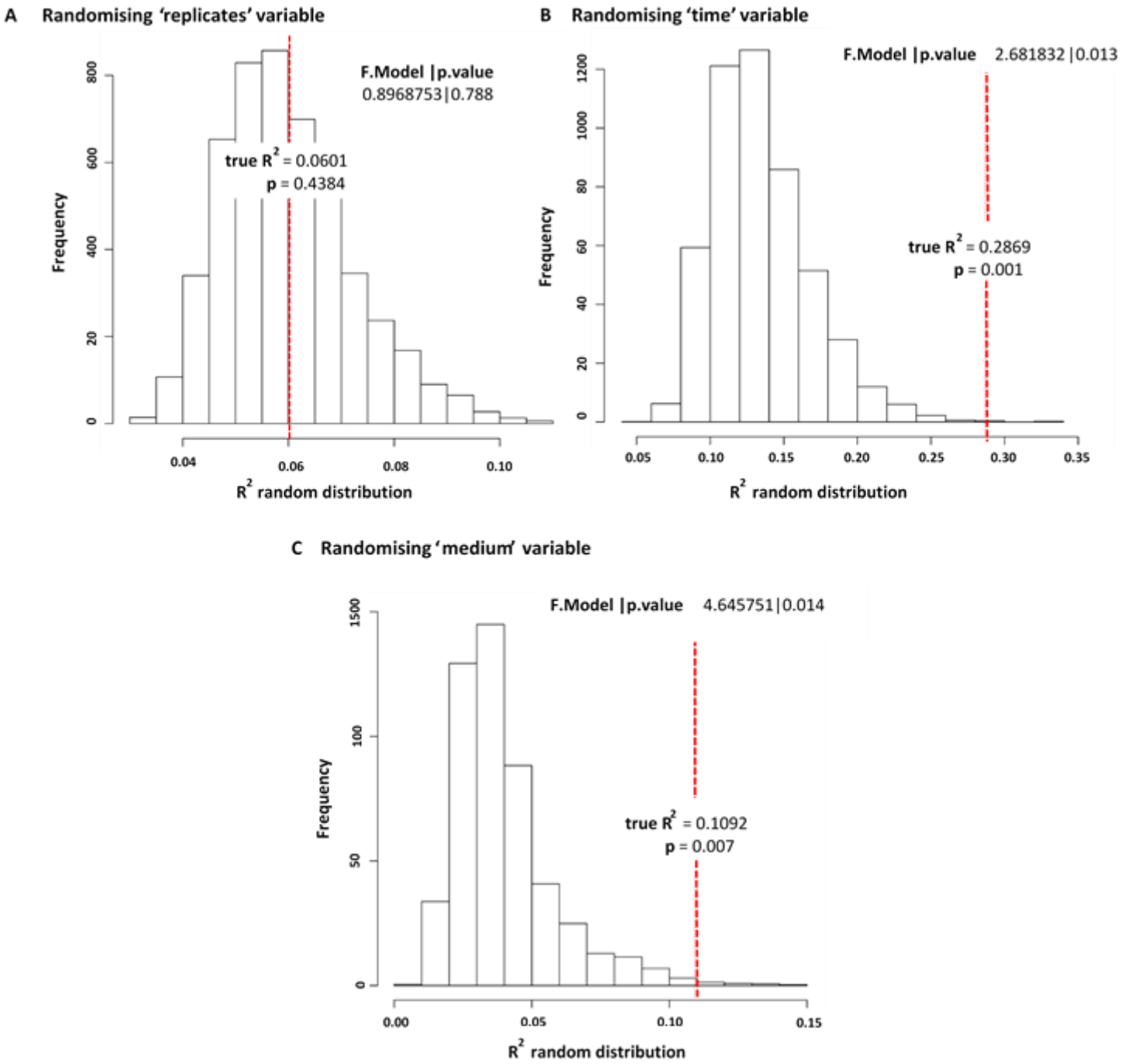
743



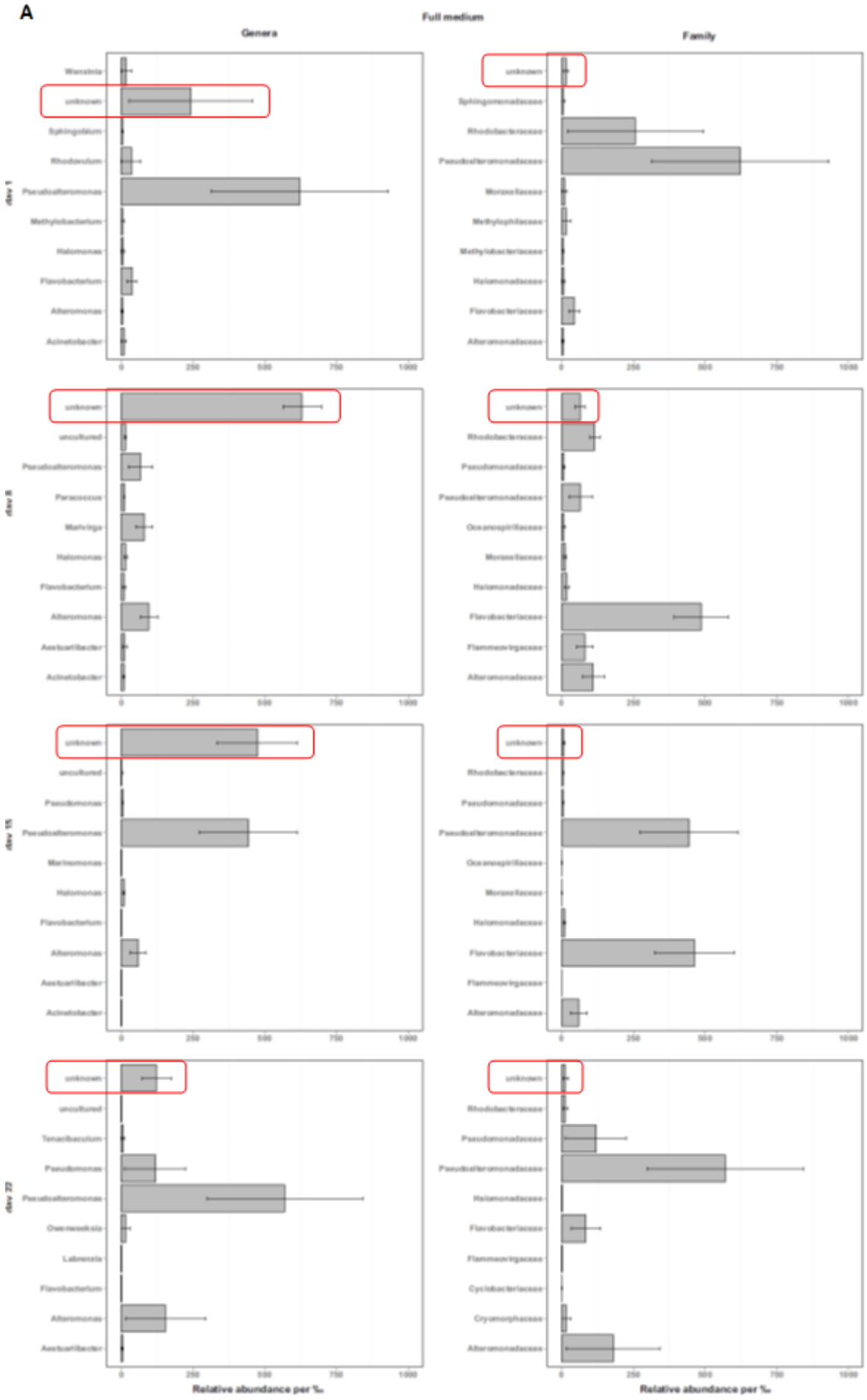
744

745 **Figure S4. Alpha diversity.** Rarefaction curves were used to evaluate the Alpha diversity in the
746 different media conditions as well as at the different time points. Species richness in both minimal and
747 complete media was ~3 000. Species richness over time remained between ~2 400 and 2 600, with
748 reduced species richness (~1 300) on Day 8 (both minimal and complete media) possibly due to
749 elevated levels of 16S *P. tricornutum* chloroplast reads which had to be omitted. Greatest species
750 richness (~ 3 000) was shown on Day 22.

751



752 **Figure S5. Beta diversity.** A modified version of PermanovaG was used to carry out permutational
753 multivariate analysis of variance using multiple distance matrices. The distance matrices [24x24] were
754 previously calculated based on the generalised UniFrac distance (Chen *et al.*, 2012), weighted UniFrac
755 and unweighted UniFrac (Lozupone and Knight, 2005) distance. The significance for the test was
756 assessed by 5000 permutations. **A** shows no significant effect between the replicates (p-value of
757 0.4384). **B** shows a significant effect for the time variable (p-value of 0.001). **C** shows also shows a
758 significant effect for the medium variable (p-value of 0.007)



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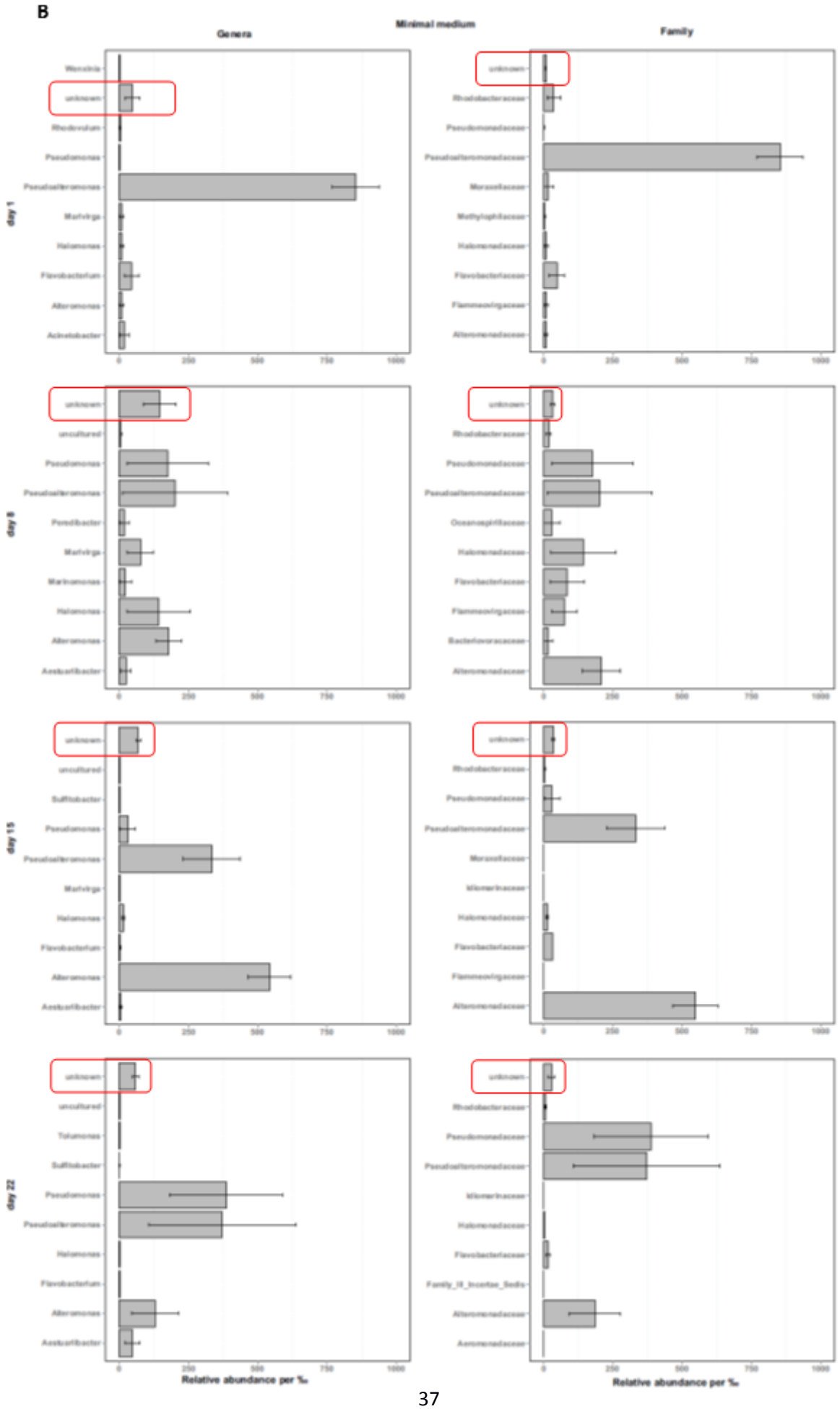

761 **Figure S6. Comparison between bacterial community at genera level and family level. A** in complete
762 media. **B** in minimal media. We show no dynamical difference within the genera that cannot be
763 observed at the family level. Encircled in red, there are a greater number of OTUs that could not be
764 assigned a taxonomy ('unknowns') at the genera level than at the family level. By investigating the
765 bacterial community dynamics at the family level, we also include taxonomical information that is
766 unavailable at the genus level.

767

Table S1. 16S V6 rRNA primer sequences. 'Max' is the complete media. 'Min' is the minimal media. 'A', 'B', and 'C' are the three replicates.

Description	Barcode Name	Adaptor A Sequence	Barcode Sequence	Forward V6 Primer Sequence	Reverse V6 Primer Sequence
Day 1_Max_A	IonXpress_071	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGAGGCTCCGAC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 1_Max_B	IonXpress_072	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAAGGCCACAC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 1_Max_C	IonXpress_073	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCTGCCTGTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 1_Min_A	IonXpress_074	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGATCGGTTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 1_Min_B	IonXpress_075	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCAGGAATAC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 1_Min_C	IonXpress_076	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGGAAGAACCTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 8_Max_A	IonXpress_077	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGAAGCGATTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 8_Max_B	IonXpress_078	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGCCAATTCTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 8_Max_C	IonXpress_079	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CCTGGTTGTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 8_Min_A	IonXpress_080	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCGAAGGCAGGC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 8_Min_B	IonXpress_081	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CCTGCCATTCGC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 8_Min_C	IonXpress_082	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TTGGCATCTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 15_Max_A	IonXpress_083	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTAGGACATTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 15_Max_B	IonXpress_084	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTTCCATAAC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 15_Max_C	IonXpress_085	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CCAGCCTCAAC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 15_Min_A	IonXpress_086	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTTGGTTATTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 15_Min_B	IonXpress_087	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TTGGCTGGAC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 15_Min_C	IonXpress_088	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CCGAACACTTC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 22_Max_A	IonXpress_090	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTAACCACGGC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 22_Max_B	IonXpress_091	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGGAAGGATGC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 22_Max_C	IonXpress_092	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTAGGAACCGC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 22_Min_A	IonXpress_093	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTTGTCCAATC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 22_Min_B	IonXpress_094	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCCGACAAGC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT
Day 22_Min_C	IonXpress_095	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGGACAGATC	MWACGCGARGAACCTTACC	CGACARCCATGCANCACCT

769 **Table S2. Generalised UniFrac distances of bacterial communities in complete and minimal media**
770 **over time.** Generalised UniFrac distance contains an extra parameter α controlling the weight on
771 abundant lineages so the distance is not dominated by highly abundant lineages. α = 0.5 has overall
772 the best power.

		DAY 1		DAY 8		DAY 15		DAY 22	
		Minimal	Complete	Minimal	Complete	Minimal	Complete	Minimal	Complete
DAY 1	Minimal	0	0.5158104	0.7195151	0.8637909	0.6668939	0.7504169	0.6655156	0.6608732
	Complete	0.5158104	0	0.7020723	0.7203046	0.7176411	0.7125498	0.7019796	0.6733559
DAY 8	Minimal	0.7195151	0.7020723	0	0.6641379	0.5844515	0.6637369	0.5905962	0.497263
	Complete	0.8637909	0.7203046	0.6641379	0	0.8558383	0.5896856	0.8340209	0.7723285
DAY 15	Minimal	0.6668939	0.7176411	0.5844515	0.8558383	0	0.6605545	0.4569922	0.5042935
	Complete	0.7504169	0.7125498	0.6637369	0.5896856	0.6605545	0	0.630498	0.5720828
DAY 22	Minimal	0.6655156	0.7019796	0.5905962	0.8340209	0.4569922	0.630498	0	0.4597439
	Complete	0.6608732	0.6733559	0.497263	0.7723285	0.5042935	0.5720828	0.4597439	0

773

774

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776

777 **References for Supplementary Figures**

778 Chen, J., Bittinger, K., Charlson, E.S., Hoffmann, C., Lewis, J., Wu, G.D., et al. (2012) Associating
779 microbiome composition with environmental covariates using generalized UniFrac distances.
780 *Bioinformatics* **28**: 2106–2113.

781 Lozupone, C. and Knight, R. (2005) UniFrac : a New Phylogenetic Method for Comparing Microbial
782 Communities UniFrac : a New Phylogenetic Method for Comparing Microbial Communities. *Appl.*
783 *Environ. Microbiol.* **71**: 8228–8235.

784 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA ribosomal
785 RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.*
786 **41**: D590–6.