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Orthogonal dietary niche enables reversible engraftment of a gut bacterial commensal

Sean M. Kearney^{1,2,3}, Sean M. Gibbons^{1,2,3}, Susan E. Erdman⁴, Eric J. Alm^{1,2,3,*,+}

¹Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, 02139, U.S.A.

²The Broad Institute, Cambridge, MA, 02139, U.S.A.

³The Center for Microbiome Informatics and Therapeutics, Cambridge, MA, 02139, U.S.A.

⁴Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA, 02139, U.S.A

Summary

Interest in manipulating the gut microbiota to treat disease has led to a need for understanding how organisms can establish themselves when introduced into a host with an intact microbial community. Here, we employ the concept of orthogonal niche engineering: a resource typically absent from the diet, seaweed, creates a customized niche for an introduced organism. In the short term, co-introduction of this resource at 1% in the diet along with an organism with exclusive access to this resource, *Bacteroides plebeius* DSM 17135, enables it to colonize at a median abundance of 1%, and frequently up to 10 or more percent, both on pulsed and constant seaweed diets. In a two-month follow-up after the initial treatment period, *B. plebeius* stops responding to seaweed in mice initially on the constant seaweed diet, suggesting treatment regime will affect controllability. These results offer potential for diet-based intervention to introduce and control target organisms.

Graphical Abstract

*Corresponding author: ejalm@mit.edu.

+Lead Contact

AUTHOR CONTRIBUTIONS













Conceptualization, S.M.K., S.E.E., E.J.A.; Methodology, S.M.K., S.E.E., S.M.G.; Formal Analysis, S.M.K.; Investigation, S.M.K., S.M.G.; Resources, E.J.A., S.E.E.; Data Curation, S.M.K.; Writing – Original Draft, S.M.K., S.M.G; Writing – Review & Editing, S.M.K., S.M.G., E.J.A; Visualization, S.M.K., E.J.A; Supervision, E.J.A., S.E.E.; Funding Acquisition, S.M.K., E.J.A.

ACCESSION NUMBERS

Data from this study have been deposited in the NCBI Short-Read Archive (SRA) and 16S rDNA gene sequences and metadata can be found under PRJNA479657 (SRP151989). The R scripts, metadata, and accompanying sequence variant table to analyze the 16S rDNA gene data can be found online at the following link: <https://github.com/microbetrainer/Seaweed>. Sequence variant tables and metadata are additionally available on Mendeley DOI: 10.17632/86xzh5c85p.1.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Diet Regime	Short Term	Long Term
 (-) seaweed-using bacteria constant dietary seaweed	 no change to microbiota	 no change to microbiota
 (+) seaweed-using bacteria constant dietary seaweed	 dietary control of bacterial growth	 no dietary control bacterial growth
 (+) seaweed-using bacteria pulsed dietary seaweed	 dietary control of bacterial growth	 dietary control of bacterial growth
 (+) seaweed-using bacteria no dietary seaweed	 no dietary control bacterial growth	 no dietary control bacterial growth

INTRODUCTION

Introducing new bacteria into an intact or disturbed microbial community is one of the primary goals of microbial therapeutics. However, we have limited knowledge about the features that govern successful colonization by introduced microorganisms. Recent work has demonstrated that the presence of functional metabolic capacity beyond that of the endogenous microbiota contributed to persistence of colonization by an introduced *Bifidobacterium* species (Maldonado-Gómez et al., 2016). A strong predictor for engraftment of microorganisms introduced by fecal microbiota transplant (FMT) is the presence of shared organisms in the donor and recipient (Li et al., 2016; Smillie et al., 2018), likely indicating that shared functional capacity mediate colonization in this case. Further, transposon sequencing of a model gut community of *Bacteroides* species identified arabinoxylan as capable of modulating the abundance of a single strain (Wu et al., 2015). Given these findings, we hypothesized that we could control the colonization of an introduced organism in an intact community by providing it with exclusive access to a resource unusable by the rest of the community.

We identified a resource unlikely to be used by microorganisms in the lab-mouse gut: the red algae, *Porphyra*, comprising the edible seaweed nori. Nori contains complex, sulfated polysaccharides including porphyran, and unlike terrestrial plants, includes vitamin B12 (Hehemann et al., 2012), which has previously been implicated as a fitness determinant for *Bacteroides thetaiotaomicron* in the murine GI tract (Goodman et al., 2009). The pathways for degradation of porphyran are rare in the human gut microbiota, and almost exclusively

found in metagenomic samples from individuals in populations known to eat seaweed (Pluvinage et al., 2018), primarily Japanese, who consume on average 5–10 g of seaweed daily (Hehemann et al., 2012). Horizontal gene transfer of the pathways necessary for breakdown of porphyrin from a marine bacterium to *B. plebeius*, a gastrointestinal commensal, suggest strong selective pressures for the acquisition of this trait (Hehemann et al., 2010). This data suggests that communities without organisms capable of metabolizing certain dietary compounds (like algal polysaccharides) may be permissive to introduction of organisms capable of directly using them (like *B. plebeius*). Recent work demonstrated this concept in a *B. ovatus* strain and two other engineered strains capable of porphyrin utilization (Shepherd et al., 2018).

Here, we confirmed the basic results of (Shepherd et al., 2018), and show that *B. plebeius* colonizes mouse guts at high levels in the presence of a preferred substrate (i.e. polysaccharides in seaweed), with no evidence of competition for this substrate from native gut bacteria. We provide a simple argument to suggest that *B. plebeius* competes with native microbes or the host for other nutrients to increase its biomass in the system. We conduct parametric analysis of a nonlinear dynamical model of the system to explain how apparently low levels of an exclusive resource can lead to abundant colonization of an introduced species. Enhanced colonization, however, comes at a cost to *B. plebeius*, with its levels depleted in the long term depending on the original seaweed treatment. We provide evidence to promote further investigation of the role of microbe-microbe and host-immune interaction in mediating these outcomes. These results provide a proof-of-principle for orthogonal niche engineering as a method for synbiotic design (i.e. controlling abundance of introduced microorganisms through manipulating resource availability) (Krumbeck et al., 2015; Panigrahi et al., 2017), but also reveal limitations to this strategy. In the context of microbial therapeutics, we show stable engraftment of a non-indigenous bacterial strain into an intact gut community in the presence of its engineered niche. However, our results also indicated that foreign microbes, when stimulated by an exclusive resource in the short term, might lose controllability in the long term.

RESULTS

Seaweed treatment does not change the composition of the mouse microbiota

We define orthogonal niche engineering as an approach involving the identification and use or engineering of an exclusive (or nearly exclusive) nutrient-species pair to introduce an organism into an intact ecosystem. This approach requires that the engineered niche (in this case through provisioning of seaweed), does not favor the growth of organisms already in a community more than the organism to be introduced (Freter, 1983). To validate that organisms in the mouse gut do not have the capacity to expand their populations significantly on seaweed-derived substrates, we introduced seaweed into mouse chow and followed the changes in the composition of the microbiota over time. We singly housed six-week old female C57BL/6 mice and randomly assigned them to two groups: (1) those receiving standard mouse chow (control) and (2) those receiving seaweed at 1% in their mouse chow (seaweed) (Fig 1A). Mice received seaweed chow continuously for 16 days, following a 32 day washout period and resumption of seaweed feeding for an additional 8

days to assess within-mouse reproducibility of the effects of seaweed feeding. Fecal samples were collected daily for the duration of the experiment, and temporally separated subsets were selected for V4 16S rDNA amplicon sequencing.

We expected that seaweed treatment would not lead to compositional changes in the microbiota. We examined the change in community structure on a seaweed diet by tracking the alpha diversity over time (Figure 1B). There were no consistent changes in Shannon diversity in the seaweed-treated mice in either the initial seaweed or late seaweed treatment time points, suggesting that seaweed feeding did not coherently alter the mouse gut microbiota in a way reflected in alpha diversity. We used Jensen-Shannon Divergence and non-metric multidimensional scaling to identify whether communities became more similar after seaweed treatment. Seaweed treated communities did not cluster separately from controls, also suggesting that this treatment led to no changes observable in the community at this level (Figure 1C). Of 289 sequence variants, none showed a significant difference in abundance (Mann Whitney U test with FDR $q < 0.10$) before and after treatment. Additionally, we found no evidence for the presence of genes involved in porphyran breakdown based on qPCR targeting the β -porphyranase gene present in the polysaccharide utilization locus (PUL) of *B. plebeius* (Hehemann et al., 2012), indicating that the genetic potential to use polysaccharides present in the seaweed was absent.

Observing limited change in response to seaweed in the gut microbiome of C57BL/6 mice, we reasoned that an organism previously reported to exploit this niche would grow in its presence in the mouse gut. Further, we wanted to pick a strain that would not engraft unless given a fitness advantage. In particular, the human-derived *B. plebeius* DSM 17135 strain carries the genes for porphyran degradation, as do at least two other (GI commensal) isolates (a BLAST search reveals porphyran degradation genes in *Bacteroides sartorii* JCM 16497 and *Porphyromonas bennonis* JCM 16335 genomes). This pathway is carried on an integrative conjugative element, and has been repeatedly transferred between gut microbial species. The positive selection on these genes, indicated by their transmission via HGT, suggests that organisms carrying these genes will be more fit in an environment rich in porphyran.

Introduction of *B. plebeius* into the digestive tract of mice

To assess the feasibility of introducing *B. plebeius* into the GI tract of mice, we ran an experiment with four groups of outbred female Swiss mice co-housed by treatment. The treatment groups were as follows: (1) mice that were gavaged at the beginning of the experiment with 10^7 CFU of *B. plebeius* (*B. plebeius* only), and mice that were gavaged with *B. plebeius* and (2) fed seaweed continuously (seaweed) or (3) delivered pulses of seaweed with four days on and four days off (pulsed), and (4) mice that were fed seaweed but not gavaged with *B. plebeius* (control) (Figure 2A). We chose outbred mice in order to reduce the likelihood of observing genotype-specific response in colonization by *B. plebeius*. Animals were co-housed by treatment to improve exchange of *B. plebeius* via coprophagy, to reduce probability of stochastic extinctions, and to select for the fittest genotype across all animals, rather than a single-animal optimized genotype.

From each mouse, fecal samples were collected daily for a 35-day period, and a subset of these samples was processed for V4 16S rDNA amplicon sequencing. Follow-up samples from 2 months after cessation of the initial seaweed feeding experiment were collected to determine whether *B. plebeius* persisted in mice feces long after the initial treatment. During the experiment we did not note any adverse affects on the mice across the treatment groups.

***B. plebeius* abundantly colonizes the GI tract of mice feeding on 1% seaweed**

We focused our sequencing efforts on the time series of 2 mice per treatment group, each randomly selected from the gavaged and untreated (*B. plebeius* only), gavaged and treated with 1% seaweed (seaweed), and gavaged and treated with pulsed 1% seaweed (pulsed) groups. In the seaweed treated groups, we expected to find enrichment for *B. plebeius* and dilution and eventually extinction in the absence of seaweed. In the pulsed treatment, we expected that *B. plebeius* levels would rise and fall to track the presence of the seaweed.

In fact, we found that the relative abundance of *B. plebeius* increased by more than two orders of magnitude ($p = 0.001$, $n = 10$ (seaweed + pulsed) mice, $n = 5$ *B. plebeius* only mice, Mann Whitney U test on qPCR-based estimates of abundance) in the seaweed and pulsed groups relative to the *B. plebeius* only group, suggesting that seaweed treatment enriches for *B. plebeius* in the GI tract of these mice (Figure 2B and Figure 2C). Indeed, at some points, *B. plebeius* rises in abundance to nearly 50% of the whole community. As expected, we observe drops (albeit with somewhat irregular patterns) in *B. plebeius* abundance that corresponded to removal of seaweed from the diet, suggesting that its ability to maintain high abundance was tied to the presence of this resource (Figure 2C). Even in mice that do not receive seaweed, the non-native, human-derived, *B. plebeius* colonizes at low levels, but frequently falls below the limit of detection, suggesting that it has limited access to additional resources in the mouse gut to enable its persistence.

Introduced organisms influence resources availability in the gut

Observing that *B. plebeius* colonizes even in the absence of seaweed in the diet, we inferred that it is capable of using resources endogenous to the mouse gut. We were surprised by the frequently high abundances of *B. plebeius* in the gut treated with seaweed, so we constructed a quantitative model of the system to accommodate these observations and facilitate further orthogonal niche engineering strategies (see Supplementary Information). In this quantitative model, *B. plebeius* can grow on either seaweed as a limiting substrate or other substrates already present in the mouse gut. Increases in *B. plebeius* abundance increase its depletion of substrates already present in the mouse gut, and reduce the availability of these substrates for growth of other organisms. Even without the quantitative model of the system, increases of biomass on carbon substrates must accompany decreases of other substrates: biomass cannot increase on a carbon substrate without commensurate consumption of substrates containing other essential elements like nitrogen.

This modeling result predicts that *B. plebeius* alters the resource pool in the gut in a way that might restrict the growth of other organisms or the host. The total abundance of bacteria in the system appears to remain unchanged in the presence or absence of seaweed as measured by qPCR (see Supplementary Figure 1). Keeping this in mind, as *B. plebeius* increases in

abundance in the system, we expect many other sequence variants to decrease, and indeed, there is an enrichment for negative correlations with *B. plebeius* in the seaweed-treated mice compared to the untreated mice even after correcting for differences in community size over time (121 negative correlations/193 total correlations with $p < 0.01$ for seaweed and pulsed groups and 1 negative correlation/22 total correlations with $p < 0.01$ for the *B. plebeius* only group, – see Methods for details). There is also a slight enrichment for negative correlations in the continuous seaweed group compared to the pulsed group.

There were few sequence variants that positively correlated with *B. plebeius* abundance. We hypothesized that the abundance of organisms with similar abundance and dynamic patterns might be constrained by the same limiting resource. We found that *A. muciniphila* significantly positively correlated with *B. plebeius* in two of the mice, one from the pulsed and one from the continuous seaweed diets (Figure 3A), and had no temporal relationship in the absence of seaweed. The remaining seaweed-treated mice both exhibited a decrease in *A. muciniphila* abundance over time, with no significant correlation to *B. plebeius*.

Because *A. muciniphila* specializes in mucin degradation (Derrien, 2004), we inferred that both organisms use mucin for growth, indicating both a resource-based niche and interaction with the mucus layer. Intriguingly, increases in *A. muciniphila* abundance was associated with even greater increases in *B. plebeius* abundance (i.e. the slope is greater than 1) (Figure 3A), suggesting that *A. muciniphila* may in some way facilitate the growth of *B. plebeius*. To test this hypothesis, we cultured *B. plebeius* and *A. muciniphila* together in the presence and absence of mucin or glucose as a limiting carbon substrate. Relative to growth on mucin alone, *B. plebeius* increases in abundance in co-culture with *A. muciniphila*. By contrast, *A. muciniphila* decreases in abundance in co-culture with *B. plebeius*, suggesting that *B. plebeius* grows at the expense of *A. muciniphila* (Figure 3B). The facilitation of *A. muciniphila* on *B. plebeius* growth is more pronounced for mucin than glucose. Such growth facilitation by and inhibition of *A. muciniphila* has been shown previously for other gut commensals, including *B. vulgatus* (Png et al., 2010).

Long-term *B. plebeius* persistence is reduced in mice constantly fed seaweed

By providing access to a limited amount of resource, we could explain the high level colonization of *B. plebeius* in the short term. We investigated whether this advantage would hold in the long term as well. We examined the presence of *B. plebeius* in mice two months after cessation of the initial seaweed-feeding period. We collected baseline samples and resumed 1% seaweed treatment in the groups that originally received seaweed. Surprisingly, although we observed blooms (to nearly 50% of the community) of *B. plebeius* on resumption of seaweed feeding in mice originally on the pulsed diet, there was no response of *B. plebeius* in the mice originally on the continuous diet, and the median relative abundance ($1.6e-5$) was just above the limit of detection for these animals (Figure 4A and Figure 4B). There was a single mouse in the pulsed seaweed group that lost *B. plebeius* entirely, but there was still a significant difference in the levels of *B. plebeius* between the constant and pulsed groups ($p = 0.02$, $n = 5$ constant mice, $n = 5$ pulsed mice, Mann Whitney U Test on qPCR-based estimates of total abundance) (Figure 4A), suggesting a

differential effect from the initial dietary regime on the long term responsiveness of *B. plebeius* to seaweed amendment.

***B. plebeius* shows increased IgA-binding when abundant after seaweed treatment**

Recalling the apparent relationship of *B. plebeius* to *A. muciniphila*, and knowing *A. muciniphila* is heavily IgA-targeted in humans and mice (Palm et al., 2014), we wondered whether IgA-targeting, and thus immune activation against *B. plebeius* might be related to its disappearance from some of the mice, by inhibiting its ability to uptake seaweed polysaccharides through steric blocking or related mechanisms that prevent biofilm formation (Moor et al., 2017). Equally, IgA-targeting may be a mechanism for retaining bacteria while simultaneously controlling their growth (Donaldson et al., 2018; McLoughlin et al., 2016).

With these considerations in mind, we evaluated whether *B. plebeius* was detected and targeted by IgA in the gut. Only in the case that *B. plebeius* generates a specific response by the immune system would we expect increased binding of *B. plebeius* relative to the rest of the microbiota, as polyreactive IgA tends not to preferentially bind *Bacteroidetes* (Bunker et al., 2017). Such a difference in IgA binding might mediate control of *B. plebeius* abundance in the constant seaweed group at late time points, and potentially explain loss of *B. plebeius*. To test this hypothesis, we flow-sorted and sequenced fecal bacteria bound by IgA as described previously (Palm et al., 2014). At the late time points, *B. plebeius* was too low in abundance ($< 1e-5$) to detect in mice in the continuous seaweed treatment group. We focused our IgA-Seq efforts on late time point samples from mice originally on the pulsed and *B. plebeius* only groups, for which *B. plebeius* was detectable by qPCR. We failed to detect a signal of differential IgA-binding of *B. plebeius* in the *B. plebeius* only mice (Supplementary Figure 2), suggesting that IgA may not be an important control mechanism for *B. plebeius* in these animals. However, in the pulsed mice that still had *B. plebeius*, there is a clear enrichment for this organism when it is present in the highly IgA-bound fraction (Supplementary Figure 2). In fact, it is more IgA-bound than any other member of the microbiota in one of these mice, suggesting that the binding was highly specific to this organism. We interpret this to mean that IgA might be an important host-side control mechanism on this organism in the presence of dietary seaweed.

Discussion

Here, we provide a proof-of-concept of diet-based orthogonal niche engineering, whereby an organism is introduced with a tailored resource to establish an orthogonal niche in an intact community. Previous studies of the influence of diet on the gut microbiota have primarily focused on the alterations in the bacterial community and functional or metabolic shifts in response to dietary perturbations (Carmody et al., 2015; David et al., 2013, 2014; Desai et al., 2016; Turnbaugh et al., 2006, 2009). It has been documented that *Bacteroidetes* in particular display a hysteretic response (i.e. memory of past dietary exposures dampens future responses) to dietary changes (Carmody et al., 2015) that may affect their future ability to respond to substrates in the diet. We provide additional evidence that previous dietary exposures affect the ability of organisms to respond to future diets.

Similar results to those found in the present paper were recently reported (Shepherd et al., 2018), confirming and complementing those in this manuscript. Shepherd and colleagues identified a *B. ovatus* strain capable of utilizing porphyran through the same PUL as *B. plebeius*, and engineered two other strains (*B. thetaiotaomicron* and *B. stercoris*) with the same capacity. In line with our results, they successfully introduce these three porphyran users into distinct, intact microbiota in Swiss Webster mice both with seaweed in the case of *B. ovatus*, and purified porphyran in water in all other experiments. They find that porphyran alone does not alter the composition of the gut microbiota in Swiss Webster mice, which mirrors what we found with seaweed in C57BL/6 mice. It is likely that differences in host genetics or the endogenous microbiota across mouse lines could cause differences in the response to dietary amendments. However, the composition of the gut microbiota in both Swiss Webster (Shepherd et al., 2018) and C57BL/6 mice (this study) is not altered by the addition of porphyran or seaweed to the diet in the absence of known porphyran users.

Shepherd and colleagues show that *B. ovatus* can colonize the colonic crypts in the presence, but not in the absence of porphyran, allowing for co-existence of isogenic strains through apparent niche partitioning. We suspect this mechanism may extend to our system with *B. plebeius*, and may contribute to the differences in responsiveness of *B. plebeius* at late time points depending on diet. One possibility is that populations of *B. plebeius* from the constantly fed seaweed group may be confined to the crypts, and so less responsive to seaweed administration because of reduced access. Recent evidence suggests that crypt colonization for *B. fragilis* is dependent on IgA (Donaldson et al., 2018), and we present evidence that *B. plebeius* is heavily IgA-bound in seaweed-treated mice, which can confine bacterial growth through enchainment and aggregation of dividing cells (Moor et al., 2017). The differences in long term responses merit further consideration as they suggest controllability of introduced organisms will depend on the history of dietary exposures, as has been observed before for the response of *Bacteroides* to dietary fluctuations (Carmody et al., 2015).

Additionally, Shepherd and colleagues show a correlation between *B. ovatus* abundance and porphyran concentration in the water and manage this control on both fiber free and fiber-rich diets. Our system administered seaweed in a fiber-containing chow and not porphyran in water. Further, our conventional mice start with more complex communities than the conventional, restricted flora mice used by Shepherd and colleagues. In all sampled mice that were originally on the seaweed diet, we observed noisy fluctuations in the abundance of *B. plebeius*, particularly in the pulsed diet, suggesting a strong dependence on this resource. These results contrast to the relatively tight control over introduced microbial abundance seen by Shepherd and colleagues, and may relate to the experimental differences outlined above.

We show that *B. plebeius* can inhibit the growth of other members of the community, particularly *A. muciniphila*, a primary mucin degrader, which has important implications for the engineering of this system. We use a conceptual and quantitative model to suggest that introduced organisms are not metabolically isolated from the existing community, but interact with it in ways that require consumption of resources in addition to their exclusive substrate. Indeed, we show that increases in *B. plebeius* during seaweed treatment do not

associate with increases in total community size, meaning that other organisms will necessarily be negatively impacted. Similarly, Shepherd and colleagues show an increase in total community size largely from an increase in the population of *B. ovatus* when treating with porphyrin on fiber-free, but not on fiber-rich diets. These results along with our own suggest that community size on fiber-containing diets is constrained by different resources than fiber-free diets, consistent with work from others on this topic (Desai et al., 2016).

The issue of resource competition will be more acute in the case of porphyrin, which does not provide a source of nitrogen for growth. If total biomass of an introduced organism increases on a carbon-only substrate, nitrogen must also be incorporated to support this biomass increase, and necessarily affects the pool of available resources either to the rest of the microbial community or to the host. Important pathogens, including *C. difficile* and *S. enterica* have been shown to use mucin-derived substrates when metabolic networks among the native microbiota are disrupted, allowing them to expand to significant abundances within the community (Ng et al., 2013). For dysbiotic communities, this consequence may be considered a desirable feature, as the introduced organism could siphon resources from microorganisms that would otherwise exacerbate disease.

These results provide additional proof for the concept of orthogonal niche engineering as an approach to introducing bacteria into complex communities. The convergence on porphyrin by us and another independent group (Shepherd et al., 2018) as a means to control introduced microbial abundance might question the extensibility of this strategy to other organism-substrate pairs, if there are few other combinations that exhibit these properties. Shepherd and colleagues manage to transfer this pathway to naïve organisms, but this strategy requires introduction of 34 genes. The evidence by Maldonado-Gómez and colleagues (Maldonado-Gómez et al., 2016), and our work suggests that synthetic strategies may not be necessary if it is possible to identify organism-substrate pairs that function in this way independent of community context. For example, through functional screens of human gut bacteria, it may be possible to reveal the nutritional preferences of specific strains to identify such pairs (Tramontano et al., 2018). Of course, a major limitation of this approach is ensuring that these strain-nutrient pairs will succeed independent of community context. As an alternative approach, synthetic nutrient-organism pairs (for example, engineering an organism to metabolize sucralose) may fulfill these design criteria. We caution that all strategies in this vein will be contingent on the universal requirement for nitrogen and other limiting resources in the gut. Rational synbiotic design will benefit from considerations of the interaction of introduced prebiotics and probiotics with other biotic and abiotic factors. In this system, there is very clear positive selection for growth on seaweed given that the genes were transferred from a marine bacterium to a gut commensal bacterium. It will be important to consider how off-target effects through species-species, species-diet, and species-host interactions can alter these responses.

Experimental Procedures

Animals

Six-week old female C57BL/6 wild type and outbred Swiss Webster mice (Taconic, Germantown, NY) were housed and handled in Association for Assessment and

Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities using techniques and diets including *Bacteroides plebeius* as specifically approved by Massachusetts Institute of Technology's Committee on Animal Care (CAC) (MIT CAC protocol # 0912-090-15 and 0909-090-18). The MIT CAC (IACUC) specifically approved the studies as well as the housing and handling of these animals. Mice were euthanized using carbon dioxide at the end of the experiment.

***B. plebeius* culture**

B. plebeius DSM 17135 was obtained from the DSMZ and cultured as specified. Briefly, colonies were obtained by the streak method on PYG (Modified) Agar incubated at 37°C in a Coy Anaerobic Chamber (Grass Lake, MI) for up to 48 hours. After the appearance of colonies, single colonies were inoculated in PYG (Modified) medium and incubated overnight prior to gavage or nucleic acid extraction. 25% glycerol stocks of *B. plebeius* were made and stored at -80°C and streaked onto fresh medium for revival and colony picking.

***A. muciniphila* culture**

A. muciniphila DSM 26127 was obtained from the DSMZ and cultured as specified. Briefly, colonies were obtained by the streak method on BHI + 0.5% (w/v) porcine type II gastric mucin (Sigma M2738) purified as described previously (Derrien, 2004) agar, and incubated at 37°C in a Coy Anaerobic Chamber (Grass Lake, MI) for up to 48 hours. After the appearance of colonies, single colonies were inoculated into liquid medium (BHI + 0.5% (w/v) mucin), and incubated overnight before use in co-culture experiments.

Co-culture Experiments

Overnight cultures of *B. plebeius* and *A. muciniphila* grown in PYG (modified) and BHI + 0.5% (w/v) mucin, respectively, were inoculated at a starting density of 10⁶ CFUs alone or in combination into medium 75 (Hoskins and Boulding, 1981) with either porcine type II gastric mucin (Sigma M2738) or glucose as the limiting carbon source at 0.5% (w/v). After 48 hours of growth, colony forming units were enumerated by plate counts on selective media (BHI + 0.5% (w/v) mucin) or PYG (modified) for *A. muciniphila* and *B. plebeius*, respectively).

Seaweed Diet Experiment

C57BL/6 mice were used in the initial studies in Figure 1 in which 10 mice were fed with a custom chow diet (Bio-Serv, Flemington NJ) containing 1% raw seaweed nori (Izumi Brand) and 10 mice had a standard control diet (Product# F3156, AN-93G, Bio-Serv, Flemington NJ). Animals were co-housed for 6 days after arrival in the MIT animal facilities, and singly housed after separation into the seaweed treatment and control groups. Fresh fecal samples were obtained within an hour daily for all animals in all groups. Fecal samples were collected into anaerobic 25% glycerol containing 0.1% cysteine, and transferred immediately to dry ice before being stored at -80°C prior to nucleic acid extraction.

***B. plebeius* Gavage Experiment**

For the *B. plebeius* gavage experiments, Swiss Webster mice were co-housed for 6 days after arrival in MIT animal facilities, and 5 mice per group were co-housed by treatment on initiation of the experiment. Fecal samples were collected for each animal for three days prior to the initiation of the experimental protocol. At day 0, mice in the *B. plebeius* gavage groups were gavaged only once with approximately 10^7 CFUs of *B. plebeius* DSM 17135 culture in 250 μ L volumes of PYG (Modified) media, and control groups were gavaged only once with 250 μ L sterile PYG (Modified) media. All groups treated with seaweed received a custom chow diet containing 1% seaweed nori at the initiation of experiments. All fecal samples were collected as described for the initial seaweed diet experiment.

Nucleic Acid Extraction

DNA from fecal samples and bacterial cultures was extracted using the MoBio High Throughput (HTP) PowerSoil Isolation Kit (MoBio Laboratories, Inc., now Qiagen) with minor modifications. Briefly, samples were homogenized with bead-beating and then 50 μ L Proteinase K (Qiagen) added and samples were incubated in a 65°C water bath for 10 minutes. Samples were then incubated at 95°C for 10 minutes to deactivate the protease. All other steps remained the same.

16S Library Preparation and Sequencing

Libraries for paired-end Illumina sequencing were constructed using a two-step 16S rRNA PCR amplicon approach as described previously with minor modifications (Preheim et al., 2013). The first-step primers (PE16S_V4_U515_F, 5' ACACG ACGCT CTTCC GATCT YRYRG TGCCA GCMGC CGCGG TAA-3'; PE16S_V4_E786_R, 5'-CGGCA TTCCT GCTGA ACCGC TCTTC CGATC TGGAC TACHV GGGTW TCTAA T 3') contain primers U515F and E786R targeting the V4 region of the 16S rRNA gene, as described previously (Preheim et al., 2013). Additionally, a complexity region in the forward primer (5'-YRYR-3') was added to help the image-processing software used to detect distinct clusters during Illumina next-generation sequencing. A second-step priming site is also present in both the forward (5'-ACACG ACGCT CTTCC GATCT-3') and reverse (5'-CGGCA TTCCT GCTGA ACCGC TCTTC CGATC T-3') first-step primers. The second-step primers incorporate the Illumina adapter sequences and a 9-bp barcode for library recognition (PE-III-PCR-F, 5'-AATGA TACGG CGACC ACCGA GATCT AACT CTTTC CCTAC ACGAC GCTCT TCCGA TCT 3'; PE-III-PCR-001-096, 5'-CAAGC AGAAG ACGGC ATACG AGATN NNNNN NNNCG GTCTC GGCAT TCCTG CTGAA CCGCT CTTCC GATCT 3', where N indicates the presence of a unique barcode).

Real-time qPCR before the first-step PCR was done to ensure uniform amplification and avoid overcycling all templates. Both real-time and first-step PCRs were done similarly to the manufacturer's protocol for Phusion polymerase (New England BioLabs, Ipswich, MA). For qPCR, reactions were assembled into 20 μ L reaction volumes containing the following: DNA-free H₂O, 8.9 μ L, HF buffer, 4 μ L, dNTPs 0.4 μ L, PE16S_V4_U515_F (3 μ M), 2 μ L, PE16S_V4_E786_R (3 μ M) 2 μ L, BSA (20 mg/mL), 0.5 μ L, EvaGreen (20X), 1 μ L, Phusion, 0.2 μ L, and template DNA, 1 μ L. Reactions were cycled for 40 cycles with the following conditions: 98° C for 2 min (initial denaturation), 40 cycles of 98 C for 30 s

(denaturation), 52° C for 30 s (annealing), and 72° C for 30s (extension). Samples were diluted based on qPCR amplification to the level of the most dilute sample, and amplified to the maximum number of cycles needed for PCR amplification of the most dilute sample (18 cycles, maximally, with no more than 8 cycles of second step PCR). For first step PCR, reactions were scaled (EvaGreen dye excluded, water increased) and divided into three 25- μ L replicate reactions during both first- and second-step cycling reactions and cleaned after the first-and second-step using Agencourt AMPure XP-PCR purification (Beckman Coulter, Brea, CA) according to manufacturer instructions. Second-step PCR contained the following: DNA-free H₂O, 10.65 μ L, HF buffer, 5 μ L, dNTPs 0.5 μ L, PE-III-PCR-F (3 μ M), 3.3 μ L, PE-III-PCR-XXX (3 μ M) 3.3 μ L, Phusion, 0.25 μ L, and first-step PCR DNA, 2 μ L. Reactions were cycled for 10 cycles with the following conditions: 98° C for 30 s (initial denaturation), 10 cycles of 98° C for 30 s (denaturation), 83° C for 30 s (annealing), and 72° C for 30s (extension). Following second-step clean-up, product quality was verified by DNA gel electrophoresis and sample DNA concentrations determined using Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific). The libraries were multiplexed together and sequenced using the paired-end with 250-bp paired end reads approach on the MiSeq Illumina sequencing machine at the BioMicro Center (Massachusetts Institute of Technology, Cambridge, MA).

16S rDNA Sequence Data Processing and Quality Control

Paired-end reads were joined with PEAR (Zhang et al., 2014) using default settings. After read joining, the complexity region between the adapters and the primer along with the primer sequence and adapters were removed. Sequences were processed batchwise using the DADA2 (Callahan et al., 2016) pipeline in R, trimming sequences to 240 bp long after quality filtering (quality trim Q10) with maximum expected errors set to 1. A final sequence variant table combining all sequencing data was generated using DADA2. Sequence variants were classified using RDP (Maidak et al., 1996; Wang et al., 2007). The resulting count tables were used as input for analysis within R.

qPCR

qPCR was carried out as described in the **16S rDNA Library Preparation and Sequencing** section. For quantification of *B. plebeius*, primers were designed to target the beta-porphyrinase A gene (BACPLE_01693) in the porphyrin degradation PUL: GH86 F: 5'-TCGAA TGTCA CAAAG CGTTC-3' and GH86 R: 5'- ATGGA CGGGA CATTCTGTTC-3'. For direct quantification of *B. plebeius* abundance, a nucleic acid standard curve was prepared using 10-fold dilutions of nucleic acids extracted from *B. plebeius* overnight cultures quantified by NanoDrop after RNase treatment. Mann Whitney U test was used to identify differences in *B. plebeius* abundance between treatment and control groups.

IgA Sorting

Pre-weighed frozen fecal samples in glycerol were thawed at 4°C and then homogenized using a handheld homogenizer and pestles (Kimble Chase Kontes) at a final dilution in sterile PBS of 100 mg per ml. Samples were processed as described previously (Palm et al., 2014). After homogenization, samples were centrifuged at 50 \times g at 4°C for 15 minutes,

then washed three times in 1 ml PBS/1% BSA at $8000 \times g$ for 5 minutes each. The pre-sort fraction was collected as 20 μL after resuspension prior to the final wash and stored at -80°C . The cell pellet was resuspended in 25 μL of 20% Normal Rat Serum (Jackson ImmunoResearch) in PBS/1% BSA and incubated for 20 minutes on ice. After incubation, 25 μL 1:12.5 α -mouse- IgA-PE (EBioscience, clone mA-6E1) was added to each sample and samples incubated on ice for 30 minutes. Samples were washed three times in 1 ml PBS/1% BSA as above, and finally resuspended in PBS/1% BSA and transferred to blue filter cap tubes (VWR 21008–948) for flow sorting. An average of 500,000 cells from the IgA-positive and IgA-negative bacteria were sorted in triplicate into sterile microcentrifuge tubes on the BD FACSAria II at the MIT Koch Institute Flow Cytometry Core (Cambridge, MA). Samples were centrifuged, supernatant removed, and stored at -80°C until nucleic acid extraction using the DNeasy UltraClean Microbial Kit (Qiagen).

16S rDNA Data Analysis

A DADA2 sequence variant table including all experiments was imported into R and analyzed using phyloseq (McMurdie and Holmes, 2013) and custom R scripts.

Spearman correlations between *B. plebeius* and all other sequence variants in the time series were determined for each of the two mice across three groups. The number of sequence variants with negative or positive correlations and unadjusted p-values less than 0.01 were determined for each group. The Fisher Exact test was used to determine whether there were differences in the proportion of positive and negative correlations between the groups for each pairwise comparison of groups.

IgA Data Analysis

We used DESeq2 to detect differences in abundance between IgA-bound and IgA-unbound fractions (McMurdie and Holmes, 2014). Using DESeq2 as applied to microbiome count data, OTUs that had an adjusted p-value of < 0.05 (Wald Test with Benjamini-Hochberg correction) between the IgA+ and IgA- fractions were considered significantly differentially abundant between the fractions.

Model Construction and Analysis

See Supplementary Information for the simulated chemostat model for bacterial growth. Code for model simulations were implemented in MATLAB R2017B.

Statistical Analysis

All statistical analyses were performed using the R statistical computing package (<https://cran.r-project.org/>), except statistics for comparison of colony forming units (CFU) for *B. plebeius* and *A. muciniphila* (Figure 3B) and total community size comparisons with qPCR (Supplementary Figure 1), which were performed in Excel. Statistically significant differences are shown with asterisks as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Number of animals (n) used for individual experiments and details of the statistical tests used are indicated in the figure legends. When quantities were expected to follow a normal distribution (as the log of total cell counts), we compared groups using a t test. Otherwise, a non-parametric (Mann-Whitney) test was used. For calculating correlations, we use

Spearman's rho to account for nonlinear relationships between two variables. IgA data was analyzed using DESeq2 and the Wald Test ($p < 0.05$) to identify significantly differentially abundant sequence variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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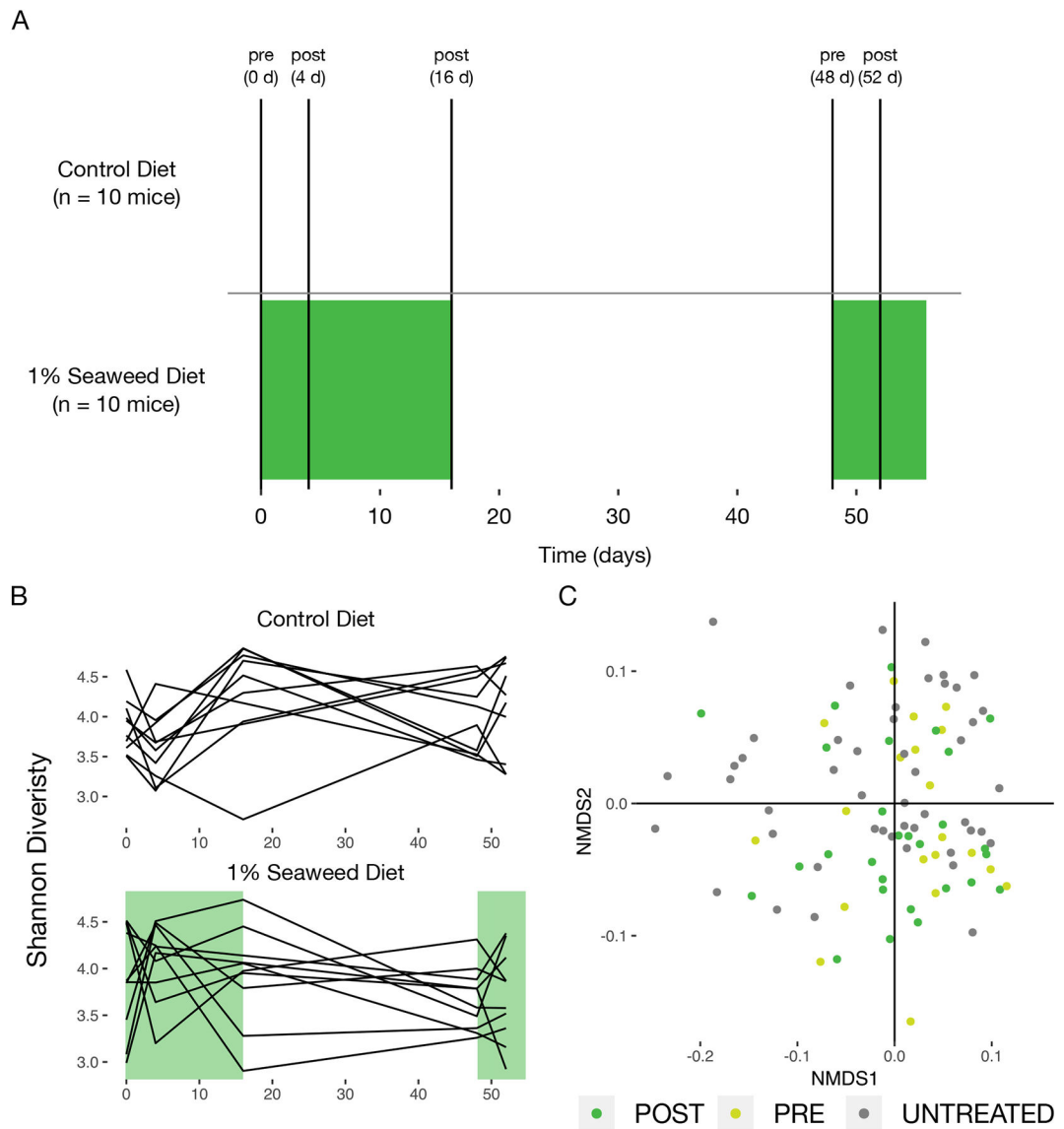


Figure 1.

Seaweed does not alter the microbiota of conventional mice. (A) Experimental design: samples were collected at the indicated time points for 16S rDNA sequencing. Green shaded region indicates period of seaweed feeding. (B) Shannon diversity over time for control animals (n = 10) and seaweed treated animals (n = 10), with green shading again indicating the period of seaweed feeding. (C) Nonmetric multidimensional scaling of the Jensen-Shannon Divergence across mice treated and untreated with seaweed and in pre- and post-time points.

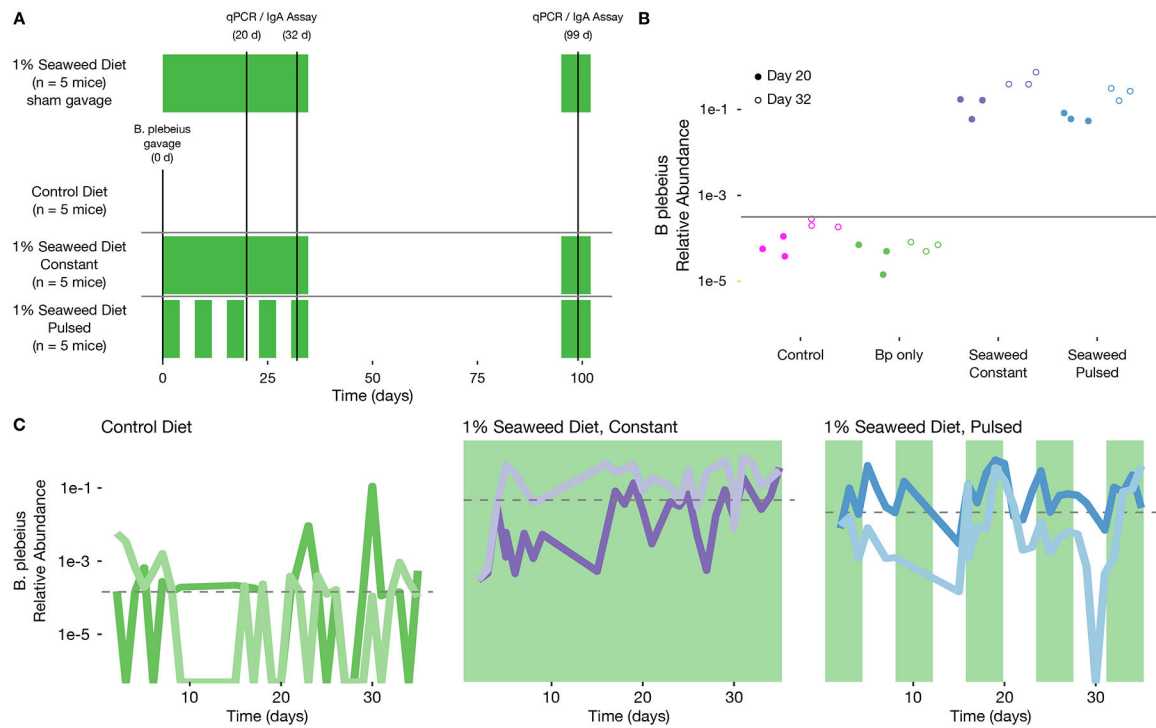


Figure 2.

B. plebeius reaches persistent high level abundance in mice consuming seaweed. (A) Experimental design: samples were collected where indicated for qPCR (n = 5 mice per group) and IgA-Sequencing; two animals per *B. plebeius* only, constant seaweed, and pulsed seaweed were sampled densely across until cessation of the first seaweed treatment window. Green shaded region indicates the seaweed dosing windows. (B) qPCR-based relative abundance of *B. plebeius* in control (n = 5), *B. plebeius* only (n = 5), constant seaweed (n = 5), and pulsed groups (n = 5) at indicated time points. The lower limit of detection from non-specific amplification is marked with a gray line. (C) Time series of *B. plebeius* in *B. plebeius* only (n = 2), constant seaweed (n = 2), and pulsed groups (n = 2), line colors distinguish individual animals, green shading indicates seaweed-dosing periods. See also Figure S1.

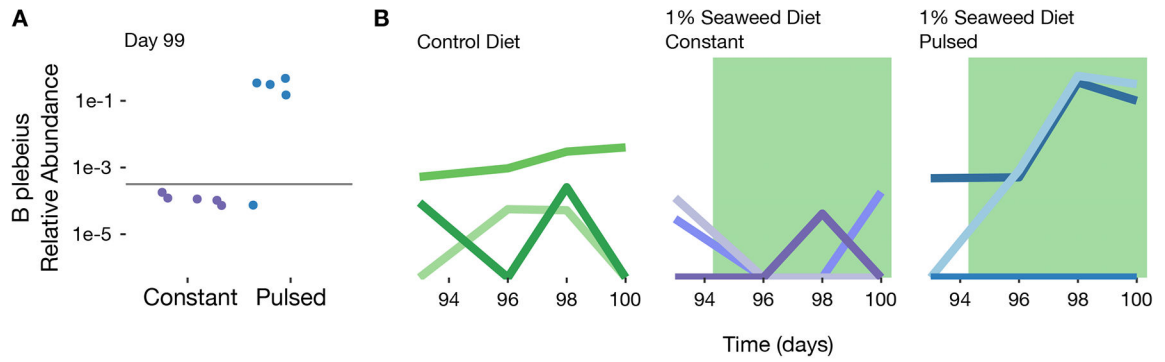


Figure 3.

Introduced commensal competes with endogenous gut commensals. (A) Scatterplot of *B. plebeius* and *A. muciniphila* abundance over time for two animals having significant correlations between these bacteria (purple = an animal from the constant seaweed group, blue = an animal from the pulsed seaweed group). (B) *B. plebeius* JCM 17135 and *A. muciniphila* DSM 26127 were cultured alone or in combination in carbon-restricted growth medium with either glucose or mucin as the primary carbon source. Bars are the standard error of the mean (n = 4 replicates/condition) (Student's t test on log-transformed abundance data: *, p-value < 0.05, **, p-value < 0.01, ***, p-value < 0.001).

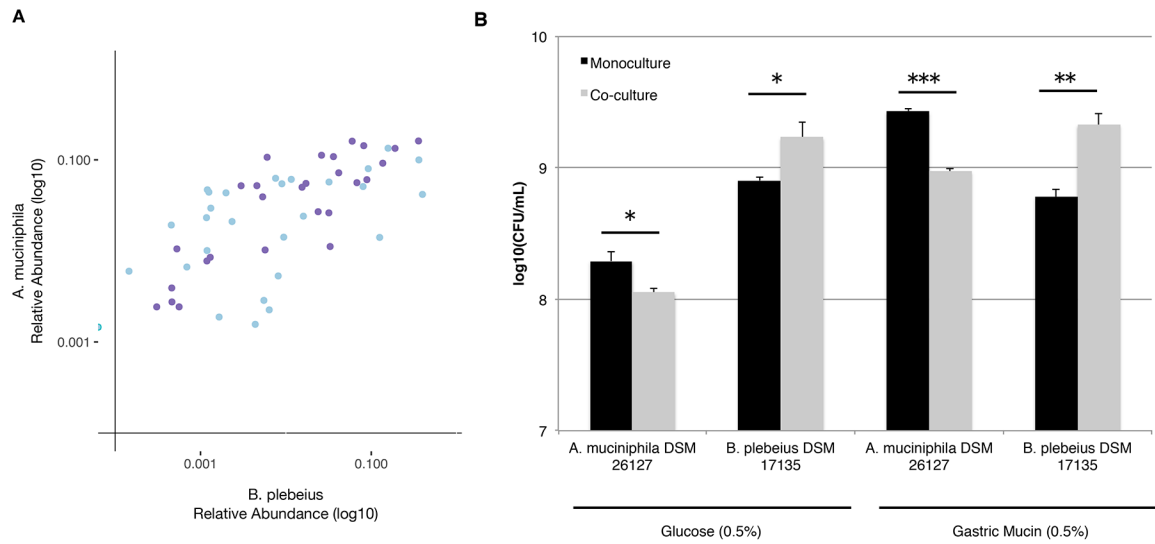


Figure 4.

Long-term persistence of *B. plebeius* depends on initial diet regimen. (A) qPCR-based relative abundance of *B. plebeius* in constant ($n = 5$) and pulsed ($n = 5$) seaweed groups at indicated time point after 1% seaweed amendment. The lower limit of detection from non-specific amplification is marked with a gray line. (B) Relative abundance of *B. plebeius* over time in late time points on resumption of seaweed treatment after washout in *B. plebeius* only ($n = 3$), constant ($n = 3$), and pulsed seaweed groups ($n = 3$).