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Pelagic ecosystem research incubators (PERIcosms): optimized incubation tanks to investigate natural communities under long term, low nutrient, and low metal conditions

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Abstract

In vitro incubations using natural marine communities can provide insight into community structure and function in ways that are challenging through field observations alone. We have designed a minimal metal incubation system for controlled and repeatable experimentation of microbial communities. The systems, dubbed Pelagic Ecosystem Research Incubators (PERIcosms), are 115 L, conical tanks designed to sample suspended, settled, and wall associated material for month long periods. PERIcosms combine some of the ecological advantages of large volume mesocosm incubations with the experimental ease and replication of bottle incubations, and their design is accessible for use by researchers without specialized training or travel to a designated incubation facility. Here, we provide a detailed description for the construction and implementation of PERIcosms and demonstrate their potential to promote replicable, diverse communities for several weeks under clean conditions using time-series results from two field experiments. One field experiment utilized coastal waters collected from Santa Catalina Island, CA and the other oligotrophic waters collected offshore of Honolulu, HI. Biomass metrics (chlorophyll a and particulate carbon) along with 16S/18S DNA based community composition assessments were conducted to show that communities contained within PERIcosms remained alive and diverse for several weeks using a semi-continuous culturing approach. We detail trace metal clean techniques that can be used to minimize external contamination, particularly for low dissolved iron environments. PERIcosms have the potential to facilitate natural community incubations which are needed to continue advancing our understanding of microbial ecology and geochemistry.

Marine microorganisms shape the world around us by altering the cycling of elements, regulating gases that impact our climate, and forming the base of the aquatic food webs (Schindler 1998; Karl 2007). In vitro incubations are a useful and often-used tool to better understand factors that control the distribution and activity of microbes in the sea. Compared to in situ observations, incubations provide the control experimentalists desire to closely monitor and quantify microbial activity under existing environmental conditions as well as to explore their response to intentional perturbations. This desire to control external variables comes with limitations since incubations are inherently simplified and therefore can be difficult to scale up to the ecosystem under study (Schindler 1998). Even so, incubations are routinely used to approximate primary productivity in natural systems (Steemann Nielsen 1952), to understand community responses to events such as altered nutrient supply (e.g., Lebaron et al. 2001; Mahaffey et al. 2012; Turk-Kubo et al. 2015; Böttjer-Wilson et al. 2021), climate change (e.g., Bach et al. 2016; Boyd et al. 2022), and anthropogenic pollutants (e.g., Menzel and Case 1977; Kuiper and Hanstveit 1984).

In order to better understand how natural communities function through incubation studies, it is important that the response of isolated communities to treatment perturbations are representative of the responses that would occur in the

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Additional Supporting Information may be found in the online version of this article.

natural environment, and that the treatment perturbations are controlled and quantifiable. The ability to maintain diverse communities for extended periods is directly related to the enclosure size for several reasons related to the characteristics of the community itself (e.g., trophic complexity, generation times) and largely unavoidable experimental conditions such as tank fouling (Chen et al. 1997; Petersen et al. 2003, 2010; Riebesell et al. 2011). Much research has gone into theoretical frameworks that dictate ideal incubation volumes and durations for specific experimental communities (see Petersen et al. 2010 and references therein). One outcome of this work is the notable benefit of large volume mesocosms that generally minimize bottle-effects and provide a more natural research setting, even under intense sampling regimes. The advantage of such studies has led to the construction of several dedicated facilities (e.g., Santschi 1985; Duarte et al. 2015; Gall et al. 2017; Båmstedt and Larsson 2018; Pansch and Hiebenthal 2019) and in situ platforms (Strickland and Terhune 1961; Menzel and Case 1977; Mostajir et al. 2013; Riebesell et al. 2013) consisting of highly sophisticated, well-tested mesocosm systems. These incubation volumes and technical systems, however, can be challenging to implement and often come at a high monetary price, meaning researchers using them often default to few treatments (e.g., Bach et al. 2016; Spisla et al. 2021), or treatments without replicates (e.g., Hoppe et al. 2008; Thomson et al. 2016; Böttjer-Wilson et al. 2021).

Small volume incubations (≤ 20 L), on the other hand, are relatively simple, more universally obtainable, and easier to perform with many replicates. However, this is offset by enhanced bottle effects and typically shorter incubation windows (Petersen et al. 1999). The trade-offs between large and small mesocosms raise the question of, what size mesocosm is optimal to promote diverse, marine plankton communities on timescales that exceed several generations of the enclosed populations, while still being small enough to be easily managed? As outlined by Riebesell et al. (2011), incubation enclosures should be as small as possible to maximize the feasible number of treatments and replicates without compromising their ability to represent the natural community they intend to simulate. An array of studies evaluating natural community incubations at a variety of scales has been conducted (Schindler 1998; Petersen et al. 2003; Petersen and Englund 2005; Spivak et al. 2011). According to theoretical and empirically derived relationships between community, mesocosm size, and experiment duration, incubations ~ 100 L (0.1 m^3) in size should suffice to sustain realistic surface ocean communities including bacteria, phytoplankton, and zooplankton for several weeks to months (Petersen et al. 2010; Riebesell et al. 2011). Here, we describe a new "PERIcosm" tank design for mid-volume incubations and show how effectively this mid-volume tank sustained marine pelagic communities using trace metal clean techniques and a semi-continuous culturing incubation approach.

A key aim of our tank design was to use components and techniques that could sustain low trace metal conditions, especially with respect to dissolved iron (Fe). Fe is an important micronutrient for all life, but Fe concentrations are generally very low in marine surface oligotrophic waters. The availability of Fe in surface water has been shown to dictate overall community productivity (DiTullio et al. 1993) and specific enzymatic functions such as dinitrogen fixation, which has an indirect control on productivity and surface ocean carbon fluxes (Mills et al. 2004; Karl et al. 2012). Evaluating the ecological role of Fe using incubation experiments is challenging because Fe contamination is hard to avoid and some ecological responses are highly sensitive to the alleviation of Fe limitation, such as photosynthetic efficiency (Behrenfeld et al. 1996), and some are relatively slow or subtle, such as diazotroph growth (Bonnet et al. 2016). While previous Fe-focused mesocosm studies have been reported (e.g., Bonnet et al. 2008; Li et al. 2015; Louis et al. 2018), we wanted to create a minimal metal system that could be operated trace metal clean under a semi-continuous framework allowing for longer incubation periods and higher volume sampling (Tortell et al. 2002).

Incubation classifications are often delineated by volume, with enclosures > 1000 L considered mesocosms, and < 1000 L as microcosms. However, this definition is not absolute and the term mesocosm has been used to describe community-based incubations with volumes exceeding ~ 1 L (Lebaron et al. 2001; Sommer et al. 2012; Stewart et al. 2012). Some authors have used the term minicosm to describe incubations 100–1000 L in size (e.g., Meyer et al. 2016; Thomson et al. 2016). We consider our newly designed, minimal metal 115 L incubation chambers mesocosms and dubbed them PERIcosms (Pelagic Ecosystem Research Incubators), and use this term throughout the remaining text. The PERIcosms were designed to be operationally simple and large enough to support complex marine microbial communities, yet small enough for treatment replication. Here we present a description of how to construct and operate PERIcosms and demonstrate their effectiveness with the results of two incubation experiments. The first experiment was conducted with coastal waters off Santa Catalina Island, CA ("Catalina" experiment) and the second with oligotrophic waters off Honolulu, HI ("Hawaii" experiment). The ability for PERIcosms to maintain representative oligotrophic and eutrophic communities of plankton for several weeks, to allow for detailed sampling regimes, and to foster good replication between treatments, all while having the potential to support trace metal clean conditions, make them a useful tool for studies of pelagic ecosystems.

Materials and procedures

Two field experiments were conducted to demonstrate the construction and performance of PERIcosms (Table 1). Trace

Table 1. Experimental details for the Catalina and Hawaii incubations. For the Hawaii experiment, four of the eight treatments are presented in the text to demonstrate the PERIcosm performance, and include the Control, +N, +P, and +NP treatments. The +NP treatment is a combination of treatments +N and +P.

Location		Catalina			Hawaii		
Starting water		Coastal. Big fisherman cove, san Catalina Island, CA USA			Offshore. North Pacific subtropical gyre, Oahu, HI, USA		
Number of tanks		3			24		
Number of treatments		3			8		
Experiment duration		14 days			29 days		
Treatment names		A	B	C	Control	+N	+P
Spike concentrations (μM)	Nitrate	2 at start, 0.2 each day thereafter	15 at start, 1.5 each day thereafter	15 at start, 1.5 each day thereafter	–	0.075 every day (except days 5 and 6)	–
	Ammonium	–	–	–	–	0.075 every day (except days 5 and 6)	–
	Phosphate	1 at start, 0.1 each day thereafter	0.8 at start, 0.08 each day thereafter	1.1 at start, 0.11 each day thereafter	–	–	0.009 every day (except days 5 and 6)
	Silicate	2 at start, 0.2 each day thereafter	15 at start, 1.5 each day thereafter	15 at start, 1.5 each day thereafter	–	–	–
Sampling dilutions		10% of tank volume daily			Twice weekly sampling of 6% or ~ 20% of the tank volume		

metal clean techniques were utilized throughout both experiments. For example, materials were acid washed and double bagged or covered in plastic, polyethylene gloves were worn during the construction and use of the PERIcosms, and the PERIcosm lids were never opened during the experiments. The Catalina experiment took place at the University of Southern California's Wrigley Marine Science Center located in Big Fisherman Cove, Santa Catalina Island, CA in September 2020, with water collected from the facility's pier. Seawater was pumped from 1 m depth using an air-driven diaphragm pump (Wilden® Part #: 00–10,001) and natural polypropylene tubing. The tubing that entered the water was placed through a rigid PVC pipe to control its depth and position within the water, and a funnel was attached to its end covered in a 100 μm mesh to limit initial mesozooplankton abundances in the experiment. Water was collected into 20 L cubitainers kept within plastic bags contained in plastic bins. The cubitainers were acid cleaned (2% citranox soak followed by 10% HCl soak and 7 Milli-Q rinses) prior to the start of the experiment, then rinsed with seawater before use and with Milli-Q water after use each day of the experiment. Polyethylene gloves were worn to handle the cubitainers and the clean ends of the sampling tube. The sample line was placed in a plastic bag when not in use and flushed with seawater prior to filling the cubitainers each day. Three PERIcosms, each with a unique treatment, were tested. PERIcosm "A" was spiked at an N : P ratio of 2 (per L seawater: 2 μmol N, 1 μmol P, 2 μmol Si) reflecting the expected macronutrient ratios in Big Fisherman Cove at the time of sampling based on our prior work at this site (unpublished data), "B" was spiked at an N : P ratio of 18 (per liter seawater: 15 μmol N, 1.07 μmol P, 15 μmol Si), and "C" was spiked at an N : P ratio of 14 (per L seawater: 15 μmol N, 0.83 μmol P, 15 μmol Si). Nitrogen was added as NO_3^- (sodium nitrate ≥99.0% BioXtra, Sigma Aldrich) and P as PO_4^{3-} (sodium phosphate dibasic anhydrous ≥99.0% ACS, VWR life sciences). Silicate was added at a 1 : 1 ratio with nitrate (sodium meta-silicate nonahydrate ≥ 98%, Sigma Aldrich adjusted to pH = 2 with trace metal grade HCl). The macronutrient stock solutions were prepared in a class 100 clean room with Milli-Q water and cleaned of potential metal contamination using Chelex-100 resin (Bio-Rad) using bulk extraction techniques. Stocks were stored double bagged in a refrigerator. The nutrient spikes were added with a large initial dose at the start of the incubation and replenished at 10% of the initial concentration each day thereafter. More detailed procedures are described below. The experiment lasted 12 d.

The Hawaii experiment took place in August–September 2021 at the University of Hawai'i Marine Center in Honolulu, Hawaii using whole seawater collected from the North Pacific Subtropical Gyre (21°05.352′N, 158°04.457′W). Seawater was collected into nine, 1000 L high density polyethylene water totes (SCHÜTZ ECOBULK 275 gal. #898821). Prior to use, the totes were sequentially washed with 2% Citranox detergent and 2% hydrochloric acid (HCl). Enough solution was added to fully cover the tote bottom, and after a set time interval the totes were tipped onto a new side so that the entire interior (except the top) could soak under each solution. For Citranox, the totes were turned after a minimum of 3 h, and after 1 day for the diluted HCl solution. Deionized water was used to rinse the tanks between the Citranox and HCl additions, and Milli-Q was used to rinse the totes after the HCl. On the ship, the cleaned totes were rinsed with seawater two more times before filling. To do so, seawater was pumped from $\sim$ 15 m water depth, well below the $\sim$ 1.5 m draught of the ship, into the top access port of the totes using an ARO 1.5″ non-metallic diaphragm pump (PD15P-FPS-PTT) with polypropylene tubing pulled to depth by a 25 lb plastic kettlebell weight. The captain maintained the ship's position so that the surface seawater flowed toward the sample tubing to prevent contamination from the ship's wake. For the rinse, seawater was splashed around the interior of the tote by rapidly moving the end of the tubing around while wearing a polyethylene glove, and that water was released using the tote drainage valves. To avoid splashing and damaging cells when the totes were finally filled, the end of the tubing was wiped with 2% HCl and inserted through the top access port to the bottom of the tote. The tubing was slowly lifted as the tote filled to avoid excess contact of the tubing with the added seawater. The totes were filled predawn and kept dark using insulated shipping jackets until the water was transferred to a PERIcosm. The entire collection took 3 h to complete, and the last four totes collected were selected to fill the PERIcosms back on shore 4–6 h later. Eight treatments, each in triplicate with 24 PERIcosms in total, were used for the experiment, but only four treatment results are presented here to illustrate performance. The control treatment had no added nutrients or other amendments, the +N treatment had 150 nmol of dissolved inorganic N per liter of seawater added as a 50 : 50 mixture of NO_3^- (sodium nitrate ≥ 99.0% BioXtra, Sigma Aldrich) and NH_4^+ (ammonium chloride ≥ 99.5% ACS, EMD Millipore), the +P treatments were given 9.4 nmol PO_4^{3-} per liter of seawater (sodium phosphate dibasic anhydrous ≥ 99.0% ACS, VWR life sciences), and +NP was the combination of +N and +P (final N : P ratio of 16). The solutions were made in a class 100 clean room with Milli-Q water. The final stock solutions were cleaned of metal contamination using Chelex-100 resin and stored double bagged in a refrigerator. The nutrient spikes were added daily at the same concentration, except for days 5 and 6 when no nutrients were added. The experiment lasted 29 d. More details for both experiments can be found in Table 1.

PERIcosm construction

PERIcosm incubation tanks were constructed according to the design illustration (Fig. 1) and parts list (Table 2 and Supporting Information S1) provided here. Their components were selected based on a desire to conduct low trace metal experiments, leading to a nearly fully enclosed design consisting of trace metal clean plastics. The experimental protocols were also created to prevent contamination from dust or other external matter and are detailed in later sections. The PERIcosms were constructed from 30 gal, natural white polyethylene inductor tanks. To light the tank, a hole was cut into the lid provided by the manufacturer leaving just the threads, and then resealed by affixing a clear acrylic disk over the hole with silicone glue. Each PERIcosm was fitted with a trap at the bottom of the tank to collect sinking particles. The trap consisted of a 4″ plastic cylinder (1.91″ OD, 1.5″ ID) between two ball valves, which can be strategically opened and closed to isolate and sample the settled material within. Trap installation made the PERIcosm length too long for the manufacturer's metal frame, so the frame was placed on 8″ x 16″ concrete blocks for the duration of the experiment. Ports were added to sample and replenish water in the PERIcosms, as described in the Filling and Sampling sections below. Prior to use, the PERIcosms were sequentially washed with 2% Citranox detergent (1 d) 2% HCl (3 d), three rinses of deionized water and three rinses of Milli-Q water. The solutions were added through the lid and used to wash the interior walls using an acid rinsed toilet brush before soaking with the lid on for the durations noted above. The lids were not opened again after the Milli-Q rinses until the end of the experiment. The PERIcosms were rinsed two more times with seawater before filling.

Water temperature in the PERIcosms was maintained by deploying them indoors, and the light was artificial. Due to their size, it was most practical to place the PERIcosms inside a climate-controlled room. For the Catalina experiments, three PERIcosms were housed in a small phytoplankton culturing room set to 20°C to reflect the local seawater temperature and was monitored daily using a separate water bath. A series of full-spectrum lights (VIRIBRIGHT BR30 Flood, Feit PAR38 Flood, and Feit GLP24ADJS Grow Light) were placed over the PERIcosms, generally above the clear acrylic lid although some light passed through the opaque walls. The final light spectrum was not determined, nor how passing through the walls modifies the light spectrum, but full spectrum lights generally emit all wavelengths of visible light. The photosynthetically active radiation (PAR) levels in the PERIcosms for this set-up averaged 100–200 μmol m^2 s^{-1} on a 12 h day/night cycle, which equates to $\sim$ 6.5 mol quanta m^{-2} d^{-1}. The PAR levels were determined with a LI-COR LI-193 Spherical Quantum Sensor connected to a LI-250A light meter, with PAR defined as visible light between 400 and 700 nm wavelengths. The sensor was inserted into the PERIcosms and moved around the interior to capture an average PAR light level. A PAR depth profile was also conducted off the sampling pier at noon during the experiment to compare with levels measured in the PERIcosms. At our 1 m sampling depth, PAR levels were

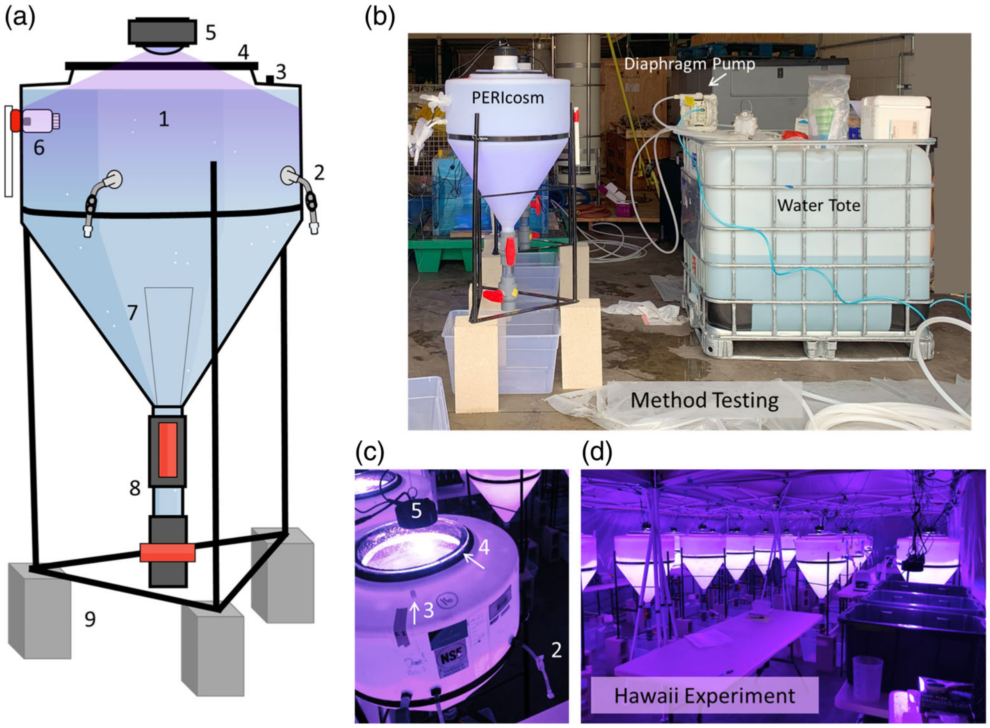

Fig. 1. Design of the PERIcosm tanks. (**a**) A cartoon depiction of a PERIcosm highlighting the purchased components required for the modifications (1–9), which are outlined in Table 2 and detailed in Supporting Information Table S1. (**b**) A picture of the PERIcosm set up for use during method testing, which includes an image of the water tote and diaphragm pump used to transfer water from the tote to the PERIcosm. (**c**) An overhead view of the PERIcosm showing the lid modification as well as the sampling arm protruding from the tank. Numbers are the same as panel a and are detailed in Table 2. (**d**) A series of PERIcosms set up for the experiment in Hawaii.

$\sim 1300\ \mu$mol m^2 s^{-1} and exponentially decreased to $\sim 200\ \mu$mol m^2 s^{-1} at the bottom of the water column, which was 12 m.

For the Hawaii experiment, we constructed a lightly insulated tent to house 24 PERIcosms. The tent was constructed out of six smaller, $10' \times 10'$ (3 m $\times$ 3 m) pop-up canopy tents

Table 2. The tank reservoir and modifications used to create the PERIcosm incubation chambers, and the recommended parts we used to complete the modifications. Capital letters correspond to the diagram in Fig. 1a and the picture in Fig. 1c.

			Manufacturer	Catalog number
1	Reservoir	Inductor tank	Den Hartog industries, Inc	INFD30-24
		Stand	Den Hartog industries, Inc	IN30/55-ST
2	Surface ports	Barbed hose fitting	McMaster-Carr	5218 K703
		C-flex tubing	Masterflex®, C-flex®	VWR # MFLX06424-81
		Pinch clamp	Halkey-Roberts®	340-TCLB
		Quick disconnect	Masterflex®	RQCF655-9024-001
3	Spike port	Socket	McMaster-Carr	51,525 K441
		Cap	McMaster-Carr	51,525 K371
4	Modified lid	Acrylic disk	SoCal plastics pro	Custom piece
5	Light	Kessil A360X refugium	Kessil	KSA360X-RF (light)
6	Mixing	Bottle (internal)	VWR	47,750–616
		Magnet (internal)	McMaster-Carr	5862 K129
		Magnet (external)	K&J magnetics, Inc.	DY08
7	Trapezoid for wall sampling		N/A	N/A
8	Trap	Valves	Grainger	32H958
		Cylinder	McMaster-Carr	4677 T24, 4677 T434
9	Risers	Concrete blocks	The Home Depot	3,306,660,000

pushed together and wrapped in 6 mil high density painters plastic sheeting. Bubble-wrap insulation was added to the exterior of the tent, and several portable air conditioning units were placed inside to cool the space. The set up was contained within a large warehouse at the University of Hawaii Marine Center. Each PERIcosm for this experiment was lit with a Kessil A360X refugium light, which prioritizes red and blue LED bulbs to increase energy efficiency. The lights were programmed for 12 h day/night cycles. Daylight output was set to full brightness using 75% blue/25% red light settings. Most of the light emitted therefore fell within the visible blue light spectrum with some red light mixed in, and largely omitted violet, green, yellow and orange wavelengths creating the overall periwinkle appearance of the tanks. More light details are available at kessil.com. We used a handheld Biospherical Instruments Inc. quantum PAR meter (AMOUR-SL-125-PAR) to quantify the light output at these settings, but since the light produced is not the full visible spectrum, caution should be taken when comparing the values to natural sunlight. The approximate average PAR inside the PERIcosm using this light setup was 200 μmol m^{-2} s^{-1}, maximizing around 300 μmol m^{2} s^{-1} near the light and dropping to $\sim$ 100 μmol m^{-2} s^{-1} near the tank margins. These settings average to about 8 mol quanta m^{-2} d^{-1}, which is similar to light levels within the summer mixed layer ($\sim$ 45 m) at Station ALOHA located 100 km north of Oahu (Letelier et al. 2004). Each Kessil light was placed on a ¼ inch PVC toilet flange in the center of the PERIcosm acrylic lid to allow heat to escape the bulb without shading its output. Other commercial light systems are also available, and PERIcosm users should customize their lighting based on experimental needs.

Setting up and conducting PERIcosms experiments

Filling

Seawater was transferred into the PERIcosms by tapping the side of each PERIcosm using a tapered national pipe threads (NPT) tap and installing water-tight, NPT to compression or barb connector fittings. The fitting threads were wrapped in Teflon tape to improve water tightness. Such ports allow water to be added from the clean water collection reservoir to the PERIcosm without exposure to the external environment, therefore avoiding contamination to the system. We employed two seawater transferring techniques to prevent damage to delicate plankton cells. On Catalina, the PERIcosms were filled gravimetrically by connecting a tapped cubitainer to the tapped port with trace metal clean C-flex tubing and lifting the cubitainer above the height of the PERIcosm. This method is relatively demanding and is recommended when only a few PERIcosms are being used. A 0.2 μm Pall AcroPak™ cartridge filter was placed in-line to add filtered water to the PERIcosm during the daily water replenishments. All AcroPak™ filters (Catalina and Hawaii) were cleaned before use. The cartridges were left filled with trace metal clean 2% HCl for several days and then rinsed with several liters of Milli-Q. Before a sample was collected, the AcroPaks™ were flushed with the sample water. Afterward, the cartridge was drained completely and refrigerated when not in use.

For the Hawaii experiment, water was pumped into the PERIcosms one at a time using an air-driven diaphragm pump in order to fill all 24 PERIcosms in a short timeframe. We recommend diaphragm pumps because they have adjustable flow rates, are designed to transport solids and semisolids (like cells) without major damage, and can be purchased with fluoropolymer parts to avoid metal contamination (Schiavello et al. 1997). We used an all plastic Saint-Gobain AstiPure diaphragm pump (PFA/ PTFE; Part # PFD3 333SI) at a flow rate of $\sim$ 9 L/min. The pumps were used inline, situated between the water collection tote and the PERIcosm. The pump was directly connected to the water collection tote with polypropylene tubing by installing a compression fitting to the tote drain valve. Water flow was directed from the diaphragm pump to the PERIcosm with polypropylene tubing connected to the tapped port on the PERIcosm, thus isolating the seawater from the external environment for the entire filling process. The PERIcosms were filled randomly by treatment starting with the first replicates and ending with the third replicates, meaning that each PERIcosm within a treatment was filled from a different tote. The amount of water added was measured using the manufacturer's volume gradations on the side of the PERIcosm. All remaining water in the collection totes was filtered using a series of all-plastic pool filters with decreasing pore sizes of 75 μm (3 M™ Micro-Klean™ part # RT20Q16G20NN), 5 μm (3 M™ Micro-Klean™ part # RT20B16G20NN), and 0.2 μm (PARKER part # PG-10110-002-01), and that water was used to replace the volume removed from the PERIcosms during each sampling event of the experiment. To clean the pool filters, they were soaked in 2% HCl inside the cartridge filter housing (PENTAIR/PENTEX part # 150166–75) for 2 days. After removing the acid, all components were Milli-Q rinsed seven times. Approximately 20 L of sample water was flushed through the clean, assembled filtration system and discarded before the remaining water for the experiment was filtered. The same plumbing system used for filling was used to add filtered water to the PERIcosms after sampling events, but it passed through an additional 0.2 μm Pall AcroPak™ cartridge filter before entering the PERIcosm.

Sampling

Several sampling ports were tapped into the side of each PERIcosm at varying depths using the same protocol as the fill port. For our research, three types of sampling were conducted, all of which utilized a short piece of C-flex tubing (MFLX06424) that remained attached to the PERIcosm sampling ports and terminated in a quick-disconnect fitting. The quick disconnect allowed for easy transitions between filling and sampling protocols. We placed clean polyethylene gloves over the C-flex tubing ends when not in use to maintain cleanliness. Flow was controlled using a plastic tube clamp (pinch style) on the C-flex tubing. The first sample type was unfiltered water collected by unclamping the tube with nothing attached to the quick-disconnect fitting. Second, 0.2 μm filtered water was collected directly from the PERIcosm by connecting a two-layer acid-washed cartridge filter (Pall AcroPak™ 0.8/0.2 μm supor filter) to the quick-disconnect. About 10″ of tubing was between the sample port and the AcroPak™ filter, which provided enough hydraulic head to filter samples by gravity even as the water level of the PERIcosm approached the height of the sampling port. A separate AcroPak™ was used for each treatment applied to the PERIcosms. Third, large volume biological sampling was accomplished by attaching a long piece of platinum-cured silicone Masterflex tubing to the quick-disconnect fitting, which passed through a peristaltic pump and terminated with Swinnex style filter holders. The type of filter used depended on the intended analysis. For our purposes, peristaltic pumps with four pump heads were used to sample clusters of four PERIcosms, and all PERIcosms were filtered simultaneously.

Sediment trap sampling was conducted using the double valve system described above. The top valve, normally kept open to allow sinking material to settle between the valves, was first closed to isolate the trap from the rest of the PERIcosm. Once isolated, the bottom valve was opened to release the contents of the trap. A wide mouth, 500 mL Nalgene container worked well to collect material from the sediment trap. How the collected, settled material was processed differed based on the goals of each experiment, but the total volume removed ($\sim$ 170 mL) and the volumes split for different analyses were recorded to properly calculate a total flux. For the Hawaii experiment, the sediment trap water was split for particulate CN and particulate trace metal analysis. The PC results are reported here, which were filtered and analyzed as reported for the suspended PC samples except with much smaller volumes (< 100 mL).

Nutrient addition

The term "spiking" is used here to refer to the addition of nutrients to the PERIcosms (spike concentrations are detailed in Table 1). Based on our experience, spikes were best applied near the center of the PERIcosm instead of through the side sampling ports. The spikes were therefore added through a small hole tapped into the top of the PERIcosm near the lid. A Luer-lock, male threaded straight socket was installed as a spike port, and a Luer-lock adapter cap was used to keep the spike port plugged when not in use. Spikes were added using a pipette inspected for rust, and the pipette was stored double bagged with the filtered pipette tips. Care was taken to not touch the spike port or any other surface with the pipette tip while administrating the spike. New pipette tips were used daily, and periodically changed within a spiking event. To better avoid cross contamination, unique spike ports could be installed for each spike applied, especially for experiments highly sensitive to Fe if Fe is to be added as a treatment. PERIcosms were mixed immediately after the addition of the spikes. The first spikes were added the same day the PERIcosms were initially filled for both experiments, referred to here as day 0, and after sampling was complete each day thereafter.

Mixing

There is not a standard protocol for mixing in biological incubations, although many mixing strategies have been tested or used. For instance, small bottle incubations can be mixed by hand (e.g., Levinsen et al. 2000; McManus et al. 2007), or left to the rolling motion of the ship in incubators at sea (e.g., Mahaffey et al. 2012; Browning et al. 2017). Larger mesocosm experiments have generally employed paddles, bubbling, or other physical means for mixing the system (Striebel et al. 2013). Physical approaches, however, are difficult to implement in ways that allow the system to remain fully isolated from external contact and thus resistant to trace metal contamination. Our goal was to design a clean mixing method that rapidly and evenly distributed the added spikes within the PERIcosms, and ensured the collection of homogenous samples that represented the entire surface suspended community. On Catalina, the PERIcosms were mixed continuously by spinning on top of automated rotating bases (2 rpm; Dynapac TS-650 Rotator), paused only to conduct the sampling and water replenishments (Supporting Information Fig. S1a). To simplify the PERIcosms for the Hawaii experiment, we devised a new mixing system which used magnets that paired an interior paddle to an exterior wand through the PERIcosm wall (Fig. 1a). The interior paddle consisted of a waterproof, 125 mL bottle with a magnet secured inside using silicone glue. The wand outside the PERIcosm consisted of a much stronger magnet attached to a short handle. By rotating the exterior magnet in a circular motion by hand around the top, vertical portion of the PERIcosm, the attached interior paddle physically disrupted and mixed the surface community. The mixing process was standardized by moving the wand in a large circle pattern (Supporting Information Fig. S1b) while going around the circumference of the PERIcosm at least twice before every sampling, and for at least 30 s after spiking. The effectiveness of both mixing procedures were tested using various qualitative assessments and quantitatively by measuring particle counts and/or chlorophyll 1) in replicates from the same PERIcosm, 2) in depth profiles, and 3) before and after mixing, all of which indicated that mixing created homogeneous conditions within the PERIcosm (examples shown in Supporting Information Fig. S2).

Water replenishments

Water replenishments using 0.2 μm filtered seawater were conducted after each large sampling event to maintain a constant volume within the PERIcosms. There is some consensus in the literature that <$\sim$ 10% of water replacement is ideal to promote continued community metabolism while preventing large disturbances, or selective pressures, to the system (Hutchins et al. 2003). For the Catalina experiment, 10% of the PERIcosm volume was exchanged with filtered seawater per day in line with the methods used by Hutchins et al. (2003). The volume removed was sufficient for all sampling needs, and any unused water was disposed of. The replenishment water was collected daily from the local pier and filtered as it entered the PERIcosm. A sample of the filtered fill water was preserved daily for nutrient analysis. The daily nutrient spikes were added after the water replenishments were completed ($A = 0.2\ \mu M$ N, $0.1\ \mu M$ P, $0.2\ \mu M$ Si; $B = 1.5\ \mu M$ N, $0.107\ \mu M$ P, $1.5\ \mu M$ Si; $C = 1.5\ \mu M$ N, $0.083\ \mu M$ P, $1.5\ \mu M$ Si, final concentrations). Relatively small contributions of dissolved inorganic nutrients were added with the replenishment water each day: $0.011 \pm 0.004\ \mu mol$ N per L of the total incubation volume measured as nitrate + nitrite and $0.012 \pm 0.001\ \mu mol$ P per liter measured as phosphate (calculated as 10% of the seawater concentration shown in Fig. 2e,g).

For the Hawaii experiment, the full sampling scheme required 20% of the PERIcosm volume to be removed in 1 day. To compensate for this large volume, samples were split into low, medium, and high frequency datasets based on the water volumes required for specific analyses. Low volume, high frequency samples were collected almost daily without water replenishment due to the small amount of water removed. Medium frequency datasets were collected twice per week, and low frequency datasets once per week. The one-time per week that just high and medium frequency samples were collected, 6% of PERIcosms volume was replaced. The one-time per week when all samples were collected (low, medium and high frequency samples), ~ 20% of the PERIcosm volume was replaced. Only the required amount of water used for sampling was removed, and the amount replaced was recorded for each PERIcosm. The daily average replenishment was < 4% using this schedule. The filtered water collected at the start of the experiment for the replenishments was filtered again with a $0.2\ \mu m$ Pall AcroPak™ as it entered the PERIcosm. An AcroPak™ filtered water sample was preserved for nutrient analysis. Nutrient spikes were added after the water replenishments were complete ($+N = 0.15\ \mu M$ N, $+P = 0.0094\ \mu M$ P, $+NP = 0.15\ \mu M$ N and $0.0094\ \mu M$ P). The replenishment water contributed an additional $\sim 0.002 \pm 0.007\ \mu mol$ N (measured as nitrate + nitrite) per liter and $\sim 0.012 \pm 0.023$ nmol P (measured as phosphate) per liter on the 20% water replacement days (measured replenishment water concentrations were $0.010 \pm 0.004\ \mu M$ N and $0.061 \pm 0.01\ \mu M$ P). This semi-continuous sampling approach has been implemented in prior natural community incubations (Tortell et al. 2002, 2008), but is less common than batch style incubations.

Wall sampling

PERIcosms had visually obvious colonies that formed on the inner wall surface over time. This material was sampled at the end of the experiments to measure nutrient flux to the walls and to determine the organisms that flourished there. Wall sampling took place immediately after the PERIcosms were drained at the end of the experiment before the biomass dried. The walls were sampled by scraping the material from a square of known area with a polyethylene gloved finger and rinsing that material out the bottom of the PERIcosm into a sampling cup using 200–400 mL of $0.2\ \mu m$ filtered seawater. The known area was traced from a trapezoidal template (4″ x 10″ x 2″) onto the outside of the PERIcosm at the bottom of the cone using a permanent marker, shown in Fig. 1a (#7). The total volume collected was measured, and the material was split onto different filters for particulate C : N and DNA analysis as described in the Analysis Methods section with the volume for each filter recorded. The sampled area was extrapolated to account for the interior area of the PERIcosm in contact with water by knowing the area sampled (193 cm^2), the total volume used for the rinse, and the entire area of the PERIcosm below the water line (11,270 cm^2). We only sampled the walls in one location near the bottom of the PERIcosm and therefore do not have a quantitative understanding for how homogenous the growth really is, especially near the top where the mixing protocol and periodic sampling likely disrupts colonization. Wall growth reported from the wall scraping method is therefore expected to be an upper constraint of the total wall growth. We recommend additional sampling to better capture wall growth variability.

Analysis methods

Analyses performed for the Catalina experiment included in vivo chlorophyll (Chl), particulate carbon (PC), nutrients, and dissolved trace metals. The in vivo chl measurements were made using a Turner Designs Trilogy Laboratory Fluorometer in vivo chlorophyll module, which measures Chl fluorescence (excitation 460 nm, emission 660–710 nm) reported as relative fluorescence units. For PC, 0.25 to 1 L of sample was filtered onto $0.7\ \mu m$ nominal pore size precombusted (450°C) glass fiber filters (25 mm diameter) and stored frozen until they could be dried, pelleted, and analyzed using a 4010 Costech Elemental Analyzer with an acetanilide standard (Xu et al. 2021). Particulate N was determined simultaneously with PC. Nutrient and dissolved trace metal samples were obtained from $0.2\ \mu m$ filtrate generated using a Pall AcroPak™ cartridge filter directly from the PERIcosm. The samples were stored in VWR® metal-free polypropylene centrifuge tubes or larger volume HDPE bottles. The nutrients were stored frozen at −20°C, and the trace metals at room temperature. Nutrients were analyzed by the SOEST Laboratory for Analytical Biogeochemistry on a Seal Analytical AA3 HR Nutrient Autoanalyzer (Armstrong et al. 1967). Nitrate and nitrite (N + N) were analyzed together using a diazo reaction based on Armstrong et al. (1967) and Hansen and Koroleff (1999), and phosphate was measured as orthophosphate using an automated procedure for the colorimetric method of Murphy and Riley (1962). Trace metals were

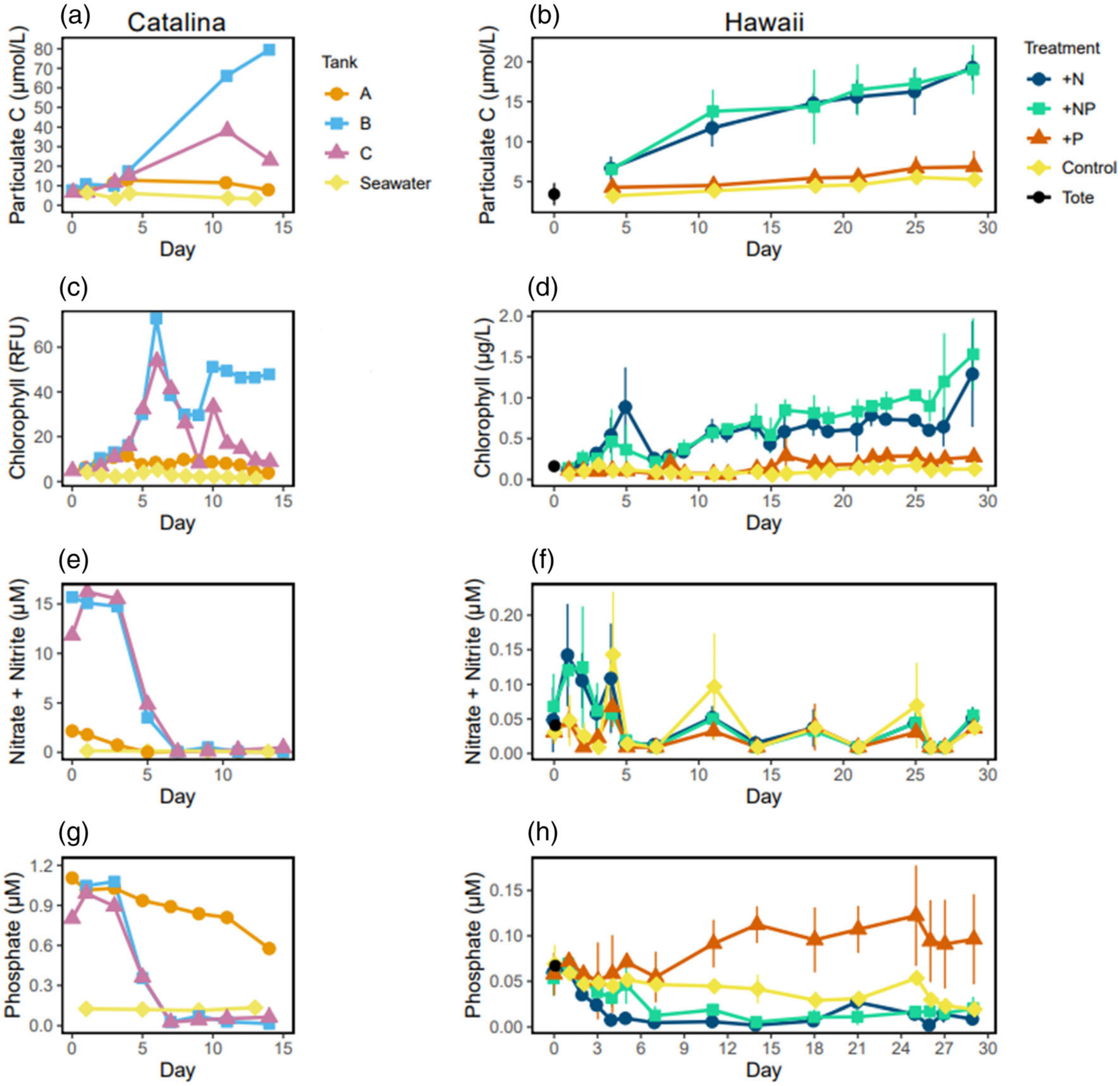

Fig. 2. (**a**,**b**) Suspended particulate carbon, (**c**,**d**) chlorophyll, (**e**,**f**) dissolved nitrate + nitrite, and (**g**,**h**) dissolved phosphate concentrations from PERIcosm incubations run on Catalina Island, California (a,c,e,g) and in Honolulu, Hawaii (b,d,f,h). Catalina treatments A (initial NO_3^- addition = 2 μM, PO_4^{3-} = 1 μM, N : P = 2), B (initial NO_3^- addition = 15 μM, PO_4^{3-} = 1.07 μM, N : P = 14), and C (initial NO_3^- addition = 15 μM, PO_4^{3-} = 0.83 μM, N : P = 18) were collected from the PERIcosms, whereas 'Seawater' represents the dilution water collected daily to replenish the tank volume after sampling. The Hawaii results depict the average ± the standard deviation for the treatment triplicates. Treatment +N received daily additions of 75 nM NO_3^- and 75 nM NH_4^+, +P received daily additions of 9.4 nM PO_4^{3-}, +NP received the same as N and P combined. The control did not receive nutrients. Tote refers to the water used to fill the PERIcosms, and its standard deviation reflects the four totes used to fill the PERIcosms at the start of the experiment.

acidified to pH 2 using hydrochloric acid and stored for at least 2 weeks. They were then concentrated using a SeaFAST automated system and analyzed by ICP-MS on a Thermo-Fisher Element 2 using isotope dilution to calculate the metal concentrations (Hawco et al. 2020).

Analyses reported for the Hawaii experiments include extracted chlorophyll a (Chl *a*), PC, nutrients, dissolved trace metals, 16S and 18S rDNA amplicon sequence variants (ASVs), imaging flow cytometry (Olson and Sosik 2007), and flow cytometry. Chl *a* and phaeopigment samples were collected in volume controlled 150 mL opaque HDPE bottles and filtered by vacuum pump onto glass fiber filters (~ 0.7 μm pore size). The pigments were extracted using 100% acetone for 7 d at −20°C and measured with either a 10-AU fluorometer (Strickland and Parsons 1972) or by a Turner Trilogy Acidification module following the manufacturer's protocol and EPA method 445.0 (Arar and Collins 1997). In both protocols, the sample was measured twice, before and after acidification, and the Chl *a* and phaeopigment concentrations were calculated based on the acidification ratio of a pure Chl *a* standard (C6144, Sigma-Aldrich Co or Turner Designs SKU: 10–850). Particulate carbon was measured the same way as the Catalina experiment, but using larger, 1–2 L sample volumes. Like Catalina, nutrients and trace metals were filtered by Pall AcroPak™ directly from the PERIcosms most days of the experiment. Once per week (days 4, 11, 18, 25, and 29), filtered water was subsampled from the peristaltic pump DNA filtrate. Nutrients were measured on a Seal Analytical AA3 HR Nutrient Autoanalyzer at UH-Mānoa as described above. Dissolved trace metals were analyzed as described above.

Samples for 16S and 18S rDNA amplicon sequencing were collected by filtering 2 L of seawater onto 0.2 μm Supor filters. Filters were placed in 1.5 mL tubes, flash frozen in liquid nitrogen, and stored at −80°C. DNA extraction was carried out using a modified Qiagen DNeasy protocol (Moisander et al. 2008). Prior to extraction, 10 ng of the genomic reference material, Takara *Thermus thermophilus* HB8 genomic DNA, was added to each sample. Following DNA extraction, amplicon libraries were prepared using 515Y-926R primers (dx.doi.org/10.17504/protocols.io.vb7e2rn). The 515Y-926R primers amplify regions of both the 16S and 18S SSU rRNA genes simultaneously, thus capturing prokaryotic and eukaryotic taxa from the same sample (Parada et al. 2016; Yeh et al. 2021; McNichol et al. 2021). PCR products were purified using Agencourt AMPure XP beads (dx.doi.org/10.17504/protocols.io.rfvd3n6) and samples were normalized and pooled. The pooled library was sequenced on two lanes of a MiSeq v3 2×300 paired-end sequencing platform at the USC Molecular Genomics Core. The bioinformatic pipeline used for the ASV analysis was carried out as described by Yeh and Fuhrman (2022; dx.doi.org/10.17504/protocols.io.vi9e4h6). The raw sequence data were demultiplexed and primers were removed using cutadapt (Martin 2011). The 16S and 18S reads were then separated from one another using the bbtools package (http://sourceforge.net/projects/bbmap/) and a custom 16S and 18S database (Yeh and Fuhrman 2022). The forward and reverse 16S reads were truncated at 210 and 180 bp, respectively, to remove low quality reads, and the resulting sequences were used to create ASVs using DADA2 in the QIIME2 software (Callahan et al. 2016; Bolyen et al. 2019). The forward and reverse 18S reads were trimmed at 210 and 170 bp, respectively, using BBduk from the bbtools package to remove low quality reads. Then, the trimmed forward and reverse 18S sequences were concatenated using bbtools and the concatenated sequences were used to create ASVs using DADA2 in QIIME2 (Callahan et al. 2016; Bolyen et al. 2019). Taxonomy was assigned to the 16S bacterial and archaeal ASVs using a 515Y-926R classifier derived from the SILVA database (v. 138; Quast et al. 2013). Similarly, taxonomy was assigned to the 16S chloroplast ASVs using a 515Y-926R classifier derived from the PhytoRef database (from PR2 v. 4.12; Decelle et al. 2015) and to the 18S eukaryotic ASVs using a 515Y-926R classifier derived from the PR2 database (v. 4.12; Guillou et al. 2013). All taxonomic assignments were carried out using the "classify-sklearn" method in the QIIME2 environment with a confidence threshold of 0.7. Contaminant ASVs were identified using the decontam package in R (Davis et al. 2018) and were removed from the final ASV tables.

The flow cytometry methods used are the same as those reported for the Hawaii Ocean Time-series, which are adapted from Monger and Landry (1993). Unfiltered samples collected for flow cytometry were preserved with 0.25% glutaraldehyde, flash frozen, and kept at −80°C until analysis on a B/D Influx flow cytometer. Forward light scatter, red fluorescence (692 nm), and orange fluorescence (580 nm) were detected and quantified. The results were then processed using FlowJo software (Tree Star) to determine the abundance of *Prochlorococcus*, *Synechococcus*, and picoeukaryotic algae.

Assessment

The PERIcosms were designed to study the response of marine ecosystems to altered nutrient supply, including trace elements, over the course of several weeks. The suitability of PERIcosms for such experiments was evaluated based on various aspects of community composition, species persistence and response, within-treatment replicability, and chemical contamination. Evaluation criteria included 1) maintenance of living biomass, 2) community diversity over long periods of incubation (up to 4 weeks), 3) reproducible community responses among treatment replicates (Hawaii experiment only), 4) the potential for trace metal cleanliness, particularly with regards to Fe contamination, and 5) practical ability to differentiate suspended communities in the PERIcosm from material collected in the sediment traps and wall-associated communities.

Assessment of living communities

To study the response of plankton communities to physical and chemical forcings, organisms must remain alive within the experimental enclosure. For an oceanographic microbial community, this means that growth is approximately balanced by mortality, including apoptosis, grazing, viral lysis, sinking, and loss during sample dilution events. We assessed the ability of the total plankton communities contained within the PERIcosms to persist and flourish using the community-level metrics of Chl, nutrient uptake, and biomass (PC) concentrations within the PERIcosms (Fig. 2).

The three nutrient additions tested in the Catalina experiment led to unique growth outcomes (Fig. 2a,c). Treatment A (2 μM N, 1 μM P, N : P = 2) showed relatively little change in in vivo chl or PC over the course of the experiment. The N addition to this PERIcosm stimulated an increase in overall biomass relative to the starting conditions (~ 2× increase in PC, Fig. 2a), but was not enough to draw down the added PO_4^{3-} (Fig. 2g). The initial 15 μM N increase in treatments B (N : P = 14) and C (N : P = 18) supported much higher in vivo chl concentrations relative to the starting condition and concentrations measured in treatment A, which reached maxima on day 6 of the experiment (Fig. 2c), and rapidly declined before stabilizing around day 9 of the experiment. The 1.5 μM daily nitrate addition to tanks B and C was undetectable on day 7 and onward (Fig. 2e), implying that the community remained active and consumed the added nutrients after the initial bloom cycle.

The four triplicated treatments presented for the Hawaii experiment also displayed unique growth dynamics, especially in response to inorganic N additions (Fig. 2b,d). Similar to the Catalina results, an initial peak in extracted Chl *a* concentration was observed on day 5 of the Hawaii experiment for the +N and +NP treatments (Fig. 2d). A second phase of growth occurred in the same treatments after the initial bloom, which was slower and led to consistently elevated Chl *a* concentrations during the second half of the experiment. The stable Chl *a* concentrations are presumed to reflect a relatively stable phytoplankton community maintained by the daily nutrient additions. The added NO_3^- was detectable prior to day 5, but not thereafter (Fig. 2f). A pattern was observed in the N + N analysis linked to how the samples were filtered, with peaks in the dataset associated with samples collected from the DNA filtrate (days 4, 11, 18, 25, and 29) on the ~ 20% water replacement days. The reason for this is unknown, but most likely due to the filtration method because the decrease is greater than the ~ 20% water replacement (high value samples were collected before the dilution took place). It is also challenging to link the N + N increase to lysed cells since the trends do not scale with total biomass—there is no obvious difference between the +N and no N added treatments—but cell lysis during filtration cannot be completely ruled out as a contributing factor. The complete drawdown of the added NO_3^- (Fig. 2f) and PO_4^{3-} (Fig. 2h) in +N and +NP further suggests continued biological uptake throughout the course of the experiment. The overall low nutrient, low biomass system that we tested makes monitoring biological activity as a function of biomass proxies a little more challenging in treatments without added N (Control and P). However, during initial experiments that we completed on Catalina to test different lighting and sampling options, a collapse of the living community was observed as a complete loss of chl after ~ 5 d and a rapid flux of PC to the sediment trap with little suspended material remaining at the end of the incubation period (data not shown). In Hawaii, both the control and + P treatments maintained relatively constant extracted Chl *a*, PC, and nutrient concentrations over time, but with slightly elevated biomass signals in +P. In fact, the PC concentrations (Fig. 2b) continuously increased in all treatments throughout the experiment. Further, the phaeopigment to Chl *a* ratios remained low (mean 0.56, interquartile range = 0.26–0.70) and similar to what is measured at Station ALOHA, the field site of the Hawaii Ocean Time-series (data available at https://hahana.soest.hawaii.edu/hot/), in all treatments. Phaeopigment is the degradation product of chl and would be expected to increase in respect to chl if phytoplankton cells were decomposed or had experienced high grazing pressure (Falkowski et al. 1988). Particulate ATP was also measured in all PERIcosms and the living percent of total PC, assuming a live C : ATP ratio of 250 (Karl 1980), remained near 30–40% (K. Björkman, results not shown here) which is similar to typical measurements from the NPSG (Henderikx-Freitas et al. 2021).

Maintenance of diverse communities

Incubation experiments cannot recreate the exact conditions of the natural environment and therefore are not expected to reproduce the exact same patterns of biological succession observed in nature, even with similar forcings. Many experimental factors can lead to succession unrelated to the treatment perturbation including the incubation volume, dilution rate, wall growth, and light/temperature. Even control PERIcosms are likely to experience a community shift over time due to species-specific responses to containment. While unavoidable, such changes do not negate the powerful inferences that can be drawn from natural community incubations such as those completed with PERIcosms when diverse community structures are maintained. Community level responses to containment within the PERIcosm were evaluated using 16S and 18S DNA results from the Hawaii experiment.

The PERIcosm community structure was described by species richness based on abundances of unique ASVs (Fig. 3a,b) and by alpha diversity based on the Simpson diversity index for each sampling timepoint (Fig. 3c,d), which was calculated using the vegan package, diversity(index = “simpson”) function in RStudio (github.com/vegandevs/vegan). Nonmetric multidimensional scaling ordination plots were used to evaluate differences between PERIcosm communities with time (Fig. 3e,f; vegan package, metaMDS[distance = “bray”] function). Community structure calculations were run separately for the prokaryote and eukaryote ASV datasets. There was an initial loss in species richness for both prokaryotes (16S) and eukaryotes (18S) during the first few days of the Hawaii experiment, although both remained relatively unchanged thereafter (Fig. 3a,b respectively). Prokaryote Simpson diversity (Fig. 3c), a measure describing the number of species present and their relative abundance, appeared unimpacted by the initial loss of richness. Eukaryotic Simpson diversity (Fig. 3d) was more variable, remaining highest in the Control and +N treatments, and displaying a marked decrease in the +NP treatment. The prokaryotic and eukaryotic community structures changed over time in all treatments (Fig. 3e,f), but treatments generally grouped together with time and separated based on whether or not they received N.

The community changes that occurred in the PERIcosms were further explored by evaluating the taxonomic composition of the prokaryotes and eukaryotes (Figs. 4, 5). The largest change in the prokaryotic community determined by 16S DNA occurred at the start of the experiment, driven primarily by a rapid decrease of *Synechococcales* and specifically

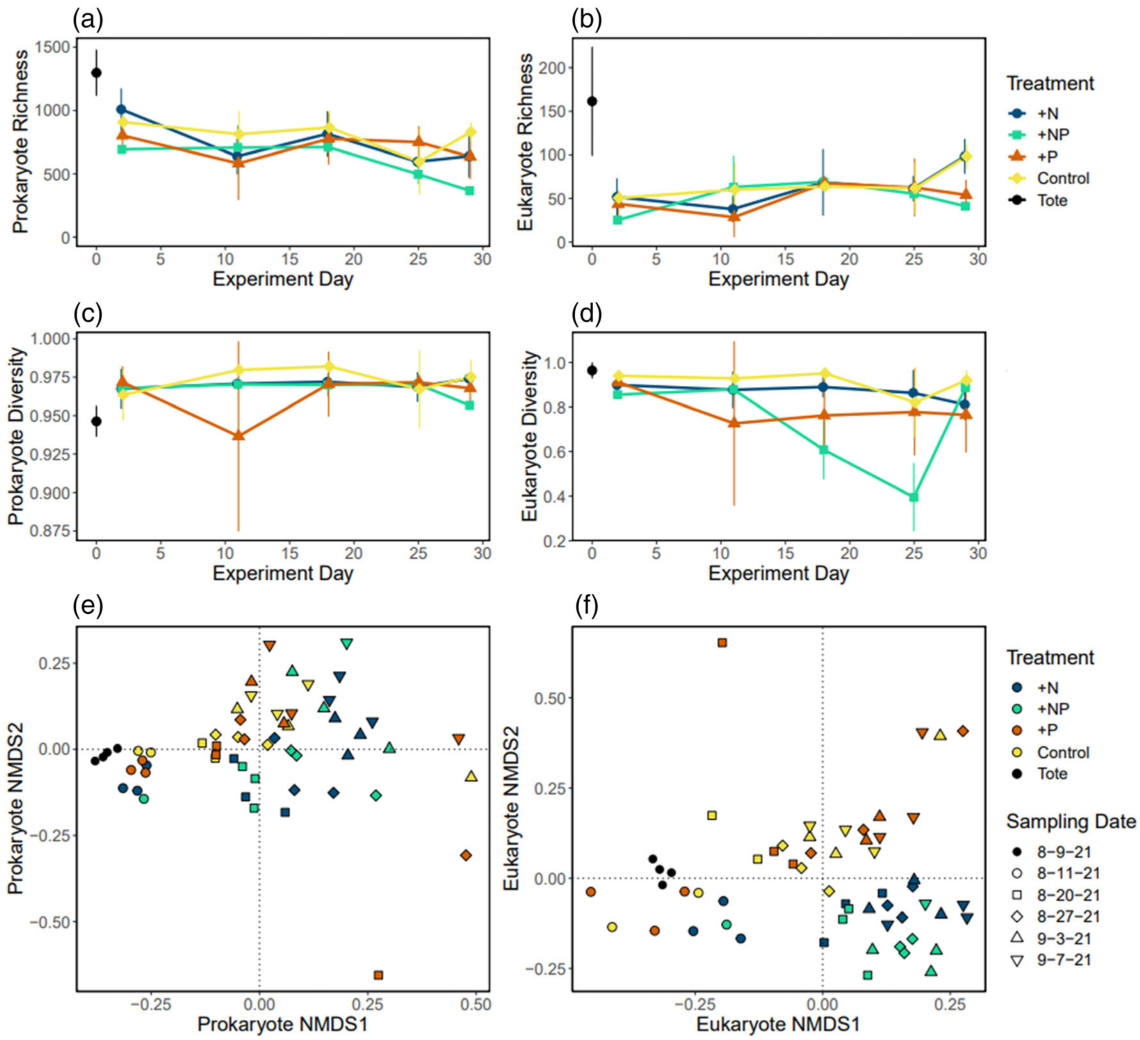

Fig. 3. Community structure maintained in the PERIcosms during the Hawaii experiment. (**a**) Prokaryote (16S DNA) and (**b**) eukaryote (18S DNA) species richness. (**c**) Prokaryote and (**d**) eukaryote Simpson diversity. (**e**) Prokaryote and (**f**) eukaryote nonmetric multidimensional scaling (NMDS) plots. The points and error bars represent the mean +/− standard deviation for the PERIcosm treatment replicates except for the tote sample, whose error reflects the four totes used to fill the PERIcosms. More information on the treatments, see the Fig. 2 caption and Table 1.

Prochlorococcus (Fig. 4). *Prochlorococcus* is an abundant microorganism in oligotrophic marine communities (Campbell et al. 1994) but is challenging to maintain in whole-community bottle incubations. Decreases in *Prochlorococcus* abundances were also quantified by flow cytometry, and were similar in magnitude and timing to previously published results from both large (60 kL) and small (20 L) mesocosm experiments conducted off the coast of Hawaii (Böttjer-Wilson et al. 2021) (Fig. 6). On the other hand, *SAR11*, an abundant oligotrophic bacterium (Eiler et al. 2009), remained at high relative 16S DNA abundances in all treatments. *Rhodobacterales*, particle-associated photoheterotrophs, drove an early community shift in the PERIcosms, as has been observed in other incubation studies (e.g., Turk-Kubo et al. 2018). Bacterial succession generally shifted from *Rhodobacterales* to *Flavobacteriales* and finally *Chitinophagales*. *Rhodobacterales* and *Flavobacteriales* are relatively common bacteria in the NPSG (Pham et al. 2008; Bryant et al. 2016). *Chitinophagales* has not been reported in many studies from the NPSG but was recently found to be a dominant group in sinking material collected there during summer export pulse events (Poff et al. 2021) and associated with marine plastic debris (Bryant et al. 2016; Vaksmaa et al. 2021). Altogether, bacterial communities and succession patterns observed during the Hawaii experiment were representative of those in the NPSG.

The taxonomic composition of the eukaryotic community (Fig. 5), characterized by 18S DNA, exhibited more variability between treatments and over time compared to the prokaryotes. However, the observed shifts generally matched expectations based on nutrient additions. Specifically, increasing the

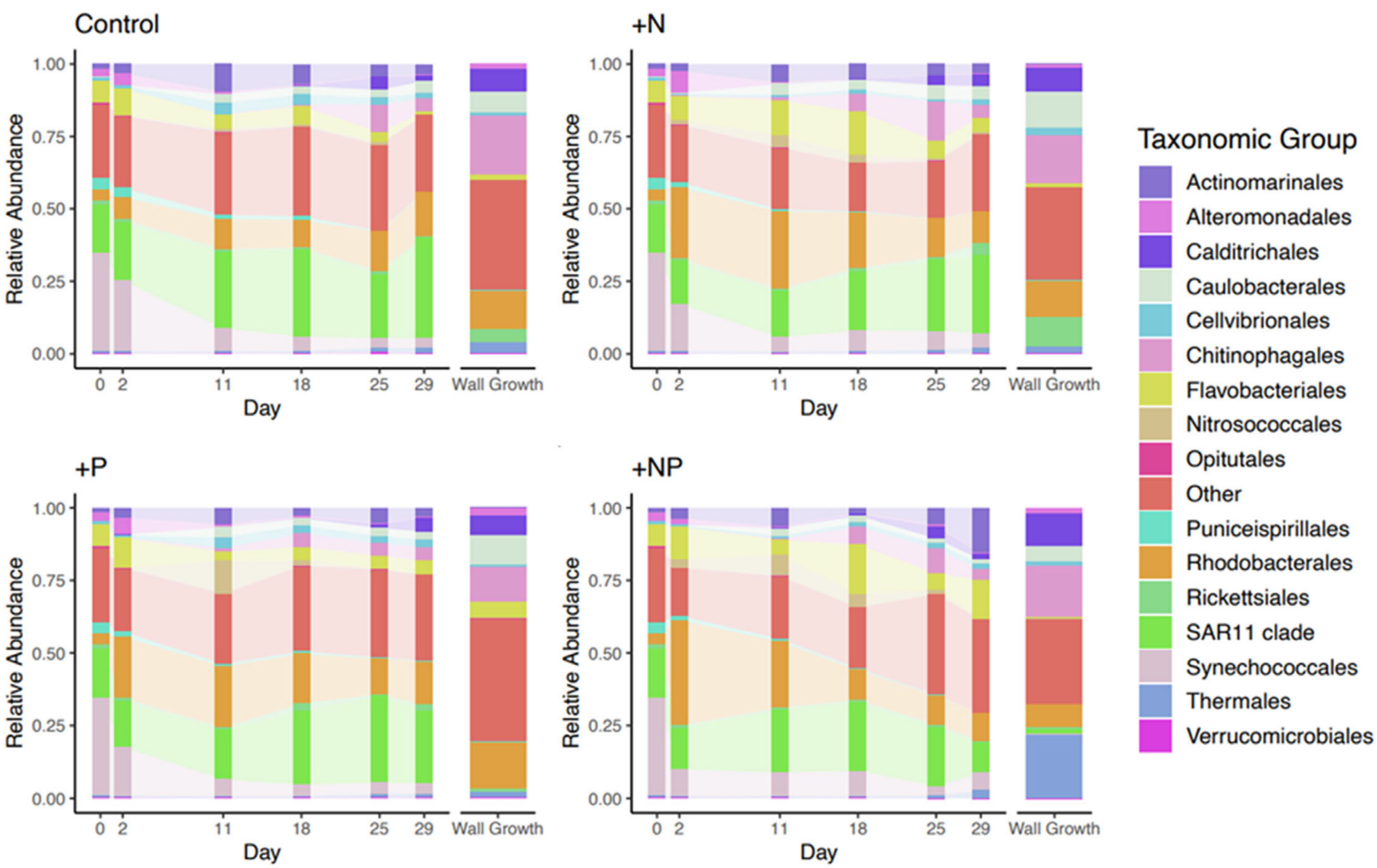

Fig. 4. Prokaryote community composition measured by 16S rDNA over time in the PERIcosm during the Hawaii experiment, as well as the community associated with the wall collected the day after the experiment ended. Each panel represents the average relative abundance of major taxonomic groups for each treatment as defined in the figure titles. Treatment information can be found in Fig. 2's caption and Table 1. Sampling dates are highlighted in the stacked bars and are shaded between to improve the visibility of the trends over time.

inorganic N supply (with or without P) led to diatoms dominating the eukaryotic community in +N and + NP, which was the largest community shift in relative abundance observed in the PERIcosms. The type of diatom that populated the PERIcosms was also treatment dependent. Species of *Pseudo-nitzschia* prevailed in the +NP treatment, but shared dominance with species of the centric diatom genera *Bacteriastrum* and *Chaetoceros* in the +N treatment. *Pseudo-nitzschia* blooms were also observed by Alexander et al. (2015) after mixing surface water from the NPSG with deep seawater, which contains NO_3^- and PO_4^{3-} near the Redfield ratio. Increases in the relative abundances of diatoms were also observed in the control and + P treatments, driven primarily by unidentified pennate species, *Pseudo-nitzschia*, and *Nitzschia*, but they remained minor components of the community. Imaging FlowCytobot results indicated that the unknown pennates could be species of *Mastogloia*, which are common bloom-forming diatoms in the NPSG (Dore et al. 2008). Dinoflagellates remained a prominent component of the eukaryotic community in all treatments, and the most abundant eukaryotic group in the control and +P treatments, as they typically are at Station ALOHA (Ollison et al. 2021). The other noticeable, consistent change among the treatments was the loss of Metazoa. It is unclear why the metazooplankton community was lost, but it could be that the enclosure size was not suitable for these large, often delicate species, or the water replenishments were at too high of a removal rate since dilutions are known to reduce zooplankton populations and therefore grazing pressures (Menzel and Case 1977; Landry and Hassett 1982). Many incubations use a mesh to remove such organisms from their incubation chambers making it difficult to compare these observations with other studies. Regardless, the eukaryotic communities maintained in the PERIcosms were representative of those found in the NPSG under both low nutrient and high nutrient conditions. We thus conclude that PERIcosm incubation chambers are a suitable research tool to address questions regarding pelagic community function under altered nutrient regimes.

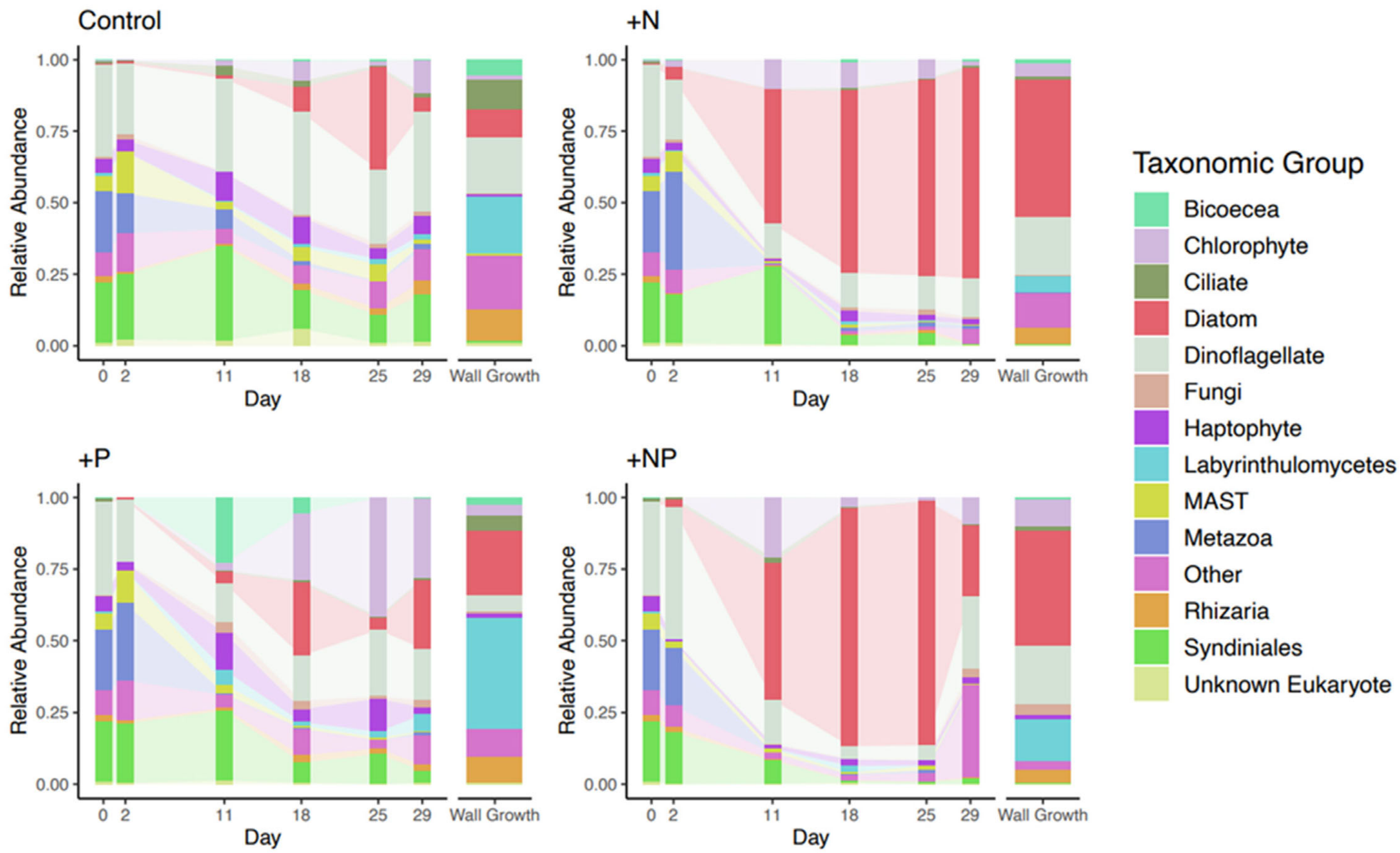

Fig. 5. Eukaryote community composition measured by 18S rDNA over time in the PERIcosm during the Hawaii experiment, as well as the community associated with the wall collected the day after the experiment terminated. Each panel represents the average relative abundance of major taxonomic groups for each treatment specified in the figure titles. Treatment information can be found in the Fig. 2 caption and Table 1. Sampling dates are highlighted in the stacked bars and are shaded between to improve the visibility of the trends over time.

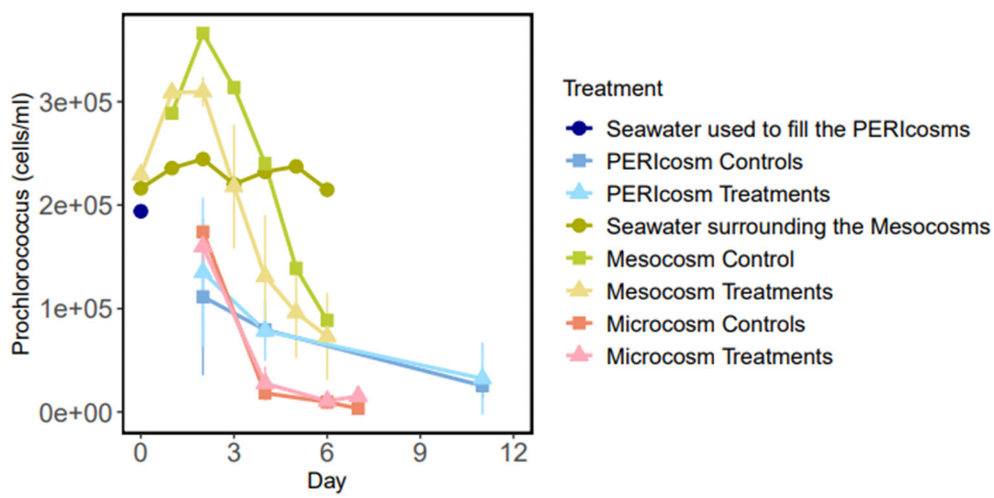

Fig. 6. *Prochlorococcus* cell counts in PERI-(115 L), meso-(60,000 L), and micro-(20 L) cosm experiments conducted near the Hawaiian Islands. The mesocosm and microcosm results are replotted from Böttjer-Wilson et al. (2021) and the PERIcosm results are from this study. The points depict the average ± the standard deviation for the treatment replicates as applicable. The treatments from the Böttjer-Wilson study were combined into one category for simplicity, as were the treatments for this study.

Reproducibility

Enclosing natural plankton communities is known to result in bottle-to-bottle variability unrelated to specific treatments or chemical amendments. Significant "bottle effects" can sometimes mask the treatment effect or lead to false interpretations of outcomes and should therefore be minimized as much as possible. Information presented thus far has demonstrated that the PERIcosm incubation volume was large enough to maintain active and diverse surface pelagic microbial communities. Here we present three evaluations that demonstrate the ability of PERIcosms to produce repeatable, significant differences between treatments using the Chl *a* and PC results from the Hawaii experiment.

First, extracted Chl *a* and PC concentrations in all PERIcosms were plotted separately to visualize the variability in response of each PERIcosm within a treatment (Fig. 7a,b). Overall, very similar trends with time are exhibited between PERIcosms given the same treatment.

Second, the coefficient of variance (CV) of Chl *a* and PC was calculated for each treatment every day that samples were analyzed (Fig. 7c,d). The CV indicates the sample variance as a ratio of the sample standard deviation and the mean. In this scenario, lower CVs indicate more-similar responses between treatment replicates. The shaded areas in Fig. 7c,d represent the range by standard deviation of Chl *a* CVs measured by Gall et al. (2017) from their Planktotron experimental chambers, which are of similar size and shape to the PERIcosms, for comparison. Gall et al. (2017) further calculated average CV of Chl *a* data from a meta-analysis of field and lab experiments using similar approaches to their incubations, which also reflect the approaches used here for PERIcosms. Those averages are represented in the solid black and gray lines, respectively, plotted in Fig. 7c,d. The majority of the PERIcosm Chl *a* CV results (72%) remained within the measured deviation of Gall et al. (2017). The Chl *a* variability was greatest in the first half of the experiment when concentrations were the lowest. Treatments with high CVs were generally driven by one PERIcosm that deviated from the rest for several days or longer, rather than anomalous values on a single day. Gall et al. (2017) did not evaluate particulate carbon CVs, so here we compared our PC CVs to the same Chl *a* results described above. All PC CVs fell within range of the Gall et al. (2017) study. The highest value was measured in the seawater totes used to fill the PERIcosms at the start of the experiment. The lower and consistent CVs measured within the PERIcosms compared to the Tote suggests that changes in PC over time were in response to the treatment specific nutrient additions and not influenced by the starting variance.

Third, an ANOVA with post-hoc analysis (Tukey's test) was used to evaluate whether the treatment Chl *a* (Fig. 8a–c) and PC (Fig. 8d–f) responses were significantly different from the control. Three groups were formed to represent the beginning (days 4 and 11), middle (days 18 and 21), and end (days 25 and 29) of the experiment for this comparison. The statistical tests were run in RStudio (version 4.3.1) using functions within the stats package, including aov() for the ANOVA and TukeyHSD() for Tukey's "Honest Significant Difference" method. The addition of N led to significant increases in Chl *a* ($p < 0.001$) and PC ($p < 0.001$) relative to the control for the duration of the experiment. Analyzed in this way, there was no significant difference between the control and the +P treatment. The responses measured during the Hawaii experiment demonstrated that the PERIcosm volume and overall experimental conditions allowed for treatment effects to outweigh bottle or handling effects, and incubations performed in PERIcosms were able to produce robust replication over long timescales, at least under the experimental conditions studied here.

Trace metal cleanliness

Another aim of the PERIcosm incubation system was to create trace metal clean experimental conditions. We evaluated trace metal cleanliness by monitoring the dissolved Fe concentrations within the PERIcosms from the start to the end of both the Catalina and Hawaii experiments. The background Fe concentrations at the nearshore Catalina site and the offshore Hawaii site are quite different. Published nearshore Fe concentrations from the Catalina site range from 1.5 to 14 nM (Pinedo-Gonzalez et al. 2014; Bolster et al. 2018); whereas, the North Pacific Gyre generally remains < 1 nM in surface waters (Boyle et al. 2005; Fitzsimmons et al. 2016). Initial Fe concentrations for the Catalina experiment were determined by sampling each PERIcosm after they were filled, averaging 11.3 ± 1.8 nM Fe. The Fe concentrations thereafter remained relatively constant or decreased (excepting treatment C on day 11) with the greatest depletion associated with greater biomass production (Fig. 9a). The large range in Fe concentrations measured during the Catalina experiment (1–15 nM) appear driven by biological uptake or particle adsorption, but are likely influenced by the daily filtered water replenishments which added on average 0.8 ± 0.2 nM Fe (10% of the "Seawater" values reported in Fig. 9a). We did not measure Fe flux to the walls, but recognize that wall adsorption has been shown to influence dissolved Fe concentrations in incubations (Fischer et al. 2007). The sampling site off Hawaii was located $\sim$ 20 km south of Barbers Point, Oahu (21°05.352′N, 158°04.457′W), and had an initial dissolved Fe concentration of 1.1 ± 0.1 nM measured in the four fill totes at the start of the incubation. The Fe concentrations measured in the Hawaii PERIcosms ranged from 0.05 to 3 nM, except for PERIcosm P3 that appeared to have been more heavily contaminated (Fig. 9b). The source of this contamination is unclear but is likely driven by operational practices rather than leaching from the materials themselves due to our minimal metal design. The range of values reported here would not suffice for studies in Fe limited regions who wish to prevent Fe contamination. Several additional steps could be taken to reduce operational contamination and achieve even lower Fe concentrations. The main two being (1) the PERIcosms should be assembled, and their interiors acid

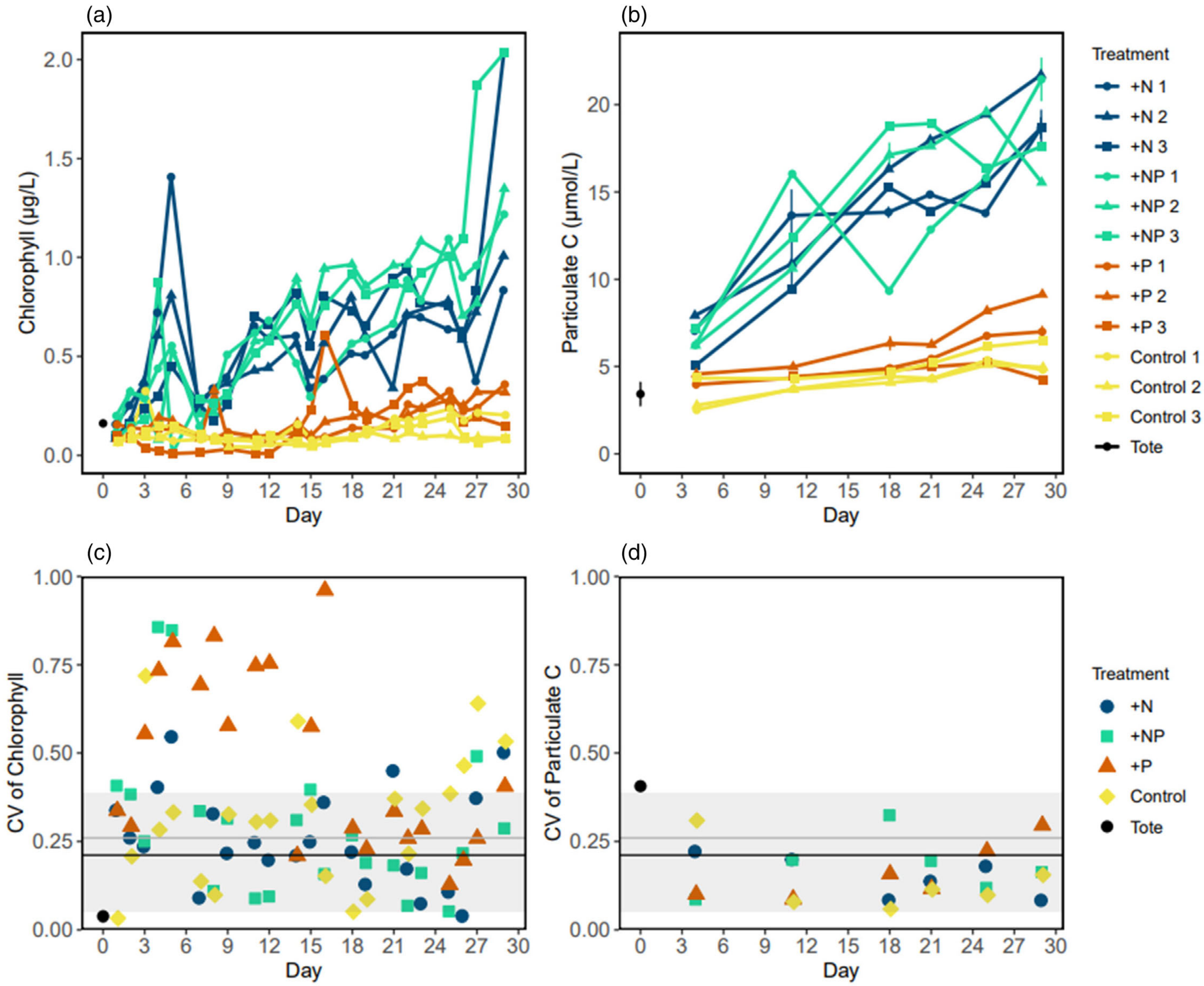

Fig. 7. The Hawaii experiment (**a**) extracted Chl *a* and (**b**) particulate carbon results from Fig. 1 are replotted to demonstrate the reproducible outcome between the PERIcosm treatment replicates. The coefficient of variation (CV) for each timepoint (treatment mean divided by the standard deviation) is plotted below the individual results for both (**c**) Chl *a* and (**d**) particulate C. The shaded area and horizontal lines in panel c and d are the same and replotted from Gall et al. (2017). The shaded area represents the range by standard deviation of Chl *a* CVs measured by Gall et al. (2017) from their Planktotron experimental chambers. The solid black and gray lines represent the average CV from Gall et al.'s meta-analysis for similarly sized incubations completed in the field (black) and in laboratory (gray) studies. For more information on the treatments, see Fig. 2 caption and Table 1.

washed, in a clean lab and never opened outside that lab space (we cleaned ours at the field sites where we did not have access to a clean lab) and (2) the incubation could be housed in a clean room, for example, a created clean space inside a shipping container.

Physical separation of sample types

In the natural environment, the composition of sinking particles notably differs from suspended particles (Fontanez et al. 2015; Duret et al. 2019) which impacts the microbial loop including carbon export (Nguyen et al. 2022). A unique benefit of the PERIcosm design and mixing protocol is that sinking material can be separated from particles that remain suspended, similar to marine snow catchers (Lampitt et al. 1993), allowing for the differences between these pools to be quantified. A small fraction of the PERIcosm suspended material ended up in the sediment trap during the Hawaii experiment for each 3–4 d sampling interval (0.1–2.8%

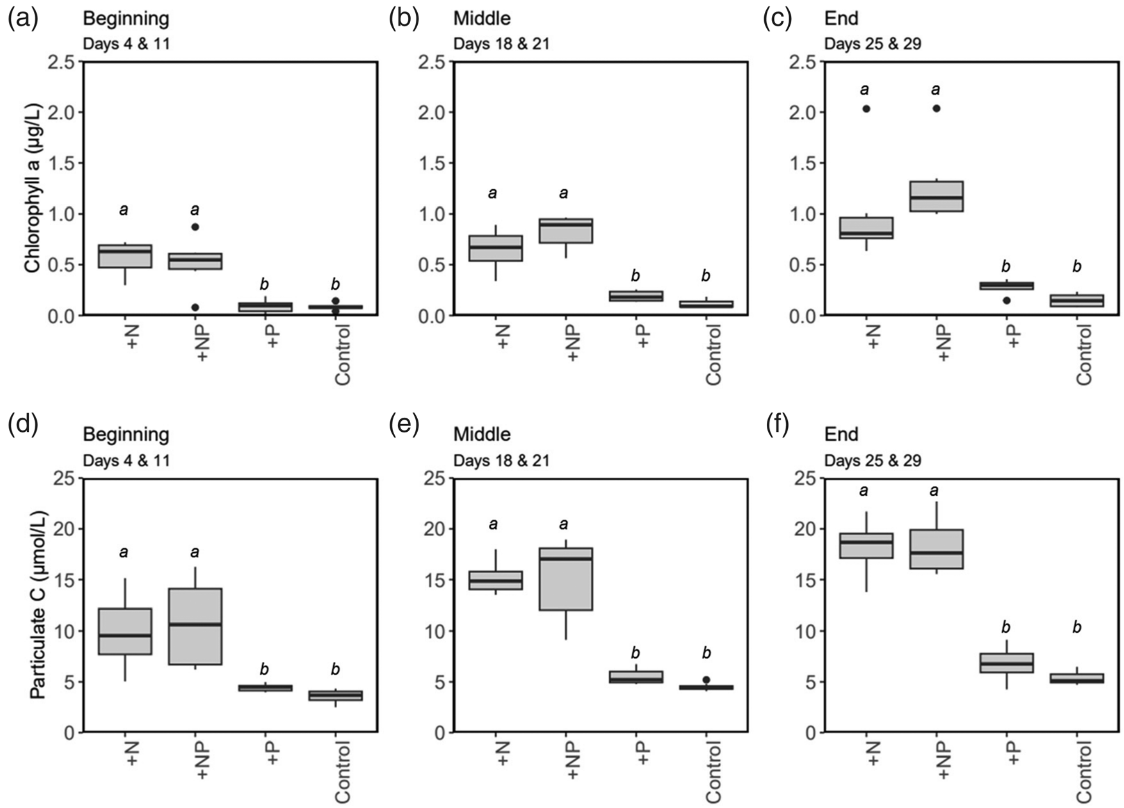

Fig. 8. Statistical differences in (**a**–**c**) Chl *a* and (**d**–**e**) particulate C between the Hawaii treatments at three distinct times of the experiment. The box and whisker plots include results from the treatment triplicates from two unique days of the experiment, as indicated in each panel title, resulting in 6 datapoints per box and whisker. The lower and upper sides of the box correspond to the first and third quartiles, and the center line represents the data mean. The whiskers extend to the furthest datapoints that are not considered outliers. Outliers were determined as values beyond 1.5 times the interquartile range and were plotted as individual circles. Italicized letters above the bars indicate significance, with statistically different results labeled with a unique letter. More information on the treatments can be found in Fig. 2 caption and Table 1.

calculated as settled g C/suspended g C) (Fig. 10). The small amount of settled material is a function of the low biomass, oligotrophic community we started with, but is also influenced by particles sticking to the walls and microbial degradation. We did not quantify the latter two but recommend using a mass balance of P to trace material fluxes, because unlike C and N, P does not have a gaseous phase mediated by microbes. Chen et al. (1997) found that over time a significant fraction (> 50%) of gross primary production in mesocosms is associated with wall-colonizing organisms, so we cannot

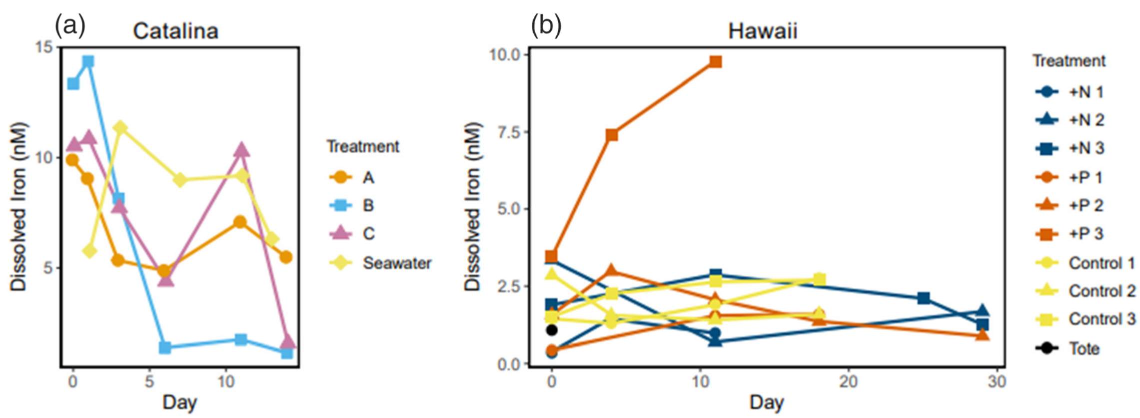

Fig. 9. Dissolved iron concentrations in each individual tank measured during the (**a**) Catalina and (**b**) Hawaii experiments. For more information on the treatments, refer to Fig. 2 and Table 1.

assume that the sampled wall material plus trap material should equal the suspended biomass and thus cannot calculate how much of the settled material remained stuck to the walls with the data at hand. The wall associated C, however, did not scale with suspended C suggesting a disconnect between those two pools attributed to wall-associated primary production, whereas patterns in the trap material did reflect patterns in suspended biomass suggesting a more direct relationship between those pools. While the sediment traps may not capture all settled material, they likely capture a representative fraction. Particle loss to walls should be considered when using the trap results to calculate sinking fluxes. Variability in the trap PC concentrations was also influenced by several factors. The relatively low particle load was near the analytical detection limit and likely influenced by biases when splitting the trap sample since the settled particles tended to be large, clump easily, and settle to the bottom of the collection container. Future studies may want to consider alternative ways of sampling and splitting the trap material, for instance, daily preserved (e.g., refrigerated) samples could be pooled to increase biomass while slowing down particle degradation rates.

The settled material had overall higher C : N ratios than the suspended particles, ranging from 6 to 105 in the settled (mean = 21) (Fig. 10d) vs. 5 to 15 in the suspended (mean = 9) (Fig. 10c). The C : N ratios were more similar between the suspended and settled material in the high biomass +N and +NP treatments, especially in the second half of the experiment when overall biomass levels were higher (C : N of 9–20 and 8–14 for settled and suspended, respectively, after day 17). The settled particles collected in the +P and control treatments had more variable C : N signatures than the +N and +NP treatments, but were still overall N deplete compared to the suspended particles (C : N of 7–105 in the settled and 5–13 in the surface after day 17). The high C : N ratios observed in the traps could also be a function of remineralization in the PERIcosms as the material sank, or remineralization in the traps as the particles accumulated over several days before collection. It is also possible that this is an analytical artifact since the relatively small amount of settled biomass resulted in signals near the detection limit, and we did not acidify the filters to remove particulate inorganic carbon, which could have been preferentially "exported" in the PERIcosms. It is not unusual for sediment trap material to have a higher C : N signature compared to suspended particles in surface waters, but in general the ratios measured here are more C rich than observations made of sinking particles in

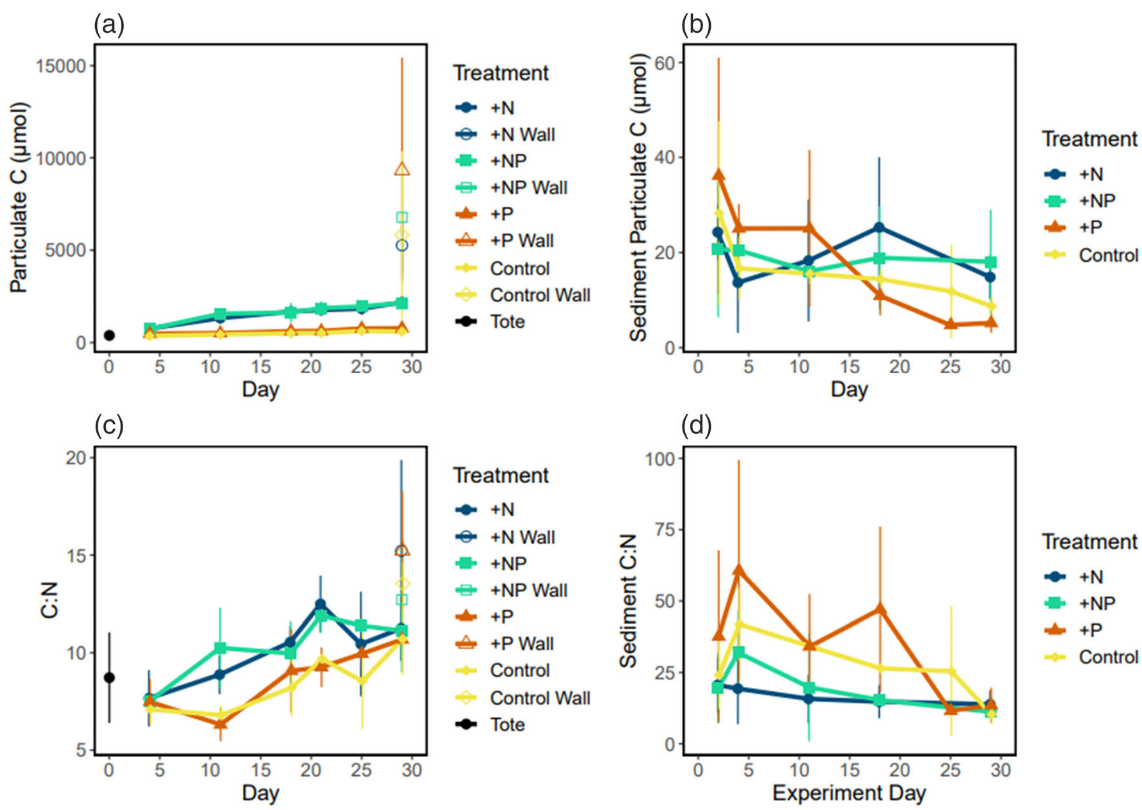

Fig. 10. Total particulate carbon (C) measured in the (**a**) surface reservoir (circles) plus what was measured associated with the walls (diamonds), and (**b**) collected in the sediment trap. Carbon to nitrogen ratios (C : N) for the (**c**) suspended particulate (circles) and wall associated (diamonds) material, and (**d**) sediment trap material. Points depict the average ± the standard deviation for the treatment replicates. For treatment information, see Fig. 2 caption and Table 1.

the NPSG, which tend to be ~7–8 (Karl et al. 1996, 2012, 2021).

All long-term incubations suffer from biological colonization of the walls, the degree of which is often related to the chamber shape and surface area to volume ratio (Chen et al. 1997). We did not physically scrape the wall interiors during the experimental incubation period for either growth prevention or to obtain samples in order to prevent trace metal contamination. For the Hawaii experiment, we calculated that 70–92% of carbon in the PERIcosm was associated with the walls at the end of the experiment (wall g C/[wall + suspended g C]) (Fig. 10a). However, this may be an overestimate since only a lower portion of the PERIcosm was sampled and we did not measure wall growth on the portion of the tank that was disturbed by mixing. DNA analysis of the wall material found that the wall-associated communities differed from those in suspension at the end of the experimental period (Figs. 4, 5). Oligotrophic, free living bacteria such as *SAR11, Synechococcus*, and *Actinomarinales* (Leu et al. 2022) were not found associated with the wall. Instead, prokaryotes generally associated with particles, marine plastic debris, or classified as parasites dominated the wall communities in all treatments including *Rhodobacterales* (Bryant et al. 2016; Duret et al. 2019), *Rickettsiales* (Duret et al. 2019), *Chitinophagales* (Poff et al. 2021; Urvoy et al. 2022), and *Caulobacterales* (Poff et al. 2021) (Fig. 4).

The eukaryotic community associated with the wall also revealed differences in relative abundances of the various taxa (Fig. 5). For instance, Labyrinthulomycetes, which are typically associated with dead or decaying phytoplankton (Raghukumar 2002) had higher relative abundances associated with the wall. Labyrinthulomycetes are often found associated with marine snow deep below the photic zone (Bochdansky et al. 2017; Bai et al. 2022) and may have found a similar niche on the carbon rich wall surface. Even though wall growth was prominent in our incubations, it did not appear to significantly impact the major experimental outcomes.

Practical application

Several aspects of the PERIcosms design make them accessible for long-term, low trace metal focused studies of microbial plankton communities. The components to construct a PERIcosm are fairly inexpensive (~ \$800 total per PERIcosm when purchased in 2020) and easy to acquire so that researchers can build these systems with basic knowledge of plumbing and construction. The design has few moving parts, making PERIcosms relatively light when empty, simple to construct and operate, and resistant to breakage. In contrast, other designs for similar or larger mesocosm systems often require intensive ship time, trained specialists to operate (e.g., Mostajir et al. 2013; Riebesell et al. 2013; Boxhammer et al. 2016), or they may require travel to a designated facility (e.g., Gall et al. 2017; Båmstedt and Larsson 2018). The all-plastic construction and fully enclosed design of PERIcosms means that they have the potential to be maintained trace metal free, even with frequent water replacements. The overall design for the experiments described here was optimized for semi-continuous incubations to take place, which extended the sampling capabilities and incubation duration window compared to similar sized batch experiments (Tortell et al. 2008). While we have successfully operated the PERIcosm systems on land, we believe they could easily be adapted for use at sea. For example, an empty 20′ temperature-controlled van would provide an ideal space to comfortably and securely operate ~ 12 PERIcosms. A clean van, or standard van with an added HEPA filter and plastic sheeting, could be used to provide further trace metal clean measures.

The practical and logistical requirements for the successful implementation of a PERIcosm incubation are like many other incubation experiments, such as access to a climate-controlled space with electricity, access to a seed community, and sampling equipment, all of which add to the costs of the overall experiment depending on individualized needs. Transforming the incubation area into a trace metal clean working environment adds extra complexities but is likely necessary to retain low Fe conditions within the PERIcosms. We recognize that finding the right space for a large experiment can be challenging, but demonstrate here that a make-shift enclosure can suffice, even in warm climates like Hawaii. We considered alternative temperature control approaches, such as using a water bath or deploying the enclosures in situ from a floating raft (e.g., Mostajir et al. 2013), but decided that keeping all PERIcosms within a temperature controlled space provided numerous advantages. For instance, all PERIcosms can be sampled simultaneously by many people, with several samples being collected from the same PERIcosm at once. This is especially important for sampling-intensive projects and time-sensitive biological samples.

While there are no constraints on what type of pelagic ecosystem could be grown within the PERIcosms (e.g., natural marine or freshwater communities, or cultured organisms), a large amount of water is required to fill and maintain their volume when many are used for several weeks. We took two approaches in this study to obtain water for incubations that illustrate this point. On Catalina, the incubations were performed at a facility located next to the ocean so that new water could be collected daily. For the Hawaii experiment, nearly 9 tons of water was collected at once in 1000 L water totes using a chartered vessel.

Discussion

Incubations are a useful tool for ecologists seeking to better understand community dynamics and function. Large volume mesocosm incubations (> 1000 L) have been used for studies investigating long-term ecological responses to physicochemical drivers because they are believed to foster more realistic communities compared to microcosms, and thus more accurately extrapolate to the environment they intend to simulate. However, the size and complexity of such large mesocosms can limit their practical use. The PERIcosms described here thus fill a unique and valuable niche for natural community incubation research based on their ability to produce realistic and reproducible microbial community responses, while remaining simple enough that scientists can build and operate them in a wide array of environments.

The potential for trace metal clean experimentation is another benefit of the PERIcosm method. While they were initially designed for studies of Fe and macronutrient effects in the NPSG, we anticipate that PERIcosms will also be well suited for research in high nutrient low chlorophyll (HNLC) regions and oligotrophic lakes. They may also be useful for studies of highly productive systems, such as upwelling regions, where the particle trap is better suited to understand particle flux dynamics due to the higher particle loads. The ability to control how nutrients are supplied to the systems means they could be used to simulate events such as dust storms, mesoscale eddies, and upwelling. Such natural events often impart community successional patterns that last days to months, which are timescales well suited for the semicontinuous PERIcosm incubation protocol presented here.

We described several key components of the PERIcosm systems that demonstrate their effectiveness for research into the behavior of plankton communities for several weeks under a controlled perturbation setting. PERIcosms bridge the experimental outcomes of mesocosms with the ease of microcosms, and their accessibility provides a research platform capable of advancing community level incubations in a novel way. The systems are constructed to be trace metal clean, are capable of replication, and promote diverse communities over ecologically relevant timescales. The PERIcosm volume allowed for high frequency collection of multiple different sample types not achievable with small volume incubations, which allowed us to capture with confidence the subtle differences in outcomes to the nutrient supply treatments. The PERIcosms therefore have the potential to facilitate studies needed to continue advancing the fundamental understanding of general microbial ecology as well as the microbial response to changing ocean conditions.

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Conflict of Interest

None declared.

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