Examining the Potential of Sandy Marine Sediments Surrounding Giant Kelp Forests to Provide Recycled Nutrients for Growth

To estimate dissolved nutrient effluxes from nearshore sediments, a closed-system, flow-through bioreactor was developed to mimic in situ conditions in sediments surrounding kelp forests. Flow-through bioreactors were built using a modified design of the flow-through plug setup developed by Roychoudhury, Viollier, and Van Cappellen (1998). In 2017, a series of five bioreactors were used to conduct sediment efflux measurements with a section of PVC pipe used as a container for the sediment core, fit between two hand-cut acrylic end-pieces that were secured using zip ties. Each bioreactor housed one sediment core sample and was designed to connect to a seawater reservoir via a peristaltic pump that would pump water through the core in a recirculating, closed-loop fashion. Bioreactors were assembled with a combusted GF/B filter on the bottom end piece of each setup to prevent sediment from eroding into the reservoirs and clogging the tubing. One bioreactor housing was used as a control containing only seawater, and the remaining four were used to house sediment core replicates. In 2018, eight acrylic bioreactors were fabricated using an 80W laser cutter (Trotect Speedy 300 Laser Engraver). These bioreactors used silicone bands to connect the PVC pipe containing the sediment core to each acrylic end-piece, creating an aligned seal (Figure 1). In 2018 and 2019, four replicates were run for both control (i.e., only seawater) and experimental bioreactors (i.e., containing both seawater and sediment).

Sediment cores to be used in the bioreactor experiments were collected from the Goleta Bay, Arroyo Burro, and Mission Creek sites in August of 2017, 2018, and 2019. At all three kelp forests, four replicate sediment cores (5 cm diameter x 20 cm long) were collected by SCUBA divers at 20 m water depth using hand corers; seawater was also collected into 2L HDPE bottles by divers from approximately 1 m above the sea floor. All samples were transported in ice-filled coolers to the laboratory. Sediment cores were stored overnight (20 hr maximum) in a flow-through seawater tank with the top section exposed to maintain oxygenation and in situ temperatures in the porewater. Seawater samples remained unfiltered and were stored overnight in a 4°C cold room.

Following overnight storage, sediment cores were removed from the seawater tank, and the top 2 cm of each replicate core was extruded, sectioned, and placed intact into a bioreactor, taking care not to compress or shear the surface of the core. The core depth was chosen to be 2 cm since that size was used in other flow-through bioreactor studies (e.g., Ahmerkamp et al., 2017; Laverman et al., 2012; Pallud and Van Cappellen, 2006), and the design aimed to sample an oxic horizon. In addition, flow through the bioreactors was designed to progress from the top to the bottom of the core to use gravity to allow porewater to percolate through the sediment. The top to bottom flow design also mimics in situ conditions, where advective flow of porewater is primarily driven by overlying wave and tidal pumping rather than underlying groundwater infiltration. Control bioreactors were assembled in the same fashion, including the filter on the bottom fitting, but without sediment.

All replicates were sealed and attached to a series of peristaltic pumps using PTFE tubing. Unfiltered seawater, which had been warmed to in situ temperature, was flushed through the bioreactors for 1 hour at approximately 5.5 mL min^{-1}. Each setup was allowed to drip freely during this period to allow existing porewater and dissolved nutrients to be flushed out. Flushing time was determined by a series of breakthrough curve analyses during which nutrient concentrations of water exiting the core were measured until they decreased to the same order of magnitude as the inflowing seawater. After flushing, each bioreactor was attached to a new reservoir of unfiltered seawater, which was held in a

15°C water bath, and the entire setup was run in a recirculating fashion for 3 hours at the same rate as the initial flushing (5.5 mL min^{-1}). This flushing rate was used, because it exchanged existing porewater approximately every 2 minutes, a rate faster than the rate of exchange measured in situ at 5 cm depth (every 10 minutes). A series of tests using an oxygen sensor confirmed that oxygen concentrations in the reservoirs were not depleted during the 3-hour period (i.e., dissolved oxygen did not decrease more than 1 mg L^{-1}). At the conclusion of each trial, an aliquot of the seawater in each reservoir was filtered through a GF/F filtered (0.7 µm, Whatman) for nutrient analyses.

Unfrozen seawater and porewater samples were analyzed for ammonium (NH_4^+) using the *ortho*-phthaldialdehyde method (Holmes *et al.,* 1999; Taylor *et al.,* 2007) and a Turner Trilogy Laboratory fluorometer (limit of detection, 0.05 µM). Standard curves were created using artificial seawater in 2016 and using low nutrient SBC seawater that had been incubated for over 6 months in 2017-2019. Frozen samples (-20°C) were thawed and analyzed for combined nitrate (NO_3^-) and nitrite (NO_2^-) via flow injection analysis on a Lachat QuickChem 8500 Series 2 Analyzer (Hach Company, limit of detection, 0.50 µM in 2017 and 0.20 µM in 2018-2019). Another subset of samples was acidified using 4N HCl, refrigerated (4°C), and analyzed for total dissolved nitrogen (TDN, limit of detection, 2 µM, precision, 1-2 µM) via high temperature combustion on a Shimadzu TOC-V Analyzer. TDN is interpreted as a combined value of dissolved inorganic nitrogen species (NH_4^+, NO_3^-, NO_2^-) and the numerous compounds considered dissolved organic nitrogen (DON). Samples were not assayed for TDN in 2017 due to an instrument malfunction and sample contamination. For all analyses, if a measured concentration fell below the limit of detection, results are reported at the limit of detection. To determine net fluxes, the rate of change in nutrient concentrations (µM) measured in the incubation reservoirs was calculated and divided by the length of the experiment (3 hr). For each bioreactor experiment, the mean rates of change by control (seawater) and experimental (sediment core) treatments were calculated, using only the replicates of each treatment that successfully completed the full 3-hour trial. For each site-year, the net change in concentration from sediment alone was determined by subtracting the mean rate of change of control incubations from the mean rate of change of experimental incubations. Net changes in nutrient concentrations were scaled to a larger surface area of benthos (1 m^2) and converted to net nutrient fluxes (µmol m^{-2} hr^{-1}) using the volume of the seawater reservoirs (250 mL) and the surface area of the sediment cores (19.6 cm^2).

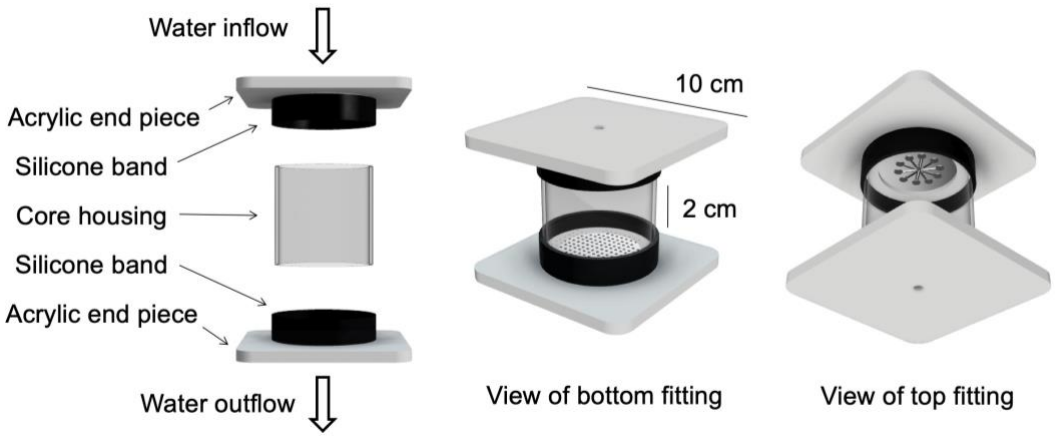

Figure 1. Flow-through sediment bioreactor design manufactured by laser cutting acrylic sheets for sediment bioreactor incubations performed in 2018 and 2019. This entire setup was connected to a seawater reservoir via PTFE tubing and run using a peristaltic pump. Bioreactor housings used in 2017

followed a similar design but were manufactured by hand. All designs are modified from the flow-through plug design developed by Roychoudhury, Viollier, and Van Cappellen (1998).

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