SCOPE 2021 Cruise Planning Remineralization Working Group Meeting
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Overarching Goals of the Theme III working group:

1) Experimentally quantify rates, stoichiometry, and controls on organic matter mineralization
   a) Mineralization stoichiometry (C, N, P, O, H, Fe, etc.) at differing depths
   b) Adsorption/desorption of specific elements onto particles
   c) Experimentally examine nutrient/energy limitation of microbial decomposition and remineralization
   d) Examine temperature-dependent changes in rates of microorganism metabolism particle decomposition.
2) Quantify the role of microorganism metabolism and growth in particle decomposition and the role of microorganisms in attenuating sinking particulate matter flux through the upper mesopelagic zone.
   a) Examine how changes in sinking particle size influence organic matter mineralization.
   b) Compare the chemical composition, caloric content, and lability of suspended versus sinking particles (operationally-defined by 53 μm particle size cut-off).
3) Conduct high-vertical resolution sampling of the lower euphotic zone and upper mesopelagic waters to quantify diapycnal and isopycnal supply of nitrogen and phosphorus pools to the euphotic zone (inorganic and organic, particulate and dissolved).
4) Examine sources and sinks (and hence turnover) of nitrite in the primary nitrite maximum, with specific focus on linking microorganisms to specific nitrite source/sink pathways.
   a) How do particles (suspended or sinking) influence N remineralization and how do these remineralization processes impact NO_2^- cycling?
   b) Do NO_3^- reducing *Prochlorococcus* contribute to elevated NO_2^- concentration near the DCM in the North Pacific?
   c) Does NO_2^- cross-feeding (within *Prochlorococcus* populations or between *Prochlorococcus* and ammonia oxidizer populations) operate at Station ALOHA?
   d) What controls the balance between nitrifier produced NO_2^- and picocyanobacterial produced NO_2^- in the mid-euphotic zone? How well do *Prochlorococcus* compete with ammonia oxidizers for NH_4^+ at different depths and light intensity?
   e) How do the different sources (ammonia oxidizers or phytoplankton) and sinks (nitrite oxidizers or phytoplankton) influence the rate of nitrous oxide (N_2O) gas production – and therefore loss of N from the ecosystem – on particles and/or in the bulk water?

Specific shipboard research plans from the Theme III working group for the 2021 cruise

1) Experiments will be conducted to examine the fate and biological reactivity of sinking particulate matter, collected from several depths through the upper mesopelagic waters. Using large diameter net traps to collect sinking particles, particles will be quantitively split and used for subsequent mineralization experiments. Types of experiments will include:

a. Paired in situ (using free-drifting arrays) and shipboard incubations where seawater (whole and grazer reduced treatments) will be amended with net trap-collected sinking particles.

Time-resolved sampling of the shipboard incubations will include quantifying changes in dissolved O_2 (for rates of respiration), concentrations of nutrients (including TN, NH_4^+, NO_2^-, NO_3^-, TP, and SRP), rates of bacterial production, and bio-optical assessment of particulate matter concentrations. Sampling frequencies will need to be at sufficient temporal resolution to quantify organic matter decay rates and respiration (e.g., hourly to daily-scale). Paired incubations to be conducted on in situ arrays will examine changes in the same elemental pools, but will focus on beginning and end-point measurements. Target depths for net trap collections include: 150, 175, 200, and 250 m.

b. To examine potential nutrient and energy limitation of microorganism mineralization of sinking particles, seawater collected from the lower euphotic zone and upper mesopelagic waters will be amended with net trap-collected sinking particles, with additional incubation treatments to include amending with potential growth-limiting organic and inorganic compounds, including inorganic and organically complexed iron, ammonium, nitrite, algal lysates, and selected amino acids.

c. Temperature-dependent mineralization rates will be examined using paired shipboard and in situ array incubations of seawater amended with net trap collected sinking particles. For these experiments, sinking particles will be collected using net traps deployed at various depths through the lower euphotic zone and upper mesopelagic waters (150-300 m). Seawater from the depths where sinking particles were collected will be incubated (in the dark) at the same depth as collection, and at depths deeper (cooler) and shallower (warmer) than the collection depth. For example, splits from sinking particles collected from 300 m will be added to whole seawater from the same depth, with incubations occurring at 150, 200, 300, and 500 m to evaluate temperature-dependent changes in rates of particle decay and microorganism metabolism.

2) a. To examine whether variations in sinking particle size influence rates of organic matter mineralization and microorganism metabolism, net trap collected sinking particles will be subjected shipboard fragmentation (via sonication). Resulting changes in particle size distributions will be quantified bio-optically. The resulting fragmented particles will be used in subsequent incubations quantifying time-varying changes in dissolved O_2, bacterial production, bio-optical particle size characterizations, and nutrient concentrations. Results will be compared to treatments using net trap collected particles without alteration of particle sizes.

b. Additional experiments will be conducted to chemically and biologically characterize differences between sinking and suspended particles. Such characterizations will include quantifying elemental stoichiometry, caloric content, and reactivity (e.g., rates of mineralization and bacterial production) of both suspended and sinking particles. Large-volume pump collections will be used to collect seawater for subsequent concentration of suspended particles from several depths in the lower euphotic zone. Suspended particles will be concentrated using cross-flow filtration, targeting concentrating suspended particles by upwards of 5000X. The resulting particle collections will be used for characterization of particle chemistry with additional particle splits used for experiments similar to those described above to quantify rates of microorganism metabolism and particle decay.

3) CTD rosette casts will be used for high-vertical resolution sampling of organic and inorganic nutrient pools in the lower euphotic zone and upper mesopelagic waters. Desired vertical resolution at scales of <5 m across depths ranging from 125 m to 300 m. Subsequent

quantification of nitrogen and phosphorus pools to include: total nitrogen and phosphorus, organic nitrogen and phosphorous, nitrite, nitrate, soluble reactive phosphorus, and particulate nitrogen and phosphorus. Potential complementary measurements of nutrient pools would include: isotope (^{33}P)-based assessment of reduced P turnover and phosphatase-based enzymatic assays.

4) Constrain the potential role of *Prochlorococcus* and nitrifiers in modulating NO_2^- concentrations and turnover in the lower euphotic zone and upper mesopelagic waters. Evaluate the impacts of these microorganisms on mineralization processes. This work would benefit from collaborative shipboard sampling and targeted experiments, including measurements focused on:

a. High-sensitivity measurements of nitrite, nitrate, ammonium, and Fe and Cu concentrations;
b. Quantitative metagenomics, amplicon sequencing and dPCR (droplet digital PCR) quantification of bulk seawater and suspended and sinking particles for *Prochlorococcus* and nitrifiers;
c. SeaFLOW based cyanobacteria abundance;
d. Metatranscriptomics of bulk water and particles to assess regulation of pathways involved in NO_2^- cycling;
b. Chemolithotrophic ammonia oxidation and nitrite oxidation rates;
c. *Prochlorococcus* rate of nitrite production (net and/or gross);
d. N_2 fixation rates to constrain "new" reduced N sources;
e. Concentrations of small organic N compounds (urea and cyanate)