Ecosystem response to an episodic nutrient entrainment event
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Open ocean eddies have been implicated in the vertical transport of deep water nutrients into the photic zone in the oligotrophic North Pacific Subtropical Gyre (NPSG; Johnson et al., 2010). During the CMORE BioLINCS cruise in the North Pacific incubations were carried out to evaluate the effects of eddy-induced episodic events on the function and biogeochemical cycles of the surface (25m) microbial ecosystem. These experiments distinguish the effects of deep nutrients alone (surface community mixed with filtered deep (130m) seawater) and biological interactions (mixed with unfiltered deep water), using the MicroTOOLs microarray (Shilova et al., 2014). The MicroTOOLs array enables metatranscriptomic analysis using 135,000 probes designed from environmental sequences targeting all three domains of life as well as viruses.

Deep-water nutrients alone resulted in higher transcription in eukaryotic phytoplankton and Prochlorococcus. Prochlorococcus and Synechococcus responded to N and Fe, respectively, within the same water mass. When microbes were present in the added seawater, eukaryotic phytoplankton decreased activity, perhaps due to competition. Deep nutrients decreased overall transcription in the blooming N2-fixing cyanobacteria Trichodesmium, Crocosphaera and UCYN-A (Fig 1A), but transcription was enhanced as a result of biological interactions. While the factors underlying the observed trends are unknown, the activities of diazotrophs were independently confirmed by measuring N2 fixation rates in each condition: rates decreased after the addition of deep nutrients alone and increased when microbes from deep water were present.

A robotic biosensor (the Environmental Sample Processor or ESP) deployed in surface waters during BioLINCS sampled a natural nutrient transport event similar to previously-observed events at Station ALOHA (Johnson et al., 2010; Fig 1B). Ecosystem-wide transcriptional profiles from experimental mixing of the surface community with unfiltered 130m water were similar to transcriptional profiles of the communities sampled in situ by the ESP (Fig 1C). Though UCYN-A and Trichodesmium were not active at the time, Crocosphaera activity increased due to this event.

While deep water nutrients fuel primary producers, nutrient sensitivities differ within and between dominant photosynthetic clades in the same water mass. Nitrogen fixation is enhanced by episodic nutrient entrainment events but apparently as a result of biological interactions rather than nutrient supply and the metabolically linked organisms remain unknown. This study demonstrates that the combination of experimentation, autonomous sampling and targeted transcriptomics enables the mechanistic understanding of organisms and processes underlying the complexities of marine microbial biogeochemistry.

**Fig 1. Effect of deep water mixing on surface microbial community in NPSG.** A) Distinct genes and organisms had up-regulated transcription in response to nutrients only (filtered deep water) and deep water with organisms. B) The ESP was used to sample vertical transport events between two eddies (top right panel in B). Nitrite concentration profile along the transect between the two eddies (bottom panel in B). Transcription samples taken with the ESP are shown: 9, 14, 15, and 16. C) Principal component analysis of transcriptional profiles shows that addition of deep water resulted in community activities similar to ESP samples 14 and 16.