Supplementary Information

Wintertime rates of net primary production and nitrate and ammonium uptake in the southern Benguela upwelling system

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Carbon fixation and nitrogen assimilation in the dark

Carbon fixation and ammonium (NH_4^+) and nitrate (NO_3^-) uptake were also measured in the dark (Figure S1). Although low, these rates are greater than zero and follow the same general depth trends as those measured in the light bottles, with a few exceptions. For example, dark carbon fixation rates at station 3 are similar to those at station 4 (Figure S1a), while in the light, net primary production (NPP) at station 4 is 3–29 times higher than at the other stations (see Figure 4a, main text). In addition, the rates of dark NH_4^+ uptake are similar to the carbon fixation rates (Figure S1b) and up to two orders of magnitude greater than the NO_3^- uptake rates (Figure S1c), which are extremely low at all stations and fairly uniform throughout the upper 30 m.

Light is a key driver of almost all phytoplankton physiological processes, including nitrogen (N) uptake (Paasche et al. 1984; Cochlan et al. 1991; Hu and Smith 1998; Planas et al. 1999; Kudela and Cochlan 2000). Many have argued that dark N uptake needs to be considered in studies of productivity as it has been observed to range from 10–100% of N uptake measured in the light (Mulholland and Lomas 2008). However, one significant complication inherent to many previous studies is that, as in the case of our southern Benguela upwelling system (SBUS) incubations, samples were collected in the light and then incubated in the dark. Because dark N uptake relies on previously stored photosynthate, it is possible that dark uptake rates measured in this way are higher than those that actually occur in the environment at night (Mulholland and Lomas 2008). Indeed, some of the earliest studies of dark NO_3^- uptake, wherein samples were both collected and incubated at night, report rates that are much lower than those estimated from daytime collections incubated in the dark (e.g. McIsaac and Dugdale 1969, 1972; MacIsaac 1978).

The general depth trends in our measured dark rates are similar to those observed in the light, suggesting that light energy acquired prior to incubation was used for photosynthesis and N consumption in the dark. Water for the incubations was typically collected three hours after dawn, such that the resident phytoplankton community had already been exposed to sunlight and had begun photosynthesizing well before the samples were collected. Once transferred to the dark incubation bottles, they were able to continue photosynthesizing, albeit far less efficiently.

Averaged across the stations, dark carbon fixation amounts to ~4% of NPP, while dark ρNH_4^+ and ρNO_3^- are 26% and 17%, respectively, of the integrated rates of light uptake. Autotrophic carbon fixation in the dark can only be supported by previously stored photosynthate, yet some amount of dark N uptake may be representative of night-time phytoplankton activity. Indeed, dark incubation experiments conducted by Probyn et al. (1990) in the inshore SBUS at night revealed that total N uptake rates by phytoplankton (<20 µm) were similar to those measured in the light. This suggests that some fraction of the N uptake that we measure in the dark may be representative of what actually occurs in the SBUS at night. The implication of this is that ρNH_4^+ and ρNO_3^- quantified in the light may underestimate total autotrophic N uptake. However, measurements of carbon fixation

help to constrain the degree to which N uptake at night may be significant since the C:N-normalised rate of autotrophic N uptake, integrated over the euphotic zone, cannot greatly exceed that of carbon fixation. In the case of the wintertime SBUS, dark N uptake may thus be significant only at station 4 where roughly one-third of the NPP is unaccounted for by the daytime NH_4^+ and NO_3^- uptake (see Figure 8, main text). However, as discussed in the main article, there are a number of other possible reasons for this. A final consideration is heterotrophic bacteria consuming inorganic N at significant rates, thereby contributing to dark N uptake decoupled from carbon fixation. Indeed, it is well known that under conditions of low dissolved organic nitrogen, most heterotrophic marine bacteria will assimilate NH_4^+ (Probyn 1985; Wheeler and Kirchman 1986) and some will assimilate NO_3^- (Middelburg and Nieuwenhuize 2000; Allen et al. 2001).

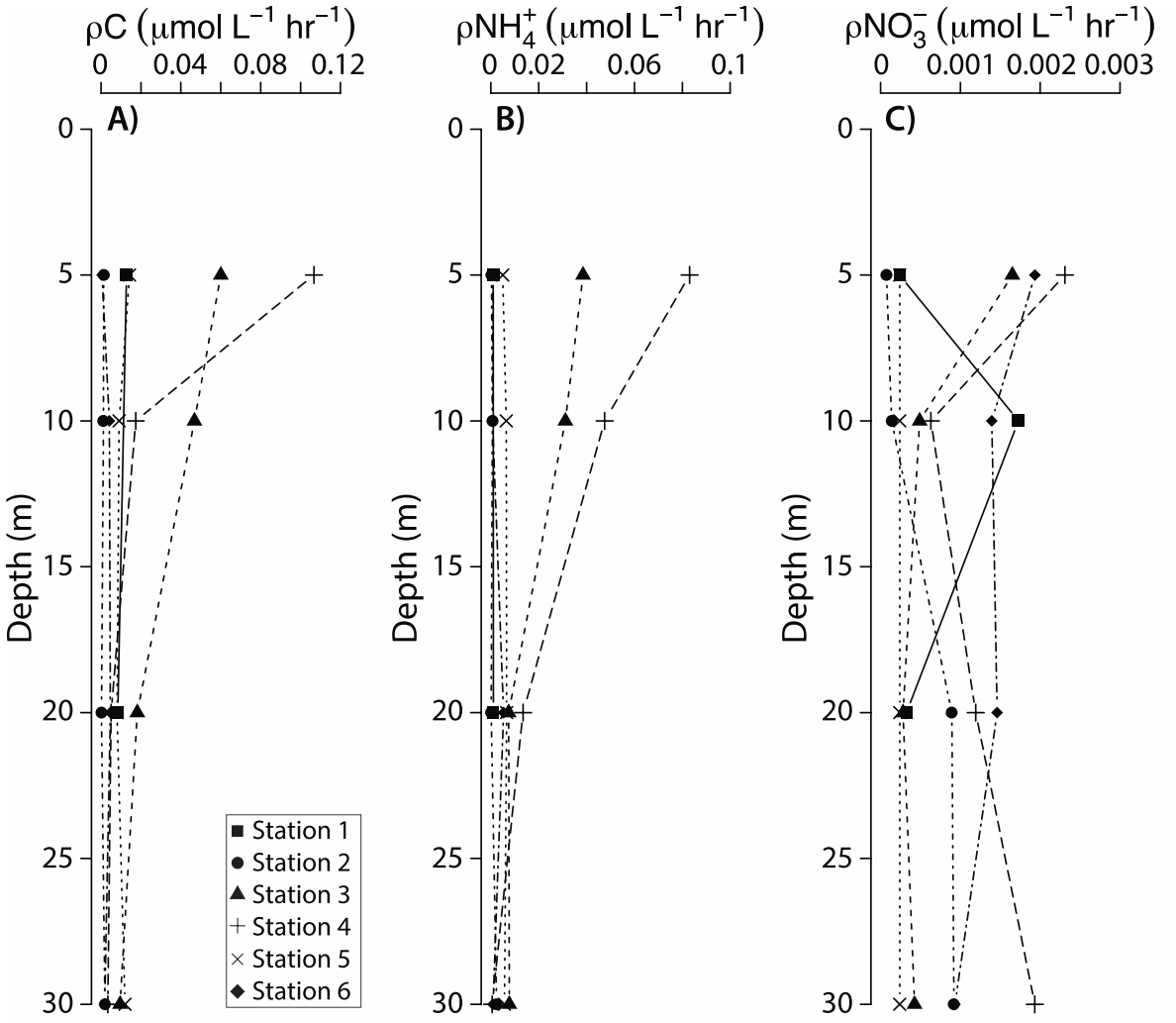

Figure S1: Results of simulated *in situ* experiments to measure net primary production and ammonium and nitrate uptake in the dark, showing the vertical profiles of A) carbon fixation, B) NH_4^+ uptake, and C) NO_3^- uptake in samples collected over the upper 30 m of the water column, at each station in the southern Benguela upwelling system, May 2016. Note the scale change for panel C relative to panels A and B

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