INTRODUCTION

Nutrient diffusing substrata (NDS) have traditionally been used to assess nutrient limitation in aquatic ecosystems. The addition of nitrogen in the form of nitrate (NO$_3^-$) or phosphorus as orthophosphate (PO$_4^{3-}$) has been shown to dramatically change the amount of biofilm that can grow in two or three weeks. We wished to determine how the rate of biomass growth changes in response to nutrient additions over a longer period of time (ie. more than 3 weeks).

QUESTIONS

- Does the limiting nutrient(s) change over time in Sycamore Creek?
- Will samples enriched with nitrogen, phosphorus, both nitrogen and phosphorus and non enriched samples reach similar biomass plateaus?
- Is Sycamore Creek autotrophic or heterotrophic and does this change over time or with changes in hydrology?

METHODS

- **Study Site:** An experiment deploying NDS with various nutrient enrichments was established in a cienega (Fig. 1) near Dos S Ranch in Sycamore Creek (Fig. 2). Biofilm accrual was determined by analysis of chlorophyll a, and biofilm metabolism [gross primary production (GPP) and community respiration (CR)] was measured.

- **Preparation:** A NDS was created by drilling a hole into the top of a poly-cup to fit a 2.8-cm frilled glass disk. Cups were then filled with 50 mL of one of four agar treatments: non-enriched (control), NO$_3^-$ enriched (+N), PO$_4^{3-}$ enriched (+P), or NO$_3^-$ + PO$_4^{3-}$ enriched (+N+P). The glass frilled disk placed on top of the agar was the only surface exposed to light and stream water. Cups were fastened to L-shaped aluminum bars and placed in Sycamore Creek.

- **Analysis:** Six cups from each enrichment type were taken from the stream at 13 days, 21 days, 35 days and 50 days. Three disks were analyzed for chlorophyll a using the hot methanol method. Three disks were analyzed for GPP and CR by reading dissolved oxygen (DO) in ambient stream water and measuring amounts after being incubated for two hours in the light or dark, respectively. Net ecosystem metabolism (NEM) was calculated as GPP-CR and represents the balance between production and respiration in a community. Results were graphed and analyzed using a two-way ANOVA.

RESULTS

Chlorophyll a:

- Nitrogen was the limiting nutrient, with significantly higher amounts of Chl a in +N (p < 0.05) and +N+P (p < 0.05) samples.
- Nitrogen enriched growth rates were several magnitudes higher than non-enriched and +P.
- There was a significant amount of growth up to day 21 (p < 0.05). However, there was not a significant difference between days 21 and 35 (p=0.132) and days 35 and 50 (p=0.858), suggesting that biofilm growth rate over time decelerated.
- A plateau point in the nitrogen-enriched samples may have been reached given a longer study time.

Metabolism:

- The large drop in GPP and CR seen on day 50 was due to a flood that occurred in the stream on day 38. Some samples were lost, and the remainder were covered in sediment.
- GPP was significantly higher in the +N+P samples (p < 0.05). GPP in +P samples were significantly lower than +N and +N+P (p < 0.05).
- Metabolism may have reached plateau, but was disrupted by the flood, where a significant difference was seen between all sampling dates except for days 21 and 35 (p=0.972)
- +N and +N+P samples were more autotrophic while non-nitrogen enriched samples were more heterotrophic.

The impact of flooding on NEM can be seen with the sharp change of N enriched metabolism to heterotrophy.

FUTURE STUDIES

In order to conclusively demonstrate that biofilm growth slows over time, the experiment should be started in early April, when risk of flooding is low. This would allow us to determine whether a similar plateau in biomass would be reached under all nutrient conditions.

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