Top-down and bottom-up control of epilithic periphyton in a tropical stream

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Abstract: Tropical forests are characterized by high tree biomass that forms a dense canopy, thereby reducing the amount of light that reaches forest streams. Forest streams are likely to receive large amounts of organic matter in the form of leaf litter and also have low primary production relative to more open forested systems. Forest clearing can, therefore, affect the in-stream carbon cycle by increasing carbon immobilization and decreasing mineralization. We addressed the question of whether heavily shaded streams are bottom-up limited by light and nutrients or top-down limited by grazers. To answer these questions, we experimentally manipulated nutrients and grazers in enclosures established in either a shaded or a partially open stretch of an Atlantic Forest stream in Brazil. Algal biomass in this stream was low (51.5 to 367.2 mg Chl *a* /m^2). Prior tree removal resulted in a 58% increase in light in the semi-open stream area, and enclosures in this area had a 2.7× increase in algal biomass and a 4× increase in primary production (from 10–40 mg C m^{-2} d^{-1}) relative to enclosures in shaded areas. Nutrient (P) addition had no effect on algal standing crop. Grazing by the caddisfly *Helicopsyche* caused a ~50% reduction in algal biomass. The addition of the most abundant predator, the stonefly *Anacroneuria*, to the enclosures did not reduce feeding by *Helicopsyche*. Substrates in the shaded and semi-open stretches differed in the dominance of some algal taxa, and grazing appeared to favor the cyanobacteria *Dolichospermum* sp. over diatoms. We conclude that algae in forested, low-order streams in the Atlantic Forest are limited by both top-down (herbivory) and bottom-up (light) factors. These findings suggest that tree removal in riparian areas can cause small changes to light availability in streams, but that even small changes can have profound effects on primary producers and, therefore, energy supply for stream consumers.
Key words: grazing, deforestation, primary production, scrapers, predator control, food webs

Low-order forested streams are heterotrophic systems fueled by litter input from the riparian zone (Abelho 2001). However, benthic algae can also have an important role in the energy balance of these streams, even at low biomass levels (Moulton et al. 2015, Neres-Lima et al. 2016, Crenier et al. 2017). Benthic algae may be particularly important in tropical systems where allochthonous organic matter is a low-quality resource for consumers because of its high tannin content and toughness and may contribute to low shredder abundance in some streams (Boyero et al. 2011, 2017, Bruder et al. 2014, Graça et al. 2015a, b). Moreover, benthic algae synthesize compounds, particularly fatty acids, which are essential for stream invertebrate growth and secondary production (Guo et al. 2016a, Crenier et al. 2017).

Primary production and periphyton biomass in low-order forested streams can be bottom-up limited by light because of dense canopy cover (Mallory and Richardson 2005) and by nutrient availability in oligotrophic systems (Hart and Robinson 1990, Hill et al. 1992, Rosemond et al. 1993, Artigas et al. 2013). Primary production in low-order

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forested streams can also be top-down limited by invertebrate and fish grazers (e.g., Feminella et al. 1989, Álvarez and Peckarsky 2005, Mallory and Richardson 2005, Taylor et al. 2006). Selective feeding by grazers can cause changes in algal assemblage structure and dominance by reducing the abundance of more-palatable algal taxa (Feminella and Resh 1991, Pan and Lowe 1994, Cibils-Martina et al. 2014). Frequently, periphyton is jointly affected by interactions among these bottom-up (nutrients and light) and top-down (consumer) effects (e.g., Hill et al. 1992, Rosemond et al. 1993, Larned and Santos 2000). Finally, predators of algal grazers may cause cascading trophic effects if they control the densities or foraging activities of grazers (Moulton et al. 2010, Álvarez and Peckarsky 2014).

Tropical forests have the highest plant and animal biodiversity and the highest plant biomass on the planet. Tropical forests also contribute disproportionately to the global carbon cycle because of their high productivity and litter decomposition rates (Huston 1979). This high tree biomass results in low streambed insolation, but tropical forests are often cleared for cattle grazing and farming (Laurance et al. 2000). In temperate systems, forest clearing results in increased light and nutrient runoff (Likens et al. 1970). However, the effect of clearing tropical forests on the global carbon cycle is poorly understood (Neill et al. 2001). Nutrient enrichment in the tropics can also be caused by agriculture and urbanization with poor sewage treatment (Mitchell et al. 2009, Ramírez et al. 2009). These environmental changes are likely to affect stream functional and structural parameters, which can contribute to the ongoing decrease in biodiversity and changes to carbon cycles.

The Brazilian Atlantic Forest is one of 25 global biodiversity hotspots and is, therefore, a conservation priority (Myers et al. 2000). It is also the most-threatened Brazilian biome. The small fraction of the original forest that remains (11–16%) is highly fragmented (Ribeiro et al. 2009). Some studies in the Rio de Janeiro Atlantic Forest have found that both nutrients and grazing limit biofilm biomass in low-order streams (Moulton et al. 2010, Lourenço-Amorim et al. 2014). These studies also found that primary production is a major energy source for stream macroinvertebrates (Neres-Lima et al. 2016). These findings are consistent with other study results in shaded tropical streams. For example, in Hong Kong forest streams, primary production was an important energy and nutrient source for aquatic food webs even when there was low algal biomass (Lau et al. 2009). The Uaimii State Forest (S. Bartolomeu, Minas Gerais, Brazil) in the Serra do Espinhaço Biosphere Reserve is one fragment of the Atlantic Forest that is part of a regional ecological corridor. Macroinvertebrate density is low in the Uaimii Forest streams (Graça et al. 2015b). Additionally, the proportion of predators is high compared with temperate systems, and these predators are often large-bodied (Graça et al. 2015b), which could limit grazers and, thus, grazer influence on algae.

To our knowledge, the importance of light for primary producers and its interaction with other factors like nutrients and grazing has rarely been addressed in the Brazilian Atlantic Forest streams, despite the uniqueness of the system. We hypothesized that primary production and periphyton biomass would be bottom-up limited by both light and nutrients in these streams because of the high proportion of predators, low dissolved nutrients, and dense shade. To address this hypothesis, we deployed 100 enclosures in a stream to manipulate herbivory pressure, light, and nutrients in different combinations, and measured how primary production responded to these manipulations. We also measured algal assemblage structure in these enclosures, to test whether these factors act as environmental filters on the local and regional species pool of algae.

MATERIALS AND METHODS

The study area

We conducted a field experiment in a ~100-m stream reach in the Mata-Pau, a 2nd-order stream that flows through the Uaimii Atlantic Forest (lat 20°17′35.56″ S, long 043°34′14.48″ W) in April–May 2016, just before the dry season. The stream bed was 1.5 to 4.0 m wide and had a maximum depth of 25 cm in the experimental stretch. The substrate consisted of coarse gravel and small cobbles (4–140 cm), and we observed no differences in substrate across the experimental reach. The forest had a closed canopy, but in a ~30-m section large trees were lacking on the north bank, which allowed sunlight to reach the stream bed directly for 3 h/d. We refer to this part of the reach as the 'semi-open' area (~70% coverage) to differentiate it from the shaded area (100% coverage).

General experimental design

We conducted field work in 3 phases. In phase 1, we sampled invertebrates to determine the identity and density of grazers and predators in the experimental stretches. We also deployed standardized tile substrates for algal colonization. In phase 2, we moved some of the colonized tiles into enclosures to conduct 2 experiments. The 1st experiment focused on interactions between light availability and trophic complexity, whereas the 2nd experiment focused on interactions between light availability and nutrients. In phase 3, we measured algal biomass and identity from tiles subjected to the different treatments in the laboratory.

During phases 1 and 2 we measured pH, conductivity (Digimed field probe; Digimed Instrumentação Analítica, São Paulo, Brazil), and current velocity (FP111 Flow Probe; Global Water; College Station, Texas) at 12 random locations in each of the shaded and semi-open areas), and discharge (velocity-area method; Hauer and Lamberti 2006) in a ~30-m downstream section with uniform width and depth. We collected water samples in a 1-L bottle for total alkalinity (titration with H_2SO_4, 0.01 N to pH 4.5; Car-

mouze 1994), total nitrogen (Kjedahl method; APHA 2005), phosphorus (ascorbic acid method; APHA 2005) and phosphate (colorimetric method; Mackereth et al. 1989) analysis. We conducted these analyses 4× during the experimental period (5, 9, 12, and 16 May). We measured photosynthetically active radiation (PAR) and temperature with Hobo® data loggers (model Pendant UA-002-64; Onset, Bourne, Massachusetts) that took measurements every 10 min in both the shaded and semi-open areas throughout the experimental period.

Algal colonization and invertebrate sampling

On 18 April 2016 we deployed 120 unglazed slate tiles (8 × 8 × 1.9 cm) on the stream bed in the semi-open area at ~6 cm depth and allowed algae to colonize and grow on the tiles for 21 days. In another Atlantic Forest stream in Rio de Janeiro, algal biomass stabilized after 15 days (Moulton et al. 2015), so 21 days should have allowed sufficient time for colonization. We ensured similar initial algal conditions for all enclosure experiments described below by incubating all tiles in the same stream stretch.

We estimated in-stream *Helicopsyche* densities from stones randomly picked up from the stream bed in the shaded (n = 10) and semi-open (n = 10) stretches (stones measured 8–12 cm along the largest axis, and individual areas were recorded). We also measured the density of invertebrates in the shaded and semi-open areas from samples (Surber sampler with 30 × 30 cm area and 0.5 mm mesh) collected at 10 locations in each area. Samples were taken by disturbing the substrate for ~45 seconds at each site within the sampling frame. We sorted the invertebrates alive in situ, preserved them in 70% ethanol, and then identified, measured, counted, and classified them by functional feeding groups based on information for both the neotropics (Bello and Cabrera 2001, Polegatto and Froehlich 2001, Baptista et al. 2006, Tomanova et al. 2006) and North America (Merritt and Cummins 1996, Cummins et al. 2005). We identified and counted taxa under a stereomicroscope (Leica M80) equipped with a digital camera (Leica IC 80 HD), and measured all specimen lengths with the software Motic Image Plus 2.0 (Hong Kong, China). Finally, we estimated specimen biomass from length-mass equations (Smock 1980, Benke et al. 1999, Johnson et al. 1999, Miserendino 2001, Stoffels and Karbe 2003).

Experimental manipulations

Experiment 1: consumers and light manipulations On 9 May (after 21 d), we created 4 replicated experimental treatments (n = 10 for each treatment in each light condition): 1) no consumers, 2) ambient density of consumers (2 *Helicopsyche*), 3) high density consumer (4 *Helicopsyche*), and 4) consumers + predators (2 *Helicopsyche* and 1 *Anacroneuria*). The stonefly *Anacroneuria* was the most abundant predator in the stream. Fish were virtually absent throughout the experiment, and during the entire study we only observed one 1-cm *Loricariidae*, a grazer. To set up treatments, we first transferred 80 periphyton-colonized tiles from the stream bed to the enclosures (40 enclosures each in the semi-open and shaded areas). Each enclosure was a 19 cm high, 18-cm-diameter plastic bucket with two 6-cm diameter windows on opposite sides. Each window was covered by 1-mm mesh that allowed water flow but kept out invertebrates (Fig. S1). We placed the enclosures at <20-cm depth and attached them to the stream bed with iron rods. We then placed 1 tile in each enclosure. During this experiment we checked the enclosures every 3 to 5 days and cleared the clays that passed through the enclosure nets and settled on the bottom of the buckets. Survivorship of invertebrates was 100% during the experimental period. After 15 days we retrieved the tiles from the enclosures to determine algal biomass and identity (see below).

Experiment 2: nutrient and light manipulation We placed a 2nd set of enclosures (n = 10) in each area with nutrient diffusing substrates (but no invertebrates) in both the semi-open and shaded areas, downstream of the 1st set. We used commercial fertilizer pellets (Pastilhas Nutritivas para Samambaias and Pastilhas Nutritivas para Flores, 2 pellets of each; West Garden, São Paulo, Brazil) as nutrients and placed them in plastic vials with 4 pinholes that allowed nutrients to diffuse into the water. The pellets (1.12–1.36 g each) contained 9% N as $(NH_4)_2SO_4$ and $NH_4H_2PO_4$, 9% P as P_2O_5, and 6% K_2O. We replaced the pellets 3× (~every 4 d) over the course of the experiment. Nutrient leaching occurred exponentially (based on 5 readings over a 4-d period measured in 3 replicate enclosures), with P increasing from 5.5 to 164.1 μg/L and total N from 46.5 to 97.6 μg/L. However, these values fluctuated based on the time since the pellets had been replaced. We used the same control tiles from enclosures with no invertebrates in this experiment as we did in experiment 1. As in experiment 1, we retrieved all tiles after 15 d and used them to determine algal biomass and identity (see below).

We also estimated gross primary production from tiles incubated in the light (shade and semi-open) and nutrient (with and without) treatments. We estimated primary production by measuring changes in dissolved oxygen concentrations (Hauer and Lamberti 2006). We placed individual tiles colonized with algae in glass flasks (1.5 L, 9-cm mouth opening) and measured changes in oxygen in the dark (aluminum foil-covered) and light. We filled all flasks with stream water and incubated them within 2 m of their enclosures (to allow flasks to be submersed) for ~4:30 h (the exact time was recorded for each bottle) in 4 runs on consecutive days, for a total of 68 measurements. We conducted each set of measurements between 09:30 and 16:30 h. We measured oxygen by inserting a YSI probe (Clark electrode; Yellow Springs Instruments, Yellow Springs, Ohio) into the

flask through a hole in the lid. We made holes in the flask lids prior to the experiment and covered them with transparent tape to stop oxygen exchange. Changes in oxygen were expressed per unit area and time (mg O_2 cm^{-2} h^{-1}), and we calculated gross primary production (GPP) as the sum of the estimated net primary production (NPP, changes in the oxygen content in the transparent flasks) and respiration (R, changes in the oxygen content in the dark flasks). We transformed oxygen values to carbon units by assuming 6 molecules of C were fixed for every 6 molecules of oxygen produced. Given that the atomic masses of oxygen and carbon are respectively 16 and 12, the release of 32 g of oxygen results in the fixation of 12 g of carbon, i.e., 0.375 g C/O_2. However, the photosynthetic coefficient is not 1.0, but 1.2 because glucose is quickly converted into other compounds (Brower et al. 1998). We, therefore, used the following equation to convert oxygen production (OP) to carbon fixation (CF):

$$CF = \frac{0.375 \times OP}{1.2} \qquad \text{[Eq. 1]}$$

Determination of algal biomass and identity

After removing tiles from the enclosures, we used a toothbrush to scrape the accumulated biofilm from the tile surface into a tray, transferred it to a 50-mL Falcon™ centrifuge tube, and adjusted it to a final volume of 45 mL. We transferred a 3-mL portion of the suspended biofilm to Eppendorf™ tubes and preserved it with 3 mL of Lugol's and 5 mL formalin (4%) for algal identification (see below). We filtered the remaining 42 mL in situ (glass fiber prefilters, AP4004700; Millipore®, Burlington, Massachusetts) and transported these samples to the laboratory in an ice chest to quantify chlorophyll *a* (Chl *a*). We measured Chl *a* by placing the filters individually in acetone in the dark for 24 h at 4°C and estimating Chl *a* concentration as:

$$\text{Chl } a\left(\frac{\text{mg}}{\text{m}^2}\right) = \frac{26.73 \times (\text{D664b} - \text{D665a}) \times \text{V}}{T \times L}, \qquad \text{[Eq. 2]}$$

where, V = extracted volume (L), T = tile area (in m^2), L = cuvette path length (cm), 26.73 = absorbance correction for 90% acetone, and D664b and D665a = optical density of 90% acetone respectively before (b) and after (a) acidification with 0.1 mL 0.1N HCl (APHA 2005).

We identified and counted the 3-mL fixed aliquot of algae from experimental tiles in Utermöhl chambers with a Zeiss inverted microscope (Zeiss, Thornwood, New York). We identified 300–500 diatom frustules per homogenized aliquot from the sample. For each sample, we first stirred the sample and then took a subsample with a dropper. If necessary, another subsample was taken to achieve the 300 to 500 frustules. We based our identifications on the taxonomic classifications proposed by Round et al. (1990) and keys by Patrick and Reimer (1966), Krammer and Lange-Bertalot (1986, 1988, 1991), and Metzeltin et al. (2005). To aid our diatom identification, we oxidized extra biofilm samples (preserved in 4% formalin; oxidized with $KMnO_4$ and HCl following Simonsen 1974) taken from the stream substrate in both the semi-open and shaded stretches. Oxidation of samples allowed us to more easily view the fine structure of diatom frustules needed for accurate identification. We then used hyrax (mounting agent) to make permanent slides, which facilitated identifying diatoms because we were able to use an immersion objective (100×) on an optical microscope (CS32; Olympus, Waltham, Massachusetts.).

Statistical analyses

We compared temperature (every 15 min along the 24 h cycle) and light intensity (every 15 min along 12 h cycle) in the shaded and semi-open areas throughout the entire 15 days of the experiment with non-parametric pairwise comparisons (Signed Rank Test). We compared the other environmental conditions with Student's *t*-test. We compared invertebrate densities, total biomass, and biomass of functional feeding groups between the shaded and semi-open stretches with either *t*-tests when the data were normally distributed or Mann–Whitney *U*-tests when data were not normally distributed. We compared Chl *a* concentration on tiles in the stream bed (freely accessible to *Helicopsyche*) and tiles in the enclosures with 2 *Helicopsyche* (stream density) with a Student's *t*-test.

In experiment 1, we assessed the simultaneous effects of consumers (4 levels: 0, 2, and 4 *Helicopsyche*, and 2 *Helicopsyche* with 1 predator [*Anacroneuria*]) and light (2 levels: shade and semi-open) on Chl *a* concentration with a 2-way analysis of variance (ANOVA), after ensuring the data was normally distributed (Shapiro–Wilk test; rank transformation was applied when the normality test failed). In experiment 2, we assessed the effect of light (2 levels: shade vs. semi-open) and nutrients (2 levels, with and without) on tile Chl *a* concentration and on primary production with 2-way ANOVA. We used the same control tiles here as we did in experiment 1 (no invertebrates).

We used algal assemblage structure data (square-root transformed) from both experiments (grazer and nutrient manipulations) to conduct both a nondimensional multidimensional scaling (NMDS) based on Bray–Curtis similarities and a 2 factorial permutational analysis of variance (PERMANOVA; PRIMER-E®) (Anderson et al. 2008). We also compared the number of taxa and cell density in the treatments with and without consumers (4 levels) and light (2 levels) with a 2-way ANOVA and a post-hoc Holm–Sidak test when the differences were significant.

RESULTS

Physical and chemical environment

The median daily light intensity in the semi-open area (3700 lux) was 58% higher than the shaded area during the day (2300 lux; $Z = 2.67$, df = 22, $p = 0.005$). However,

during the 3 h direct sunlight reached the stream bed, light intensity was ~10× higher than in the shaded area (Fig. 1). The temperature was identical in both reaches (mean = 16.7°C in both areas; shaded area range = 15.0–18.3°C; semi-open area range = 14.9–19.5°C). During the experiment, the pH (7.4 ± 0.1; mean ± SE), conductivity (31.6 ± 2.6 μS/cm), turbidity (3.1 ± 0.1 NTU), total alkalinity (283 ± 16 μEq/L), oxygen (9.4 ± 0.3 mg/L), N-total (46.5 ± 3.0 μg/L), P-PO_4 (3.1 ± 0.4 μg/L), and P-total (5.5 ± 0.2 μg/L) remained relatively stable. The mean current velocity in the stream was 0.27 m/s and was significantly higher in the semi-open (median = 0.28) than in the shaded stretch (median = 0.16; $U = 414.5$, df = 46, $p = 0.009$). Within the enclosures the current velocity was near zero. Finally, the discharge ranged from 0.09 to 0.17 m^3/s, with a decrease toward the end of the experiment.

Invertebrate densities and biomass

The mean densities of *Helicopsyche* on natural stones were similar between shaded (1.6 ± 0.4 SE /stone) and semi-open (1.7 ± 0.2 SE /stone) stretches ($U \geq 42.5$, df = 18, $p \geq 0.570$). These estimates extrapolate to approximately 310 *Helicopsyche* m^{-2} under the assumption that the entire stream bed was covered with stones. The densities of *Helicopsyche* on tiles left in the stream bed during the experiment were 3.0 $tile^{-1}$, or 240 m^{-2}.

The overall density of the 31 observed taxa was 175 (±27 SE) individuals m^{-2}, and the total biomass was 348 (±149 SE) mg (DM) m^{-2}. We found no differences in density or total biomass between the semi-open and shaded areas ($t = 0.797$, df = 18, $p = 0.44$; and $U = 64$, df = 18, $p = 0.31$; Table 1). Predators made up 61% of the invertebrate biomass (26% in number of individuals), followed by filtering-collectors (31%), grazers and shredders (3% each), and gathering-collectors (2%; Fig. 2). A large proportion of predator biomass in the shaded area was contributed by just 5 specimens of *Corydalus* (Megaloptera, Corydalidae). If these 5 specimens are excluded from analyses, the biomass totals change to 68% filtering-collectors, 16% predators, 6% each grazers and shredders, and 4% gathering-collectors. The total biomass in shaded and semi-open areas did not differ significantly among any of the functional feeding groups (U = 41–89, df = 18, p = 0.054–0.970).

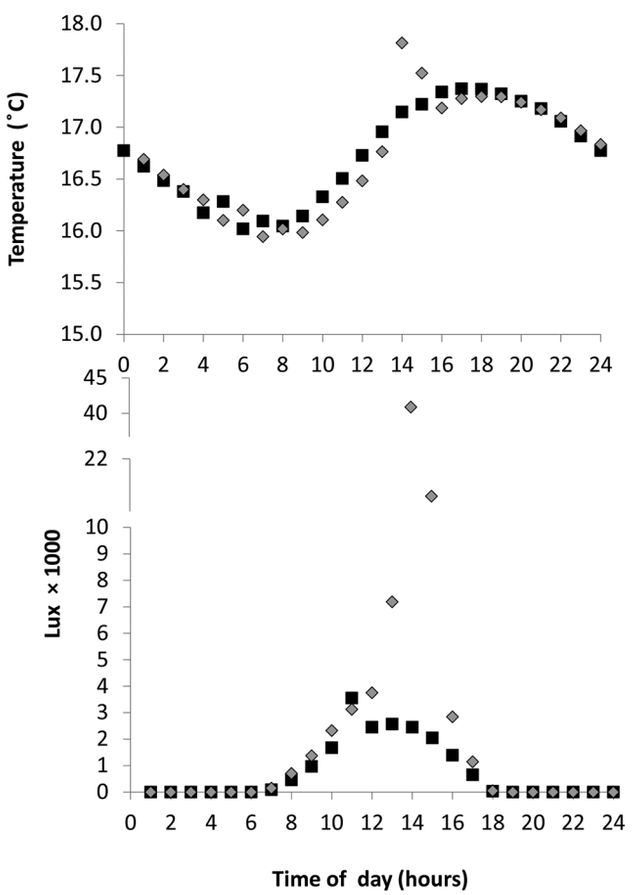

Figure 1. Average hourly temperature (°C) and light (lux) in the shaded (black squares) and semi-open (gray diamonds) areas in the Mata Pau Atlantic Forest stream (n = 72 d × 24 h = 1728 observations).

Experiment 1: consumer and light manipulations

Both light and consumer density affected algal biomass ($F > 12.99$; $p < 0.001$; Table S1; Fig. 3). Algal biomass on tiles with no consumers was significantly higher in the semi-open area (343.5 ± 48.7 mg Chl *a* m^{-2}) than in the shaded area (128.4 ± 13.3 mg Chl *a* m^{-2}). The presence of *Helicopsyche* at densities of 2 and 4 $tile^{-1}$ caused a 50 to 60% reduction in algal biomass in the semi-open and shaded areas, respectively. The presence of predators did not cause decreased algae on the tiles (Fig. 4).

Experiment 2: nutrient and light manipulation

Algal biomass differed among treatments ($F > 4.66$, $p < 0.04$), but algal biomass was significantly reduced in the presence of nutrients in the shaded, but not in the semi-open area (Fig. 5; Table S1). Gross primary production expressed as fixed C ranged from 0.009 to 0.417 μg C cm^{-2} h^{-1} (Fig. 6) and was significantly higher in the semi-open than the shaded area ($F = 31.38$, $p < 0.01$; Table S2). Gross primary production was not affected by nutrients alone but nutrients did interact with light such that gross primary production was lower in the shade when nutrients were present.

Algal assemblages

Twenty-seven algal taxa colonized the tiles (Table 2). All algae were in the Bacillariophyceae except for *Dolichospermum* sp. (Cyanobacteria) and *Ulothrix* sp. (Chorophyceae).

We found no difference in number of taxa across treatments ($F_{1,43} < 2.415$, $p > 0.127$), but the number of algal cells

Table 1. Mean (±SE) benthic invertebrate abundance in terms of number (No.) of individuals and dry mass (DM) m^{-2} in semi-open and shaded areas of the Mata Pau stream, Minas Gerais, Brazil.

			No. individuals m^{-2}		mg (DM) m^{-2}	
Family/other	Genus	Feeding group	Semi-open	Shaded	Semi-open	Shaded
Oligochaeta		gathering-collector	1 ± 1	0 ± 0	0.1 ± 0.1	0.0 ± 0.0
Planariidae		predator	7 ± 2	12 ± 5	0.2 ± 0.1	0.3 ± 0.1
Hydracarina		predator	0 ± 0	1 ± 1	0.0 ± 0.0	0.0 ± 0.0
Baetidae	*Americabaetis*	gathering-collector	6 ± 2	4 ± 2	1.1 ± 0.6	0.3 ± 0.1
Baetidae	*Baetodes*	grazer	0 ± 0	1 ± 1	0.0 ± 0.0	0.1 ± 0.1
Baetidae	*Paracloeodes*	grazer	4 ± 2	1 ± 1	0.5 ± 0.3	0.1 ± 0.1
Baetidae	*Zelusia*	gathering-collector	1 ± 1	0 ± 0	0.4 ± 0.4	0.0 ± 0.0
Leptophlebiidae	*Farrodes*	grazer	4 ± 2	13 ± 4	0.2 ± 0.1	1.5 ± 0.5
Leptophlebiidae	*Hagenulopsis*	grazer	2 ± 2	18 ± 5	0.7 ± 0.7	2.5 ± 0.9
Leptophlebiidae	*Hermanella*	filtering-collector	1 ± 1	1 ± 1	0.1 ± 0.1	0.1 ± 0.1
Leptophlebiidae	*Hylister*	filtering-collector	0 ± 0	3 ± 2	0.0 ± 0.0	0.9 ± 0.6
Leptophlebiidae	*Thraulodes*	grazer	23 ± 5	22 ± 4	4.1 ± 1.8	5.9 ± 1.5
Leptophlebiidae	*Tricorythodes*	gathering-collector	1 ± 1	0 ± 0	0.1 ± 0.1	0.0 ± 0.0
Leptophlebiidae	*Campylocia*	filtering-collector	0 ± 0	1 ± 1	0.0 ± 0.0	0.2 ± 0.2
Perlidae	*Anacroneuria*	predator	12 ± 6	29 ± 10	11.6 ± 9.1	11.8 ± 4.6
Perlidae	*Kempnyia*	predator	3 ± 2	3 ± 2	3.3 ± 2.9	17.4 ± 16.4
Coenagrionidae		predator	4 ± 4	10 ± 5	0.3 ± 0.3	1.1 ± 1.0
Megapodagrionidae		predator	0 ± 0	1 ± 1	0.0 ± 0.0	0.2 ± 0.2
Corydalidae		predator	0 ± 0	8 ± 3	0.0 ± 0.0	377.4 ± 282.1
Elmidae		gathering-collector	3 ± 2	3 ± 2	7.2 ± 3.3	1.9 ± 1.2
Psephenidae		grazer	15 ± 5	9 ± 5	0.0 ± 0.0	0.0 ± 0.0
Calamoceratidae	*Phylloicus*	shredder	1 ± 1	0 ± 0	11.9 ± 11.9	0.0 ± 0.0
Helicopsychidae	*Helicopsyche*	grazer	35 ± 17	20 ± 6	3.0 ± 1.8	1.5 ± 0.5
Odontoceridae	*Marilia*	shredder	8 ± 4	7 ± 3	4.2 ± 3.4	1.1 ± 0.7
Hydropsychidae	*Smicridea*	filtering-collector	9 ± 7	7 ± 2	56.2 ± 44.1	80.9 ± 47.8
Hydropsychidae	*Leptonema*	filtering-collector	2 ± 2	1 ± 1	47.7 ± 31.9	22.1 ± 22.1
Polycentropodidae	*Cyrnellus*	filtering-collector	1 ± 1	0 ± 0	2.6 ± 2.6	0.0 ± 0.0
Ecnomidae	*Austrotinodes*	filtering-collector	1 ± 1	2 ± 1	2.6 ± 2.6	2.4 ± 1.9
Chironomidae		gathering-collector	8 ± 4	14 ± 9	0.4 ± 0.3	0.5 ± 0.2
Simuliidae		gathering-collector	1 ± 1	4 ± 2	0.1 ± 0.1	0.9 ± 0.7
Tipulidae		shredder	1 ± 1	2 ± 1	1.4 ± 1.4	1.2 ± 0.8
Tabanidae		predator	0 ± 0	2 ± 2	0.0 ± 0.0	6.5 ± 5.7

cm^{-2} was significantly higher in the semi-open (155 ± 26) than the shaded area (53 ± 12; $F_{1,43}$= 6.683, p = 0.013). Assemblage structure was significantly different between the semi-open and shaded areas regardless of grazer presence (pseudo $F_{35,1}$ = 2.018, p = 0.036). The algal taxa *Dolichospermum* sp. and *Eunotia bilunaris* both had higher relative abundances in the semi-open than the shaded areas (12 vs 2% and 20 vs 13%, respectively). Conversely, *Fragilaria* sp. was less abundant in the semi-open than in the shaded areas (17 vs 32%; Table 2). However, when grazers were present *Dolichospermum* relative abundance increased from 3.5 to 38.9% in the semi-open area, and from <0.1 to 10.5% in the shaded area (Table S2). In contrast, we found no interaction effect of light and nutrients on assemblage structure (pseudo $F_{15,1} \leq 1.8875$; $p \geq 0.052$). In the nutrient only treatment *Gomphonema apiculatum* was more abundant (7.7 vs 3.1%), whereas *Navicula accomoda* was less abundant (6.9 vs 31.2 %, Table S1).

DISCUSSION

Light and grazing both limited periphyton biomass and gross primary production, whereas nutrient additions did not. Some of these findings, especially biomass limitation by grazing, were unexpected because we anticipated the large proportion of predators would keep grazer at densities

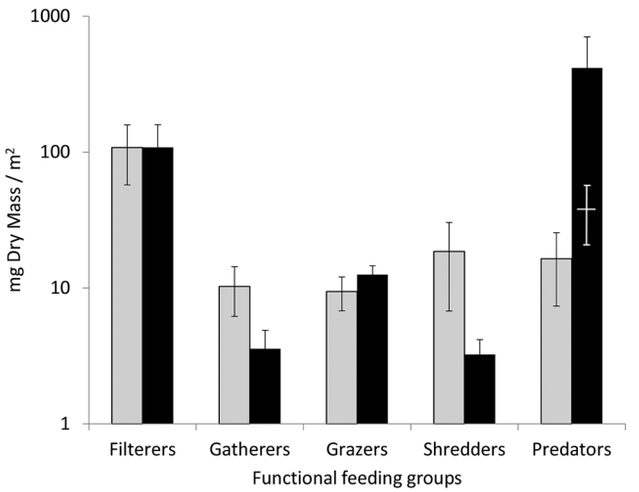

Figure 2. Mean (±SE) biomass of invertebrate functional feeding groups in shaded (black) and semi-open (gray) areas of the Mata Pau stream. In the column that represents biomass of predators in the shade, the area above the white line indicates the contribution of 5 specimens of *Corydalidae*.

too low to influence the algal biomass as they did. Our findings have important implications for forecasting the ecosystem impacts of ongoing anthropogenic changes in these Neotropical Atlantic Forest streams.

Nutrients

Nutrients can limit primary production in streams (Hart and Robinson 1990, Artigas et al. 2013, Lourenço-Amorim et al. 2014). The Mata Pau stream total nitrogen was 46.46 ± 2.71 μg/L, which is at the extreme low end of the range reported for other tropical rivers and streams with low or no nutrient input (35–230 μg/L range; Brito et al. 2006, Moretti et al. 2009, Agra 2014, Guo et al. 2016b). The P-PO_4 concentration of ~3.1 μg/L is also at the low end of the range reported for other tropical streams with low anthropogenic impacts (2.7–470 μg/L range; e.g., Rosemond 1993, Flecker et al. 2002, Encalada et al. 2010, Ferreira et al. 2012, Moulton et al. 2015). Consistent with our expectations based on the low nutrient levels, algal biomass was low in these streams. However, it was within the range of values reported for other low-order streams (e.g. Rosemond 1993, Álvarez and Peckarsky 2005, Lourenço-Amorim et al. 2014, Garcia et al. 2015, Moulton et al. 2015, Guo et al. 2016b).

Here, we found that nutrient addition did not result in increased algal biomass. This finding is contrary to the results of some previous studies (Hart and Robinson 1990, Artigas et al. 2013, Lourenço-Amorim et al. 2014), but it is consistent with some other systems that have low ambient N and P concentrations (e.g., Mallory and Richardson 2005). In nutrient-limited systems where algal biomass is not affected by nutrient additions, algal growth limitations are generally attributed to other limiting factors, particularly light (Larned and Santos 2000, Lourenço-Amorim et al. 2014, Guo et al. 2016b). Streams in this area of the Atlantic Forest, though generally nutrient poor, are probably N- rather than P-limited, as reported for other Atlantic Forest streams in Rio de Janeiro state (Lourenço-Amorim et al. 2014, Moulton et al. 2015).

Nutrient addition to the shade treatment had an unexpected inhibitory effect on both primary production and algal biomass. This could be a result of nutrient toxicity, as has been reported in other studies (e.g. Burrows et al. 2017). This is unlikely, however, because we did not use extremely high nutrient concentrations in our experiment. Biomass accrual inhibition in our experiment could be a consequence of the replacement of some taxa or changes in the activity of key remaining taxa (see below). The interactive effects of light and nutrients on primary production warrant further investigation.

Light

Light can often limit primary production in streams and rivers (e.g. Hill et al. 1995, Mallory and Richardson 2005). We found that the removal of only a few trees in 1 area of 1 stream bank caused an overall 58% increase in light in that area, and resulted a 2.7× increase in periphyton biomass relative to fully shaded sections of the stream. We also found that primary production increased in the semi-open area compared with the shaded area. Our estimates for gross primary production under shade conditions (10 mg C m^{-2} day^{-1}) are at the low end of the range reported for other shaded tropical streams (5–113 mg C m^{-2} day^{-1}) (Davies et al. 2008). The 58% light increase in the semi-open area resulted in a 4× increase in primary production (to ~40 mg C m^{-2} day^{-1}). However, it is unlikely that the 58% increase in light fully eliminated light limitation, because the increased rate of primary production was still lower than the ~90 mg C m^{-2} day^{-1} value reported by Moulton et al. (2015) in another low-order Atlantic Forest stream receiv-

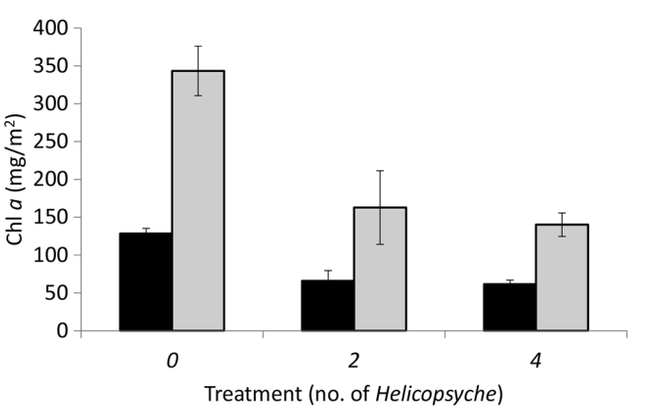

Figure 3. Mean (±SE) Chl *a* on tiles incubated in enclosures with 0, 2 and 4 *Hydropsyche* $tile^{-1}$ placed in the shaded (black) and semi-open (gray) areas of the stream.

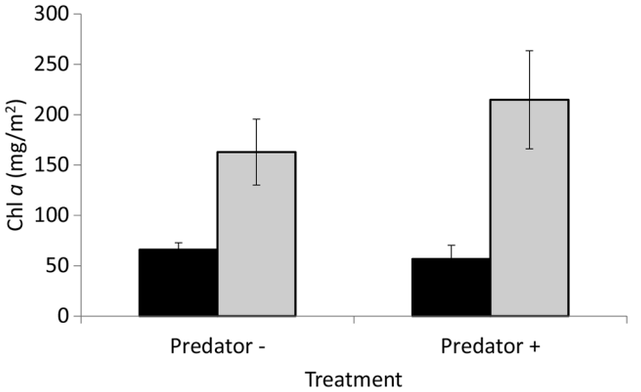

Figure 4. Mean (±SE) Chl *a* on tiles incubated in enclosures with 2 *Hydropsyche* in the absence (Predator −) and presence (Predator +) of 1 *Anacroneuria* in the shaded (black) and semi-open (gray) areas of the stream.

ing direct sunlight. Our primary production values could be underestimates of production occurring in the stream, because of the lack of current and continuous nutrient renewal in our experimental buckets. Even so, algal biomass changed consistently in the enclosures, and we think the observed differences across treatments is a good indicator that light is limiting primary production in these closely shaded streams.

Other studies have found evidence for light or nutrient limitation (e.g., Mallory and Richardson 2005) or co-limitation (e.g., Larned and Santos 2000; light and P co-limitation). We also found an interaction of light and nutrients, with unexpectedly lower algal biomass under nutrient enrichment in the shade relative to algal biomass in semi-open conditions. Under shade conditions, 3 algal taxa differed between the control (no nutrients) and nutrient enriched conditions. *Eunotia bilunaris* constituted 12.7% of the cells in control treatments, but only 4.5% in enriched conditions. *Fragilaria capucina* also decreased between the control and enriched treatments, from 53.4 to 43.1% In contrast, *Eunotia minor* increased from 5.0 to 21.6% in the control and in the nutrient enrichment treatment, respectively. Regardless of the mechanism behind the changes in the abundance of a few algal taxa, the decrease in C fixed by primary producers under nutrient enrichment in the shade may reinforce the heterotrophy of these systems.

Herbivory

Helicopsyche is the main grazer in the Mata Pau stream, and reached densities of ~310 individuals m^{-2} on colonizable stone surfaces during this experiment. These densities are within the lower range reported for other systems (e.g., from 200 to ~2000; Pan and Lowe 1994, Guo et al. 2016b). We did not expect these grazers to reduce algal biomass, because their densities were so low compared to previously reported densities for the same streams, and because of the high proportion of predators. We found, however, that even though grazers were not numerically abundant they substantially lowered algal biomass. This finding refuted our hypothesis that top-down control of algal biomass by herbivores is unimportant in our system.

Grazer pressure was more intense in the semi-open than in the shaded stream area. In the shaded area grazers removed 33% of the algal biomass, whereas in the semi-open area grazers removed 53%. It is plausible that consumers grazed more efficiently in semi-open than in shaded areas because of high resource concentrations in semi-open areas. Thus, grazers in shaded areas would need to move more to find algal cells, and *Helicopsyche* growth rates might be faster in the semi-open areas than in the shaded ones. The results of our grazer experiment contrast with previous studies in which the grazing snail *Juga silicula* consumed 90% of the primary production under low-irradiance conditions, and <15% under high irradiance conditions (Lamberti et al. 1989). However, a meta-analysis that examined the controls on benthic algae found that while both light and herbivory are important, herbivory has a greater effect under high light conditions (Hillebrand 2005). This pattern can occur if high light favors algal types that grazers can easily digest (Hillebrand 2005).

Our results also contradict reports from other Atlantic Forest streams (Rio de Janeiro; Lourenço-Amorim et al. 2014) in which algae responded more to nutrient enrichment than to herbivore exclusion. The authors suggested the weak top-down effect may be associated with seasonal changes in the density of consumers and the small size of the dominant grazers (mainly Baetidae).

Predation

During our experiments, predators made up 26% of all invertebrates in terms of biomass in the Mata Pau stream, which is typical for nearby streams (33%; Graça et al. 2015b) and for streams elsewhere in the Atlantic Forest (31%; dos Santos and Rodrigues 2015). Predators were expected to exert a strong influence on herbivores and, thus, herbivory. However in the enclosures, the presence of the *Anacro-*

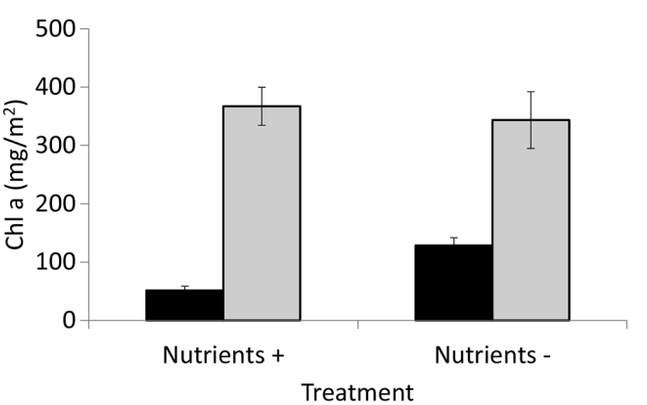

Figure 5. Mean (±SE) Chl *a* on tiles incubated in enclosures with and without additional nutrients in the shaded (black) and semi-open (gray) areas of the stream.

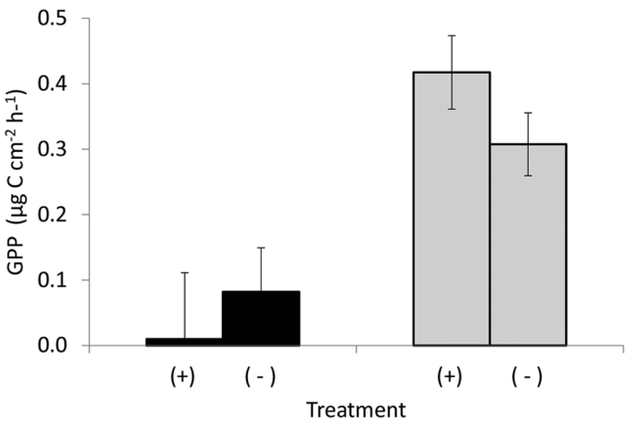

Figure 6. Mean (±SE) gross primary production in terms of carbon fixation on tiles incubated with (+) and without (−) nutrients in the shaded (black) and semi-open (gray) areas of the stream.

neuria predator did not reduce herbivory. Most likely, the sand-covered *Helicopsyche* case is an effective protection against predation by *Anacroneuria*. In this study area, therefore, the most abundant predator does not appear to control grazing by the most abundant grazer by either direct consumption or reducing grazer activity (e.g. McIntosh et al. 2006, Álvarez and Peckarsky 2014). The abundance of other, more vulnerable, herbivores such as Baetidae (low in the study system) might be controlled by predators. However, this hypothesis remains to be tested. Finally, fish, which may be abundant in other low-order Atlantic Forest streams, may consume *Helicopsyche* and trigger trophic cascades down to the periphyton (Moulton et al. 2010).

Periphyton community structure

Diatoms are the dominant periphytic taxa in shaded Atlantic Forest streams (Felisberto and Rodrigues 2010, Lobo

Table 2. Mean (±SE) numbers (cells) of algal taxa and their relative abundances (%) on tiles incubated in semi-open and shaded areas in the Mata Pau stream.

	cells / cm^2		%	
Taxa	semi-open	shaded	semi-open	shaded
Aulacoseira granulata (Ehrenberg) Simonsen	0.10 ± 0.33	0.37 ± 0.84	0.1	0.4
Cyclotela meneghiniana Kützing	0.02 ± 0.10	0.05 ± 0.15	<0.1%	0.1
Diatoma mesodon (Ehrenberg) Kützing	0.71 ± 1.22	0.67 ± 1.17	0.5	0.8
Dolichospermum sp.	18.96 ± 31.84	2.02 ± 3.45	12.2	2.4
Eucyonema sp.	0.06 ± 0.17	0.12 ± 0.39	0.0	0.1
Eunotia bilunaris (Ehrenberg) Schaarschmidt	30.40 ± 28.30	11.35 ± 9.90	19.6	13.4
Eunotia minor (Kützing) Grunow	17.80 ± 16.40	10.68 ± 11.94	11.5	12.6
Eunotia flexuosa (Brébisson ex Kützing) Kützing	0.10 ± 0.22	0.35 ± 1.33	0.1	0.4
Fragilaria intermedia (Grunow) Grunow	0.31 ± 0.77	0.16 ± 0.78	0.2	0.2
Fragilaria capucina Desmazières	26.47 ± 40.81	26.67 ± 36.54	17.0	31.5
Fragilaria ulna (Nitzsch) Lange-Bertalot	6.28 ± 7.03	1.69 ± 11.49	4.0	2.0
Gomphonema acuminatum Ehrenberg	3.16 ± 5.04	0.46 ± 0.88	2.0	0.5
Gomphonema apiculatum Ehrenberg	4.28 ± 5.87	3.06 ± 4.66	2.8	3.6
Gomphonema gracile Ehrenberg	8.54 ± 17.06	2.69 ± 4.34	5.5	3.2
Gomphonema intricatum Kützing	10.32 ± 14.66	5.84 ± 5.59	6.6	6.9
Gyrosigmail sp.	1.35 ± 2.57	0.97 ± 2.56	0.9	1.2
Navicula accomoda Hustedt	20.25 ± 15.91	8.02 ± 9.06	13.0	9.5
Navicula lanceolata Ehrenberg	3.41 ± 6.17	1.99 ± 3.09	2.2	2.4
Navicula tridentula Krasske	0.90 ± 1.81	1.90 ± 3.35	0.6	2.2
Navicula sp4.	0.14 ± 0.45	0.19 ± 10.43	0.1	0.2
Nitzschia sp.	0.02 ± 0.10	0.09 ± 0.35	<0.1%	0.1
Pinnularia lanceolata Heiden	0.31 ± 0.83	0.21 ± 0.78	0.2	0.2
pinnularia sp2.	0.06 ± 0.20	2.87 ± 7.55	<0.1%	3.4
Pinnularia viridis (Nitzsch) Ehrenberg	0.75 ± 2.57	1.65 ± 2.08	0.5	1.9
Placoneis clementis (Grunow) E.J.Cox	0.12 ± 0.52	0.46 ± 1.56	0.1	0.5
Surirella sp.	0.10 ± 0.36	0.00 ± 0.00	0.1	<0.1%
Ulothrix sp.	0.31 ± 1.18	0.09 ± 0.44	0.2	0.1

et al. 2010). As in other stream studies, in our study grazing resulted in fewer diatoms and more cyanobacteria (e.g. Rosemond 1993, Rosemond et al. 1993), likely because cyanobacteria are less palatable or have fewer nutrients for grazers compared with diatoms (Martin-Creuzburg et al. 2008). Cyanobacteria lack highly unsaturated fatty acids like 20:5ω3, which are fundamental for grazer development (Müller-Navarra et al. 2000, Crenier et al. 2017). Regardless of the mechanism, we found that grazing was an important factor that structured algal communities.

Conclusion

Our findings from the Uaimii subsection of the Atlantic Forest indicate that light also may be a major factor limiting primary producers in other remains of the Brazilian Atlantic Forests. In particular, we showed that small changes in canopy cover may have a profound influence on in-stream energy sources and algal assemblages. The Brazilian Atlantic Forests have been so highly fragmented that ½ of the remaining Atlantic forest is <100 m from an edge (Ribeiro et al. 2009). This fragmentation results in increased light penetration to streams and in higher periphyton levels now than in the past. Our estimates were that a 58% increase in light resulted in a ~4× increase in gross primary production and ~2.7× increase in algal biomass. If our estimates can be extrapolated to other areas of the Atlantic Forest, fragmentation may thus be enriching streams with higher-quality resources (algae vs leaf litter), leading to changes in stream structure (invertebrate and algal assemblages) and function including decreased heterotrophy and increased autotrophy.

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Author contributions: MASG and MC designed the study and supervised the experiments; MASG, MC, JELB, KRF, JF, and JFGJ performed the experiments; JF analyzed water samples; KRF identified the invertebrates; JELB identified the algae; MASG analyzed data and organized the writing of the manuscript. All authors contributed to writing the manuscript.

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