

LETTER

The root of the problem: Direct influence of riparian vegetation on estimation of stream ecosystem metabolic rates

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Scientific Significance Statement

Whole-system estimates of ecosystem respiration and gross primary production of aquatic systems are generally thought to reflect biological processes occurring in the water. However, living roots of plants growing next to the system might alter metabolism, and little is known of how important they are. We show that respiration by roots of riparian vegetation can substantially influence dissolved oxygen dynamics in a small stream, and do so with a diurnal pattern that mimics those caused by in-stream photosynthesis. We show that the intimate relationship between riparian vegetation and streams goes beyond leaf inputs, shading, and leaching of dissolved materials from terrestrial to aquatic habitats.

Abstract

Abundant living roots can be found in some streams and other shallow marine and freshwater habitats. A reach of a small Brazilian forested stream had 28% cover by live roots and exhibited diurnal trends in dissolved oxygen that could be attributed to gross primary production, but we hypothesized that activity of riparian tree roots in the channel caused this pattern. During sunny periods, trees transpire deoxygenated water from roots to the canopy but not in the dark, resulting in diurnal cycles of dissolved oxygen. Whole-stream shading experiments showed that photosynthesis in the stream is not responsible for the pattern. Sealed chamber measurements showed living roots of riparian vegetation had substantial respiratory activity and ammonium and nitrate uptake, and rates per unit area were greater than sand and less than silt (the other two dominant substrata), indicating roots can substantially alter in-stream biogeochemistry.

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Data Availability Statement: Data are available through the Konza Prairie LTER portal at <http://www.konza.ksu.edu/knz/datasets/TextFiles/BrazilRoot/>.

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Whole-system metabolism (ecosystem respiration, ER, and gross primary production, GPP) is a central aquatic ecosystem function and is often used as an index of stream ecosystem health (Fellows et al. 2006; Correa-González et al. 2014) and the trophic state (Dodds 2006, 2007). Metabolism is affected by natural features of the aquatic and terrestrial ecosystems (e.g., biofilms, light availability, canopy cover and rainfall) as well as by the impacts from anthropogenic activities (Wang et al. 2003; Frankforter et al. 2010). However, we know of little work on an additional biological component in some streams, living tree roots in the open channel.

Streams and other aquatic habitats (e.g., lake shores, estuarine environments) can have exposed living roots associated with nearby vegetation. These roots and their associated biofilms might be an important component of benthic metabolism but their importance to whole systems is not well

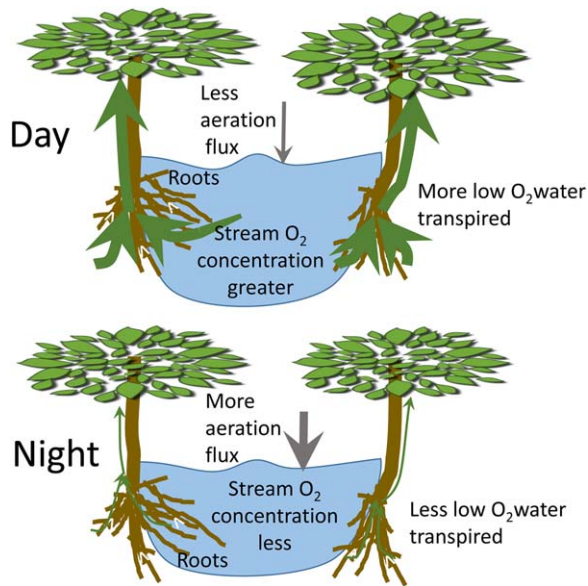


Fig. 1. Conceptual diagram of how diurnal changes in riparian vegetative evapotranspiration and water transport could influence dissolved oxygen concentrations in streams.

known. We found a diurnal swing in O_2 in a small tropical stream that could be GPP-related. However, little light reached the stream bottom and there was a very high mass of live tree roots lining the edges of the channel and streaming into the main flow. We suspected the roots might be involved in the diurnal O_2 swings. Evapotranspiration of plants is greater during the day than during the night, moving more water from the roots into the leaves during the day (Lambers et al. 1998). Thus, we hypothesized stream O_2 was related to evapotranspiration through roots (Fig. 1), and that tree roots were significant players in biogeochemical processing.

In the day, evapotranspiration rates increase, moving water from the roots into the aboveground plant tissues and at night evapotranspiration decreases and there should be less water movement. Roots continuously respire and consume O_2 . During the day the O_2 -depleted intercellular water in the roots tends to move up into the plant, lessening apparent O_2 demand in the stream. At night, all the O_2 demand needs to be met from O_2 diffusing into the stream from the atmosphere. Additionally, these roots could be very important to whole-stream metabolism and biogeochemical cycling. We tested these hypotheses by: (1) measuring light and dark metabolism of the roots and other substrata from two streams in sealed recirculating chambers to rule out photosynthesis, (2) we covered 100 m of a stream in dark plastic for 24 h to more convincingly rule out photosynthesis using whole-stream measurements, and (3) we tested N uptake rates of substrata in chambers using ^{15}N -ammonium or ^{15}N -nitrate to assess activity of tree roots and other substrata commonly observed in a stream.

Methods

We used two sites located in relatively pristine watersheds dominated by Cerrado vegetation and dense and diverse tropical forest streamside vegetation in São Paulo State, Brazil. Point transects within the studied reaches ($n = 30$) indicated sand, silt, and roots were the dominant cover types in these streams. The primary site is Espraiado Stream (S 21° 58.825', W 47° 52.42'). This narrow and deep stream (average depth 24 cm, average width 67 cm) had a discharge of 14 L s^{-1} during our experiments. We made secondary measurements of root metabolism at a similar stream, Broa (S 22° 11.569', W 47° 53.879') about 20 km from the Espraiado Stream. This stream had a discharge of 35 L s^{-1} , with channel average depth and average width of 28 cm and 84 cm, respectively.

Metabolism and N isotope uptake measurements were made in recirculating chambers (Rüegg et al. 2015). The root measurements were made with a modified top to the chambers and other substrata with the original chamber top. For roots, we added an acrylic stand tube about 8 cm diameter and 5 cm high just upstream of the working area of the chamber (Fig. 2). Living roots were sealed into the chamber from the atmosphere using a latex glove with some fingers chopped out and rubber bands to tighten the glove to the roots and the wrist of the glove put around the stand tube.

Silt and sand were taken with minimally disturbed cores of 160 cm^2 , to a depth of 1 cm and placed gently in trays in the chambers. Roots were left attached to the plants. Two incubations at Espraiado were made with a common streamside understory riparian plant (the nonnative *Hedychium coronarium*) that could easily be separated from the bank with the root systems intact. The remaining tree roots (species could not be determined given the forest diversity and intermingling of roots) were measured in submerged chambers.

Once substrata were sealed in the chambers, they were darkened and O_2 was logged every 30 s with an YSI ProODO O_2 meter (Yellow Springs Instruments, Yellow Springs, Ohio) with an optical sensor (± 0.1 mg L^{-1} accuracy). After at least a 0.03 mg O_2 L^{-1} decrease or 20 min, dark plastic was removed and chambers were incubated an equal amount of time in the light. All measurements were made during the daytime. The probe reads to 0.01 mg O_2 L^{-1} and a drop of only about 0.03–0.05 mg O_2 L^{-1} allows a reliable linear estimate of metabolic activity in many substrata.

Following incubations, substrata were removed and returned to the laboratory. Sand and silt samples were shaken with water and poured off to leave behind the large inorganic particles. The samples were then suspended and subsampled for dry mass, ash-free dry mass (Eaton and Franston 2005), and N isotope composition. Roots were dried and weighed.

Nitrogen uptake rates of individual substrata were measured in light incubations. We used stock solutions of ^{15}N

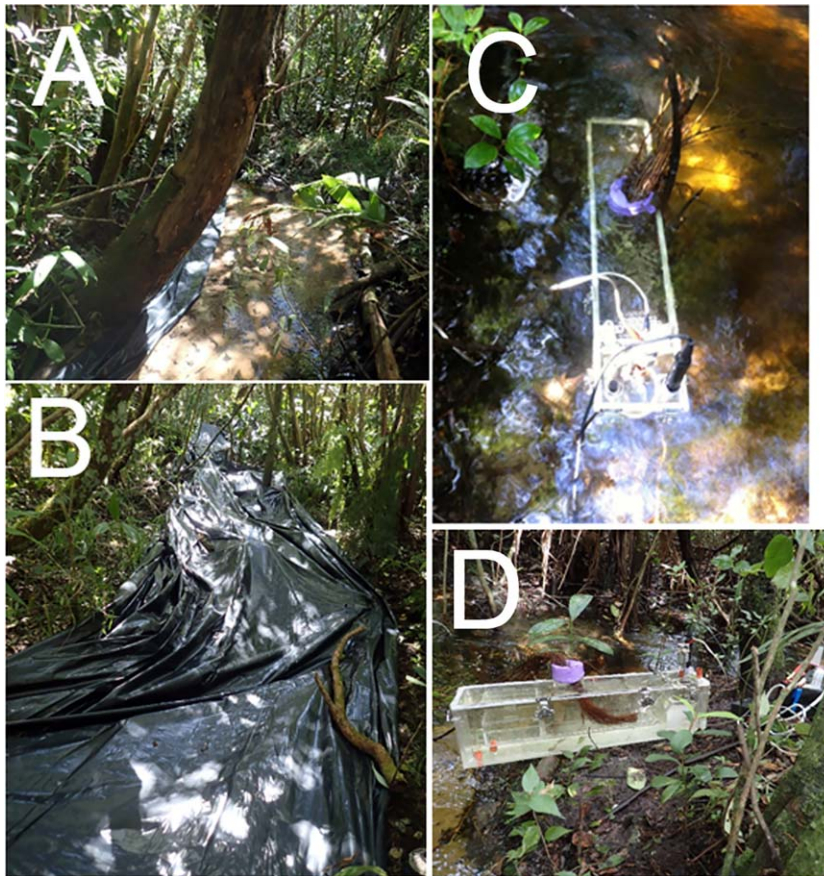


Fig. 2. Stream before plastic (A), after covering by plastic (B), chamber in stream with roots (C), and chamber outside of stream with roots still attached and going into the stream at the left behind the chamber (D).

NaNO_3 (98+% ^{15}N Isotec Company) of 108.6 mg L^{-1} of 0.1 N HCl (to preserve solutions in the field) and ^{15}N NH_4Cl (98+% ^{15}N , Aldrich) of 21.6 mg L^{-1} of 0.1 N HCL. We added $50 \mu\text{L}$ stock ^{15}N ammonium or nitrate stock solution per chamber with a 1.2- to 1.5-fold increase in ^{15}N for nitrate and an 11- to 40-fold increase for ammonium. The acid addition was too small to substantially alter the pH of the $\sim 10 \text{ L}$ chambers. We sampled water chemistry before and after each incubation; samples were filtered in the field (Whatman GF/F) into acid-washed bottles, returned to the laboratory and frozen for analyses of nitrate (Eaton and Franson 2005) and ammonium (Mackereth et al. 1978). Incubations were terminated by gently rinsing substrata in label-free water in the field and filtering (sand and silt samples, see above) or drying (roots) immediately upon return to the laboratory. Additional samples were also collected for natural abundance background levels. Dried samples were ground and filters were analyzed directly for ^{15}N content and % N at the SIMSL facility at Kansas State University.

The flux of ^{15}N per unit area was calculated from the change in ^{15}N content of substrata over time and the mass

of N per unit area. The total uptake of N was then scaled by the ratio of the $^{15}\text{N}/^{14}\text{N}$ in the chamber water. This ratio was calculated from the mass of ^{15}N added, the measured ammonium or nitrate concentration in the chamber, and the volume of the chamber. Equations for these calculations were taken from Dodds et al. (2000).

We covered a reach of 100 m of stream with black plastic (> 99.9% absorption) to darken the channel and directly establish that in-stream GPP was not responsible for the observed diurnal O_2 trends. Following 2 weeks of sunny and dry conditions, we monitored the uncovered stream for 24 h (starting 23 April 2016), placed 100 m of plastic covering over the stream for the next 24 h, and then removed the plastic for the last day. Plastic was not touching the water surface (Fig. 2), to avoid altering aeration rates. We monitored light above and at the bottom of the stream, water O_2 and temperature at the upstream end and at the bottom of the plastic-covered reach (two MiniDO₂T above and one Onset logger and one MiniDO₂T below), and depth (Hobo depth logger). Atmospheric pressure at surface was taken from a nearby monitoring station to correct depth readings.

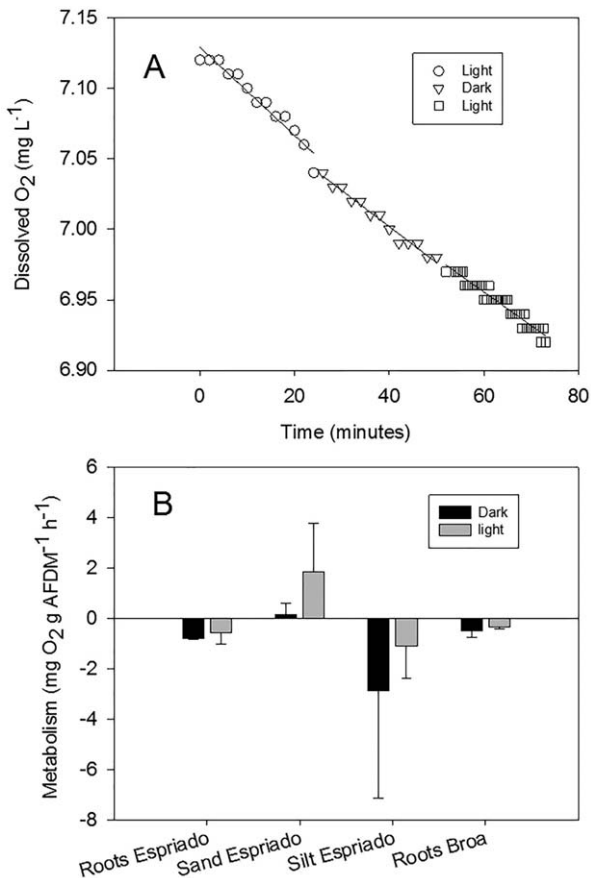


Fig. 3. Dissolved oxygen trace from a single root experiment alternating from light to dark and back to light from Broa stream, with regression slopes not significantly different (**A**) and metabolic rates of stream compartments from Espraiado and Broa normalized to ash free dry mass, in the light and in the dark (**B**). Error bars are one standard deviation.

Probes were calibrated against air-saturated water before and after deployment to account for drift during the 3-day deployment. We estimated canopy cover with a spherical densiometer (Jennings et al. 1999).

We measured aeration and discharge in Espraiado for modeling of metabolism and estimation of the “footprint” of each O₂ probe (Hall et al. 2016). Aeration was measured by releasing a solution of 238 g NaCl in 1 L of H₂O with 60 mL of SF₆ that had been equilibrated overnight, at 22 mL min⁻¹ with a metering pump (FMI, New York). Conductivity was monitored at the bottom of the reach until plateau was reached. Gas was sampled along the reach and analyzed by gas chromatography (Hall and Tank 2005). Stream widths were taken at 20 transects across the reach. Discharge was measured with a pulsed addition of NaCl (750 g NaCl in 5 L H₂O) (Kilpatrick and Cobb 1985) metered with a logging conductivity meter at the bottom station (Hanna Instruments, Limena, Italy). Metabolism in the experimental reach

was modeled for the day before, the day of, and the day after the plastic was placed on the stream with a nonlinear parameter estimation program (Riley and Dodds 2012).

Analysis of variance (ANOVA) was used to test for differences in metabolism and ¹⁵N enrichment in Espraiado chamber experiments (Statistica 10.0, Statsoft, Tulsa, Oklahoma)

Results

Ambient light in the stream or streamside had little influence on metabolic rates (GPP or ER) of roots or other compartments in the stream as measured in recirculating chambers (Fig. 3). Canopy cover at Espraiado was 66% and at Broa was 72%. A light probe placed in the bottom of the Espraiado channel had about 6% of the light on average (when the stream was not covered with plastic) as compared to a probe above the water but below the canopy (Fig. 4). A single chamber run in the Broa stream that started in the light, was covered, and then reopened had no difference in regression slopes of O₂ vs. time ($p > 0.05$) suggesting no effect of light on net production and therefore no measurable GPP (Fig. 3A). Similarly, ash free dry mass-specific rates of ER (Fig. 3B) were not significantly influenced by light. Two-way ANOVA on Espraiado rates indicated that substrata had a significant effect on rates ($p = 0.023$) but light did not ($p = 0.139$).

In Espraiado Stream, transects put sand (31%), roots (28%), and silt (21%) as dominant cover types. However, the mass of organic material was dominated by silt followed by roots (Table 1).

When we scaled rates of respiration to the entire ecosystem, they were dominated by silt followed by roots (Table 1). This is because more organic material was associated with fine sediment and the rate of respiration per mass of dry sediment was greatest (Fig. 3). Still, respiration rates associated with roots and their attached biofilms were measurable and made up a substantial portion of respiration by the dominant substrata.

For the ¹⁵N-ammonium and nitrate additions two-way ANOVA indicated that the amount of label differed significantly by substratum ($p = 0.040$) and treatment ($p = 0.042$), with a marginal interaction between substrata and treatment ($p = 0.054$). Uptake rates of nitrate and ammonium by organic material in sand did not differ significantly from zero (Table 1). Roots were more active in nitrate uptake than silt or sand, but uptake rates of ammonium were greater for silt than roots.

The darkening of the stream channel did not influence the daily oscillation of O₂ in Espraiado Stream (Fig. 4) and reduced light in the stream channel below detectable limits. While O₂ was always below saturation, there were consistent increases in O₂ during lighted periods. The peak O₂ concentration preceded the peak light, which preceded peak temperature.

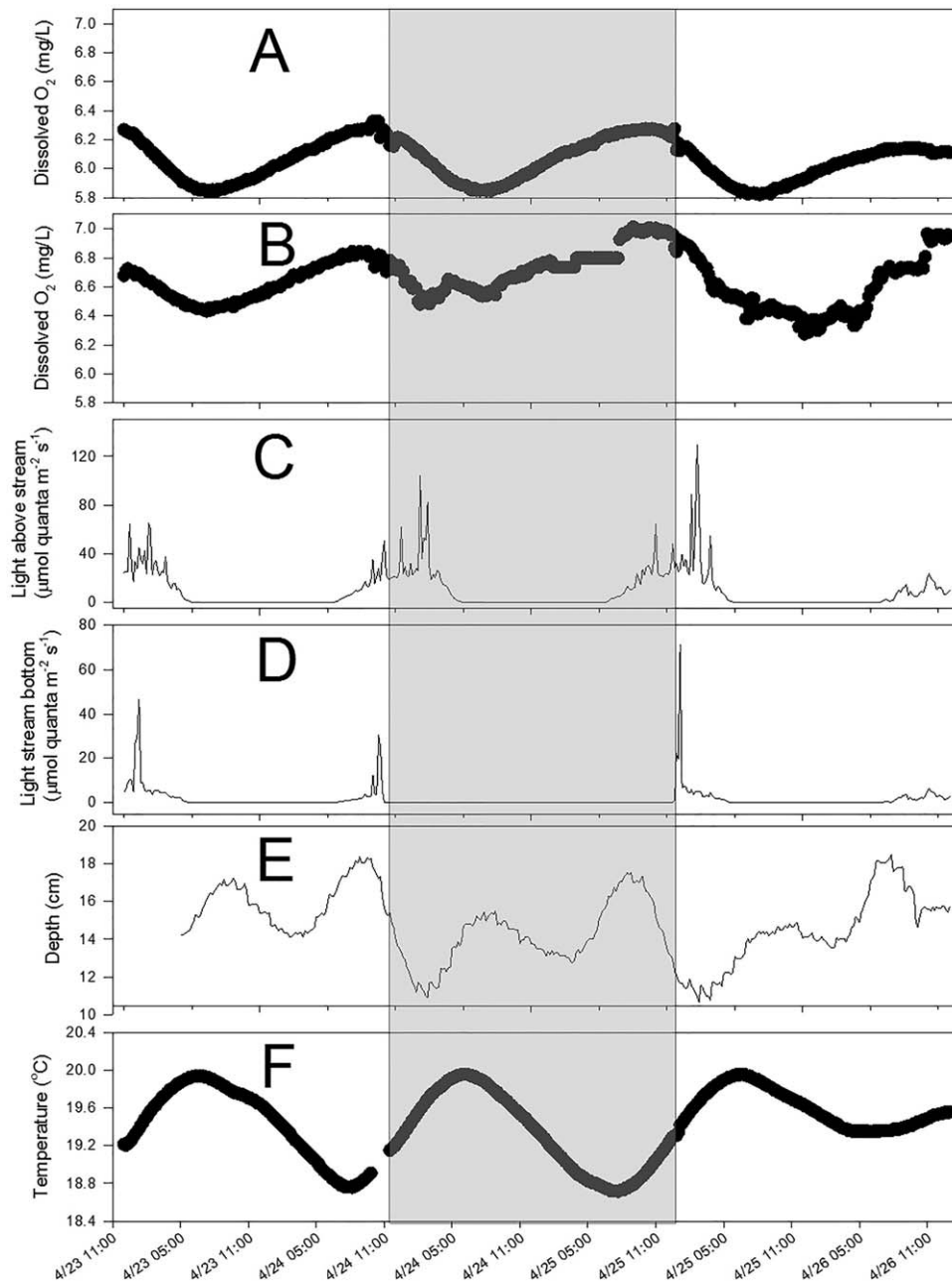


Fig. 4. Environmental conditions in Espraiado Stream before, during and after 24 h of stream being covered with black plastic. The time period with the plastic cover is indicated by the gray shading. **(A)** O₂ at bottom of treatment reach, **(B)** O₂ at top of treatment reach, **(C)** light above stream, **(D)** light at bottom of stream (note expanded scale relative to **C**, **E**) stream depth, and **(F)** stream temperature.

The ~ 7% difference in concentrations at peak O₂ and minimum O₂ during the shaded portion of the experiment suggests roughly 7% of the discharge would need to be transpired (assuming anoxic water is removed through the roots) to account for the concentration difference. The maximum diurnal effect on depth was a 42% decrease, meaning the water loss from the channel could more than account for the concentration difference. The differences between the

upstream and the downstream probe during the shaded portion of the experiment were 0.4 and 0.7 mg L⁻¹ O₂ during day and night, respectively. The diurnal difference of 0.3 mg L⁻¹ O₂ that developed over the 100 m stretch of stream each day is greater than the accuracy of the O₂ probes suggesting the shaded region was long enough for our experiment.

The first 2 days of the experiment were done under clear skies. The third day was partly cloudy with somewhat lower

Table 1. Mass of material per unit area and rates of respiration, nitrate and uptake per unit mass and per unit stream area, and incubation nitrogen concentrations for dominant compartments (by area) in the Espraiado Stream. Where negative values for uptake are reported, they are essentially below detection as our method did not allow determination if mineralization exceeded uptake, likewise with a positive value for respiration.

| Measurement | Units | Roots | | Silt | | Sand | |
|---|--|-------|------|-------|------|-------|------|
| | | Mean | SD | Mean | SD | Mean | SD |
| Total mass | g m^{-2} | 103 | 57 | 119 | 106 | 47 | 20 |
| Ash free dry mass | g m^{-2} | 68 | 37 | 44 | 39 | 17 | 7 |
| Respiration | $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ | -1.25 | 0.05 | -2.51 | 1.80 | 0.03 | 0.18 |
| NO_3^- -N conc. | $\mu\text{g L}^{-1}$ | 38.1 | 18.4 | -1.6 | 2.6 | -0.2 | 0.1 |
| NO_3^- -N areal uptake | $\text{mg m}^{-2} \text{ h}^{-1}$ | 38 | 18 | -236 | 382 | -97 | 79 |
| NO_3^- -N mass-specific uptake | $\mu\text{g g}^{-1} \text{ min}^{-1}$ | 6.2 | 3.0 | -33.1 | 53.5 | -34.4 | 27.9 |
| NH_4^+ -N conc. | $\mu\text{g L}^{-1}$ | 184 | 92 | 1970 | 1818 | -536 | 379 |
| NH_4^+ -N areal uptake | $\text{mg m}^{-2} \text{ h}^{-1}$ | 30 | 15 | 276 | 255 | -190 | 134 |
| NH_4^+ -N mass-specific uptake | $\mu\text{g g}^{-1} \text{ min}^{-1}$ | 38 | 18 | -236 | 382 | -97 | 79 |

SD, standard deviation.

light at the stream surface (Fig. 4) and had a lower O_2 peak. This is consistent with the fact that diurnal oscillation in O_2 is not evident under completely cloudy and rainy conditions at other times of year (data not shown).

The plastic did not influence temperature or oscillations of depth in any obvious fashion. The difference in temperature from upstream and downstream did not change with or without plastic on the stream (data not shown).

Estimated GPP rates (as modeled by Riley and Dodds 2012) using the 2-station method across the experimental reach were $0.43 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, $0.72 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, and $0 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ on days 1–3, respectively. The highest GPP estimate was on the day the plastic was covering the reach and the lowest on the cloudiest day where evapotranspiration would be expected to be lowest. Therefore, the model incorrectly indicated measurable GPP in a completely dark reach. The rates of ER for these 3 days were $15.7 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, $17.3 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, and $15.3 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ on days 1–3, respectively, exceeding the weighted and scaled up sum of the rates from chambers by about fivefold.

Discussion

Our data show that living riparian roots that are in the stream channel can have a substantial impact on N uptake and O_2 dynamics in a small tropical stream. They were the second most important site of respiration and N uptake. We know of no other published measurements of metabolism or N uptake of roots and their associated biofilms while they are still attached and in the stream. We assume this lack of measurement is because of the technical difficulties that we solved with our modified chamber design. While tree roots in lotic environments can be important animal habitat and

alter hydrology (e.g., Fritz et al. 2004), their biogeochemical role is less well defined.

There are several published examples of N uptake by roots from streams, or the nearby hyporheic. For example, natural abundance isotopic data shows that marine-derived nitrogen from anadromous salmon enters riparian vegetation (e.g., Mathewson et al. 2003). Also, a stream ^{15}N addition to a desert stream indicated that N moved from the stream into riparian vegetation (Schade et al. 2005). In-stream measurements of N uptake into different stream compartments based on ^{15}N addition experiments (e.g., Dodds et al. 2000) have not assessed the importance of living tree roots to N uptake to our knowledge. Our experiments suggest that roots can be an important N sink in stream nutrient budgets. We do not know if biofilms on the roots were responsible for the uptake, or the roots themselves. Macrophytes in European streams can form important substrates for biofilms that dominate N uptake (Levi et al. 2015), and biofilms on roots could be important as well.

Ammonium uptake rates were greater than nitrate uptake rates per unit biomass for roots and silt. Nitrate uptake can provide the bulk of the uptake in temperate forest trees (Nadelhoffer et al. 1984), but this might not be true for roots in our stream. If the nutrient taken up by the roots enters the trees, it might not be directly available for other stream compartments (but we did not separately estimate uptake by epiphytic biofilms). Tree leaves can fall back into the stream channel, and tree roots can be broken down by the microbial community in tropical and subtropical streams (Bloomfield et al. 1993; Fritz et al. 2006) and presumably enter the stream food web, so all the N entering the roots might not be lost to aboveground vegetation.

We find no other explanation for diurnal changes in stream flow and O_2 concentrations other than O_2 -depleted

water from within and around the roots moving up into the riparian vegetation. The main water sources for plant transpiration and for streamflow have been assessed for several ecosystems (e.g., Penna et al. 2013). Evapotranspiration by streamside trees can significantly influence discharge of streams, as mediated by groundwater and stream water depletion (Constantz et al. 1994) although riparian vegetation does necessarily use water from the stream (Dawson and Ehleringer 1991). Curiously, we observed two peaks and troughs of water depth per day and cannot explain this pattern, but the data indicate that diurnal cycles do drive changes in discharge. Tree roots can also facilitate O₂ transport to sediments through the vascular system of the plant and even pressurized transport can occur through the plant stem's intercellular vascular system (Grosse et al. 1992), but we suspect these processes are too slow to explain the observed O₂ patterns.

Chamber and shading experiments clearly ruled out photosynthesis in the stream channel as the causative factor of O₂ increases during the day. Only the sand exhibited positive (but nonsignificant) O₂ net production (Fig. 3). Given the low light in the stream channel, and that stream shading did not alter O₂ trends, or 2-station estimates of GPP, primary producers in the stream almost certainly did not cause the observed O₂ patterns.

Measured aeration rates can be used to estimate zone of upstream influence (Hall et al. 2016). Using this approach, the 80% O₂ turnover distance was 1.2 km, indicating that the diurnal trends in O₂ probably occurred over relatively long pieces of stream channel. However, O₂ dynamics can be influenced by processes relatively close to the point of measurement (e.g., Dodds et al. 2013). If measured water velocity characterizes upstream velocities, the expected travel time for this distance is 4.7 h. This distance would lead to substantial observed lags in the response of photosynthesis to light and tend to push the observed O₂ peak later than solar noon if in-stream photosynthesis caused the peak.

Our data suggest that increased evapotranspiration by the trees during sunny days move more O₂-poor water from the roots up into the plant than during the dark, leading to an observed increase in O₂ concentration in the stream channel. Temperature increases should decrease O₂ as saturation concentration decreases and respiration rates increase. We observed the opposite trend.

Some of the strongest evidence for our evapotranspiration hypothesis comes from the observation that the O₂ peak preceded peak sunlight, not the expected delayed peak that occurs after solar noon (Chapra and Di Toro 1991). The earlier peak is consistent with an explanation linked to plant evapotranspiration. The seasonally dry tropical tree *Simarouba glauca* had leaf hydraulic conductance that peaked mid-morning but was depressed mid-day to conserve water (Brodribb and Holbrook 2004). While we do not have data on

tree species in the Cerrado area where our streams are located, our results are consistent with this general explanation. Saturation of photosynthesis is not a likely cause of the peak in O₂ preceding the peak in sunlight as the onset of saturation of photosynthesis for periphyton in streams is generally at substantially greater irradiances (if it occurs at all) than observed in the stream channel (Dodds et al. 1999).

Our results open the broader question of how important are roots in other aquatic habitats? Buried root activity is probably mostly ascribed to sediment respiration, and diffusive processes should average out the effect over time leading to less diurnal O₂ fluctuation. Exposed roots tend to occur where water movement precludes sedimentation, although some cases of exposed roots (e.g., floating vegetation islands or vines from trees hanging over still water) may occur in more quiescent waters. However, shallow waters in streams, wetlands, and perhaps estuarine areas can all have exposed roots. When living roots are in the water we expect the strongest effects occur when water depth is relatively shallow, high aeration does not flatten the diurnal curve (e.g., high energy streams), and the relative areal cover of living roots is fairly high.

In conclusion, tree roots can play a large role in biogeochemical processing in streams, overriding the temperature influence on O₂ solubility and respiration rates. Nighttime rates of ER should most strongly reflect total respiration occurring in the stream channel, as little water is moving into the trees in the dark. This particular stream had a very high biomass of active roots in the stream channel, so probably represents an extreme in the continuum. We know of no other measures of activities of roots and their associated biofilms in streams while they are still attached to living plants and they can be important in stream ecosystem function.

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