



## THE INFLUENCE OF LAND COVER ON PERIPHYTON COMMUNITIES IN STREAMS IN NORTHERN GUATEMALA

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### ABSTRACT

Oil palm cultivation and the conversion of tropical forests to pastures are impacting freshwater tropical systems. This study examines periphyton biomass, richness, diversity, and community composition in streams affected by forests, pastures, and oil palm plantations, with and without forest buffer strips. Streams shaded by forests or riparian buffers exhibited more canopy cover, lower water temperatures, and reduced light, while those in pastures and unbuffered plantations had higher periphyton biomass, indicated by elevated chlorophyll-a levels. Periphyton richness and diversity were higher in pasture streams compared to forested ones and streams through oil palm areas. Common periphyton taxa differed among stream depending of land cover. Streams in buffered and unbuffered palm plantations featured taxa such as *Navicula* and *Gyrosigma*, whereas pasture streams commonly included were characterized by genera such as *Cymbella*, and *Gonatozygon*, and forest stream communities featured *Phormidium* and *Eunotia*. Pasture streams displayed altered taxa richness and diversity compared to the other land uses. There were no significant differences in periphyton communities between the two oil palm cultivation types, indicating that conservation buffers may not effectively protect periphyton communities in these settings. This research highlights the need for further studies on the impacts of agricultural practices on aquatic primary producers.

**Keywords:** benthic algae, land cover change, pasture, palm oil.

### RESUMEN

**La influencia de la cobertura del suelo en las comunidades de periphyton en arroyos del norte de**

**Guatemala.** La conversión de bosques tropicales por palma aceitera y potreros impactan en las comunidades acuáticas. Estudiamos los cambios en la riqueza, diversidad y comunidades de perifiton en arroyos de bosques, potreros y plantaciones de palma aceitera, con y sin franjas ribereñas. Los arroyos en bosques o plantaciones con franjas ribereñas mostraron mayor cobertura de dosel, bajas temperaturas de agua y menor entrada de luz que los arroyos en potreros y plantaciones sin franjas ribereñas. Estos últimos presentaron mayor biomasa de perifiton, evidenciada por altos niveles de clorofila-a. La riqueza de taxa y diversidad fueron significativamente mayores en arroyos de potreros en comparación con los de bosque y palma aceitera. En los arroyos de plantaciones de palma predominaban taxones como *Navicula* y *Gyrosigma*. En los de potreros *Cymbella* y *Gonatozygon*, y en los de bosque, *Phormidium* y *Eunotia*. No se encontraron diferencias significativas en las comunidades de perifiton entre los dos tipos de cultivo de palma aceitera, lo que indica que las franjas de conservación pueden no proteger eficazmente a las comunidades de perifiton en estos entornos. Destacamos la necesidad de realizar más estudios sobre los impactos de las prácticas agrícolas en los productores primarios acuáticos.

**Palabras clave:** algas bentónicas, cambio de cobertura terrestre, pastos, aceite de palma

### INTRODUCTION

Tropical forests provide essential ecosystem services and are home to more than half of the species on earth (Myers, 1988). Despite their importance, tropical forests have been rapidly declining due to deforestation caused by agricultural expansion (Giam, 2017; Laurance et al., 2014) and pasture lands



(Kaimowitz, 1996). Oil palm cultivation (*Elaeis guineensis* Jacq.) is a primary driver of deforestation in the tropics and is the fastest-expanding crop in the world (Davis et al., 2020; Vijay et al., 2016). In Latin America, pastureland has also significantly replaced tropical forests, making it one of the most prominent changes in land cover (Graesser et al., 2015; Wassenaar et al., 2007). Pasture and palm cultivation homogenizes habitats, driving the loss of biodiversity (Meijaard et al., 2018; Reiners et al., 1994) with negative impacts documented for insects (Fitzherbert et al., 2008; Kruess & Tschardt, 2002).

Oil palm cultivation and pastures can also significantly impact aquatic ecosystem function and aquatic biodiversity particularly through the modification of riparian vegetation (Rojas-Castillo et al., 2024a). Riparian vegetation plays a crucial role as a buffer to streams subject to land cover conversion, mitigating the adverse effects of changes in the surrounding environment on aquatic ecosystems (Naiman & Décamps, 1997; Reichenberger et al., 2007). Land conversion in the riparian zone can affect in-stream sedimentation rates (Koren & Klein, 2000), water quality parameters such as dissolved oxygen, temperature, and nutrients (Chellaiah & Yule, 2018), and the availability of allochthonous food sources and microhabitats in streams (Gonçalves et al., 2014). When riparian buffers are completely removed, intense light can penetrate streams (Osborne & Kovacic, 1993), and surface runoff can enter streams carrying high concentrations of nutrients (Kennedy, 1984; Kuriata-Potasznik et al., 2020). Research has shown that the conversion of land to oil palm cultivation can significantly alter macroinvertebrate (Luiza-Andrade et al., 2017), and fish communities (Chua et al., 2020). Conversion to pasture can influence leaf litter decomposition (Lemes da Silva et al., 2020) and ambient nutrient concentrations (Neill et al., 2001). Relatively little work has focused on photosynthetic microorganisms such as periphyton in oil palm plantation streams (Rojas-Castillo et al., 2024a). Though some work has considered the impact of conversion to pasture on periphyton communities, there is still much to be learned (Tromboni et al., 2019).

Periphyton communities are essential components of stream ecosystems, supporting food webs (Wu, 2017) and biogeochemical cycling (Hagerthey et al., 2011). Periphyton communities, found abundantly in various stream ecosystems, exhibit remarkable diversity and demonstrate swift adaptability to shifts in water quality (Li et al., 2010). They respond to changes induced by shifts in land cover and environmental conditions, manifesting alterations in both their structural composition and functional dynamics. For instance, streams draining pasture, or agriculture (e.g., coffee plantations), have shown greater taxon richness and diversity compared to forested streams (Vázquez et al., 2011). Additionally, increases in algal biomass

are common when land conversion opens the canopy, allowing more light, and potentially more nutrients, to enter streams (Quinn et al., 1997; Von Schiller et al., 2007; Tromboni et al., 2019). The traits of community members can also shift in response to land conversion, moving towards communities dominated by tolerant taxa (Bere & Tundisi, 2011; Mangadze et al., 2015; Tromboni et al., 2019). Collectively, the patterns suggest that periphyton communities may be a valuable, but underutilized bioindicator for monitoring changes in streams impacted oil palm plantations, providing deeper insights into the agricultural impacts on aquatic ecosystems.

Our study was conducted in Guatemala, the sixth major oil palm producer in the world (IndexMundi, 2024), which has also experienced a significant increase in land allocated to pasture in recent years (Carr, 2004). Conversion to pasture land and oil palm cultivation are interconnected, as the expansion of oil palm plantations in the country has primarily occurred at the expense of pastures, in addition to land conversion from other crops and forests (Furumo & Aide, 2017). To evaluate the effects of land-cover change and the expansion of the oil palm monocrops on periphyton communities in Guatemalan streams, we conducted a comparison of periphyton communities in streams draining pastures, forests, and two types of oil palm plantations -those that implement riparian buffers as a mitigation strategy and those that do not. We aim to answer (i) how does land use relate to stream algae biomass (measured as benthic chlorophyll-*a* concentrations)? and, (ii) what are the impacts of land-use change on periphyton richness, evenness, and community composition? We expect that streams with high canopy cover (forest and oil palm with buffer strips) would have reduced algae biomass, species richness and diversity compared to open canopy streams (pasture and unbuffered oil palm streams) due to light limitation and lower temperatures. Additionally, we expected that the community composition in open canopy streams would be characterized by taxa associated with greater light input, temperature, nutrients, and turbidity compared to the closed canopy streams, as riparian vegetation is also expected to reduce nutrient and sediment runoff.

## METHODS

### Study site and experimental design

We worked in the Lachuá Ecoregion of northern Guatemala, which is a low-lying, karst-dominated landscape. The average temperature is 25.3°C, and annual precipitation in the region is greater than 2500 mm with two seasons predominating: dry (February to May) and rainy (June to October) (CONAP, 2003). The ecoregion comprises the Laguna Lachuá National Park

(LLNP), declared a RAMSAR site of international importance for wetland conservation (RAMSAR, 2004). It is also considered one of Guatemala's last remnants of tropical rainforests composed of dense vegetation of at least 76 plant families (CONAP, 2003). Surrounding the LLNP, land cover is mixed. Approximately 55% of the forest cover has been replaced by pastures, human settlements, roads, and annual crops (Quezada et al., 2014). Agriculture practices consist of subsistence crop production, such as corn, beans, and chili, and also included larger plantations of cardamom, coffee, cocoa, rubber, and oil palm (MAGA, 2012; Quezada et al., 2014). Oil palm cultivation in the Lachuá Ecoregion began in 2006 (MAGA, 2012), and often involved deforestation or the replacement of previously intervened lands, primarily pastures (Furumo & Aide, 2017), and currently northern Guatemala has the largest area of oil palm plantations in the country (GREPALMA, 2019). The study was conducted during the rainy season between July and August of 2021 when nutrient and sediment runoff was expected to be greater. We collected samples in 19, first and second-order streams draining tropical forest (FO;  $n = 7$ ), pasture (PA;  $n = 6$ ), and oil palm plantations with riparian buffers (OPB;  $n = 3$ ) and without riparian buffers (OP;  $n = 3$ ) (Fig. 1).

#### Catchment and stream characteristics

We measured land cover, water temperature and light input, and water quality variables in all study streams. To estimate land cover, we employed the stream catchment areas previously delimited by Rojas-Castillo et al., (2023). The percentage of each land cover (i.e., tropical forest, palm oil, pasture, secondary vegetation or roads) was estimated using Google Earth 2021 satellite images (Google Earth engine, 2021) by manually delimiting polygons and then transforming into shape files in QGIS (QGIS Development Team, 2019) to calculate the area ( $m^2$ ) of each type of land cover. The estimation of the canopy cover density was obtained from Rojas-Castillo et al. (2023).

Water temperature and light input were measured using HOBO® Pendant MX Temp/Light data loggers in each stream. The loggers were programmed to measure temperature ( $^{\circ}C$ ) and light (lux) every 30 minutes over a month. Physico-chemical parameters were measured once during the sampling period, i.e., one deployment of the probes and one water sample that was collected during the study. Variables measured included dissolved oxygen (DO mg/L), conductivity ( $\mu S/cm$ ), turbidity (NTU), and pH, that were measured using a multiparametric probe (Model 6000; YSI, Yellow Springs, OH, USA), pH-meter (ecoTestr pH2) and turbidimeter (Eutech-100). We also collected and analyzed samples for Biochemical Oxygen Demand (DBO; mg/L),  $NO_3$  (mg/L),  $NH_4$  (mg/L), inorganic nitrogen,  $SiO_2$  (mg/L), and  $PO_4$  (mg/L) using a single, 2-liter water sample from each stream using

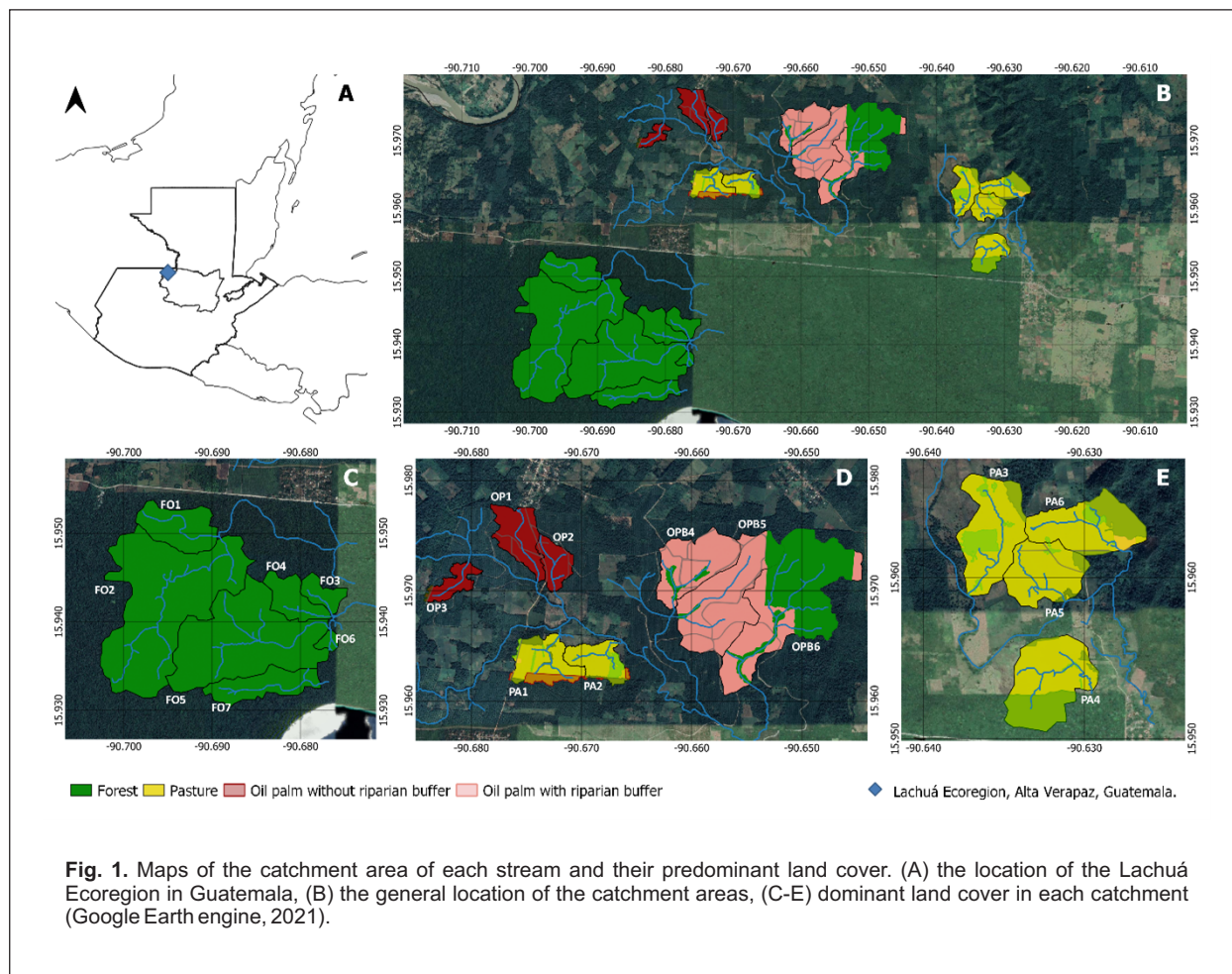
acid-washed plastic jars. Samples were refrigerated until analysis in the Analytical Solutions laboratory Chemical ( $SiO_2$  only) and the Environmental Research Laboratory (LIQA; the rest of the analytes).

#### Periphyton biomass: benthic chlorophyll-a concentrations

The concentration of benthic chlorophyll-a ( $mg/m^2$ ), a proxy for algal biomass, was measured on stones and in sediment following Jacobsen et al. (2016). Replicate samples were collected at four points in the streams, at least 20 m apart. The total number of replicates collected was: FO = 28; PA = 24; OP = 12; OPB = 12. Each replicate consisted of the collection of 3 stones and 3 core sediments. Stones were collected manually, and cores were obtained from the superficial layer of sediments using a 55-cc syringe. The samples were placed in bottles with 96% ethanol (Pápista & Böddi, 2002) and stored in the dark in a refrigerator for 48 hours until the chlorophyll was extracted. To complete the extraction, the ethanol from samples was filtered by gravity with Whatman® glass microfiber filters, grade GF/F, 0.7  $\mu m$  pore. The solvent obtained was stored in aluminum-lined bottles under refrigeration for 24 hours. The solvent was processed in HACH DR 6000 spectrophotometer at 665 nm and 750 nm. Once we obtained the values from the spectrophotometer, we calculated the surface area of the sediments and rocks. The sediment's surface area was equivalent to the diameter of the syringe (seven  $cm^2$ ), and the rock surface area was obtained from the formula of Jacobsen et al. (2016) that includes the measurement of the length, width and height of the stones. The benthic chlorophyll-a concentration was quantified spectrophotometrically by the method described by Søndergaard & Riemann (1979) using the specific coefficient of absorption of chlorophyll in ethanol (Jacobsen et al., 2016).

#### Periphyton communities

To evaluate periphyton community composition, we collected periphyton from three locations (0m, 50m, and 100m) in each stream reach (Rojas et al., 2022). The total number of replicates collected from each habitat type was: FO = 21; PA = 18; OP = 9; OPB = 9. Substrates were not distributed evenly among streams, so in pasture and oil palm streams, we collected on rocks, when possible, but we also collected samples from wood when rocks were not available. To delineate the sample area on the hard surfaces, we used a four-centimeter diameter PVC ring, and gently scraped from the surface using a brush. We washed the scraped material with stream water and fixed in 4% lugol (Stevenson & Bahls, 1999). Samples were stored in dark bottles until they were analyzed in the laboratory. The initial sample volume of 100 mL underwent 24-hour sedimentation, followed by volume reduction to 50 mL through vacuum filtration.



Subsequently, it underwent an additional 24-hour sedimentation, resulting in a further reduction in volume to 18 mL (Bellinger & Sigee, 2010). We conducted the volume reduction process at room temperature, on a horizontal surface and out of direct sunlight in order to be able to identify and quantify organisms in a smaller volume (20 mL) than the initial one (100 mL) (Karlson et al., 2010).

We identified the soft algae and diatoms in wet mounts to the genus or morphospecies level using a Labomed Lx400 Phase Contrast Microscope. The same taxonomic resolution was used to estimate taxa richness and the community composition in each sample by counting the first 200 organisms in each sample (Stevenson & Smol, 2015). We used keys of E. Bellinger & Sigee (2015); Bicudo & Menezes (2006); Cox (1996); Prescott (1978); Wehr & Sheath (2003). After completing the identification process, we preserved the samples in 2 mL of 10% formalin to ensure they remain intact for an extended period (Karlson et al. 2010). An adaptation of the Arriola et al. (2015) and Biggs & Kilroy (2000) formulas were used to obtain the relative density estimate for each sample (formula 1).

#### Formula 1

$$\text{Organisms density } cm^{-1} = \frac{\text{Counted organisms} \times \mathbf{Volf}}{(\mathbf{Voli} \times \mathbf{Volc}) / (\mathbf{Diameter})}$$

Where,

**Counted organisms:** total number of organisms counted in each sample.

**Volf:** concentrated volume (18 mL).

**Voli:** collected volume (100 mL).

**Volc:** volume counted to reach 200 organisms.

**Diameter:** Sampling diameter, according to PVC ring (4 cm x 5 substrates = 20 cm).

#### Statistical analysis

To assess whether differences in water chemistry could be documented among land cover types, we used PERMANOVA, followed by Pairwise-Permanova when needed (Table 1) (Gentleman et al., 2008). Using the raw data from the periphyton counts, we calculated the observed and expected species richness (S), and Simpson index (D) with the iNEXT package in R using the ChaoRichness, and ChaoSimpson with 95% confidence intervals (Hsieh et al., 2016). For obtaining Pielou Evenness (J) we used the formula  $J = \text{Shannon-Weiner} / \log(\text{Richness})$  (Alatalo, 1981). For our

**Table 1.** Catchment and canopy cover showing mean and standard deviation in parenthesis of each land use (FO = Forest, OPB = Oil Palm with riparian buffer, PA = Pasture, OP = Oil Palm).

Land cover	Land cover					Canopy cover (%)
	Forest (%)	Pasture (%)	Oil palm (%)	Secondary vegetation (%)	Roads (%)	Rojas-Castillo et al., (2023)
FO	100 (0)	0 (0)	0 (0)	0 (0)	0 (0)	83 (2.5)
OPB	0 (0)	0 (0)	71 (30.3)	27 (32.1)	2 (2.1)	81 (0.9)
PA	5 (12.9)	72 (28.9)	3 (6.3)	18 (12.9)	0.33 (0.5)	14 (17.5)
OP	0 (0)	0 (0)	96 (1)	1 (2.3)	2 (1.5)	37 (12.3)

subsequent analyses, we used the Expected Richness and Expected Simpson values derived from the iNEXT package, as these indices (S and D) tend to be sensitive to undetected presence of rare species and the impact of sampling effort (Colwell et al., 2012). To estimate the effect of land use on S, D, J, and chlorophyll-a concentration, we conducted analysis of variance (ANOVAs) on linear mixed-effect models, with stream as a random effect (Bates et al., 2015). In cases when land cover significantly impacted a response variable, we also ran a post hoc Tukey-Kramer test to evaluate differences among treatments (Herberich et al. (2010); Code in supplementary material as 01Biodiversity and 03Chlorophyll).

We ran a non-metric multidimensional scaling (NMDS) with the Bray-Curtis Index using the periphyton density to evaluate differences in community structure for each stream (Code in supplementary material as: 04Community). To test the relative influence of the environmental variables on periphyton communities, we used the function *envfit* from the *vegan* package (Gentleman et al., 2008). We then completed a PERMANOVA and a pairwise PERMANOVA to compare differences in the observed group in the NMDS (Anderson, 2017). To identify the representative taxa from each of the groups identified through NMDS, we performed an indicator species analysis using the *multipatt* function from the *indicspecies* R package (De Cáceres & Legendre, 2009). Since buffered and unbuffered streams in oil palm were not statistically different (PairwisePermanova;  $r^2$  0.237,  $p = 0.400$ ), we used only three groups in the indicator species analysis: forest, pasture, and oil palm.

## RESULTS

### Land cover and physicochemical parameters

The study catchments had at least 70% of their area covered by the designated land cover, i.e., forest, oil palm plantation or pasture (Table 1). All forest catchments on Laguna Lachuá National Park were characterized by 100% forest cover. In contrast, pasture catchments had more variability, but pasture

cover in all watersheds exceeded 72%. The catchments dominated by oil palm (OP1-OP3) were covered by 96% palm cover, while the other palm sites (OP4-OP6) had 71% palm cover and 21% secondary vegetation.

Of the parameters measured, light, temperature, dissolved oxygen, and turbidity were significantly different among land cover treatments. Light input was significantly higher in pasture and unbuffered oil palm (PERMANOVA  $p = 0.001$ ). In pasture streams, the temperature was almost 4°C higher (PERMANOVA  $p = 0.001$ ) and dissolved oxygen was almost 3 mg/L lower compared to streams in all other land cover (PERMANOVA  $p = 0.002$ ). Palm oil streams with and without a buffer strip had the highest turbidity compared to forest and pasture streams (PERMANOVA  $p = 0.001$ ). Relative to pasture and forest streams, oil palm streams with no buffer had two times and four times water turbidity, and oil palms streams with buffers had three times and seven times greater turbidity, respectively (Table 2). Phosphorus ( $PO_4$ ) concentrations were below detection in all study streams and nitrogen forms ( $NO_3$  PERMANOVA  $p = 0.157$ ;  $NH_4$  PERMANOVA  $p = 0.156$ ; and inorganic N (PERMANOVA  $p = 0.163$ ) and  $SiO_2$  (PERMANOVA  $p = 0.233$ ) did not show a statistical difference.

### Benthic chlorophyll-a

Chlorophyll-a in sediment samples ( $mg/m^2$ ) was significantly impacted by land cover (ANOVA;  $F = 5.64879$ ,  $p = 0.0085$ ; Fig 2). The greatest concentrations were in unbuffered oil palm streams (mean = 10.5,  $sd = 10.3$ ), followed by pasture (mean = 9.9,  $sd = 8.6$ ), buffered oil palm (mean = 1.3,  $sd = 0.8$ ) and forest (mean = 1.1,  $sd = 0.7$ ). Post hoc tests revealed the strongest differences in the comparison of pasture with forest and buffered oil palm streams and with unbuffered oil palm and forest (Fig. 2. Supplementary material 1). Though there was variation among treatments in benthic chlorophyll estimates collected from stones ( $mg/m^2$ ), the difference was not statistically significant (ANOVA;  $F = 3.090275$ ,  $p = 0.059$ ; Fig. 2). Despite no significant difference was shown by the test, the stones exhibited a comparable pattern to sediment samples: greatest concentrations in pasture (mean =

**Table 2.** Stream environmental characteristics, showing mean and standard deviation in parenthesis of each land use (FO = Forest, OPB = Oil Palm with riparian buffer, PA = Pasture, OP = Oil Palm). Variables with significant statistical difference (PERMANOVA) in bold and with the p value in parentheses. Post hoc test (Pairwise PERMANOVA) differences are shown with letters, e.g., A, B, C, AB. All PO<sub>4</sub> concentrations were no detectable (ND).

Land cover	Nutrients				
	SiO <sub>2</sub> (mg/L)	Inorganic Nitrogen (mg/L)	NH <sub>4</sub> (mg/L)	NO <sub>3</sub> (mg/L)	PO <sub>4</sub> (mg/L)
PERMANOVA	p = 0.199	p = 0.163	p = 0.146	p = 0.051	
FO	14.37 (5.6)	0.05 (0.04)	0.04 (0.04)	0.01 (0.0)	ND
OPB	9.23 (1.3)	0.06 (0.08)	0.06 (0.08)	0.00 (0.0)	ND
PA	13.67 (3.8)	0.08 (0.03)	0.05 (0.02)	0.03 (0.03)	ND
OP	10 (2.9)	0.09 (0.05)	0.07 (0.04)	0.03 (0.01)	ND

Land cover	Physicochemical variables					
	Light (lux)	Temperature (°C)	DO (mg/L)	Turbidity (ntu)	Conductivity	pH
PERMANOVA	p = 0.001	p = 0.001	p = 0.002	p = 0.001	p = 0.097	p = 0.137
FO	74.6 (49.8) A	24.7 (0.4) A	5.4 (1.6) A	6.7 (4.1) A	52.8 (26.1)	6.7 (0.3)
OPB	5.2 (4.8) AB	24.6 (0.5) A	5.1 (0.9) A	47.3 (27.4) B	98.6 (29.3)	6.7 (0.2)
PA	991.8 (269.3) C	28.4 (1.5) B	1.8 (0.4) B	14.1 (6.9) A	106.4 (50.5)	6.9 (0.2)
OP	778.9 (864.9) BC	24.7 (1.4) A	5.2 (1.1) A	28.7 (4.5) AB	68.1 (29.5)	6.6 (0.4)

15.9, sd = 18.6), followed by unbuffered oil palm (mean = 9.2, sd = 8.3), forest (mean = 5.9, sd = 9.1), and buffered oil palm (mean = 0.9, sd = 1.2).

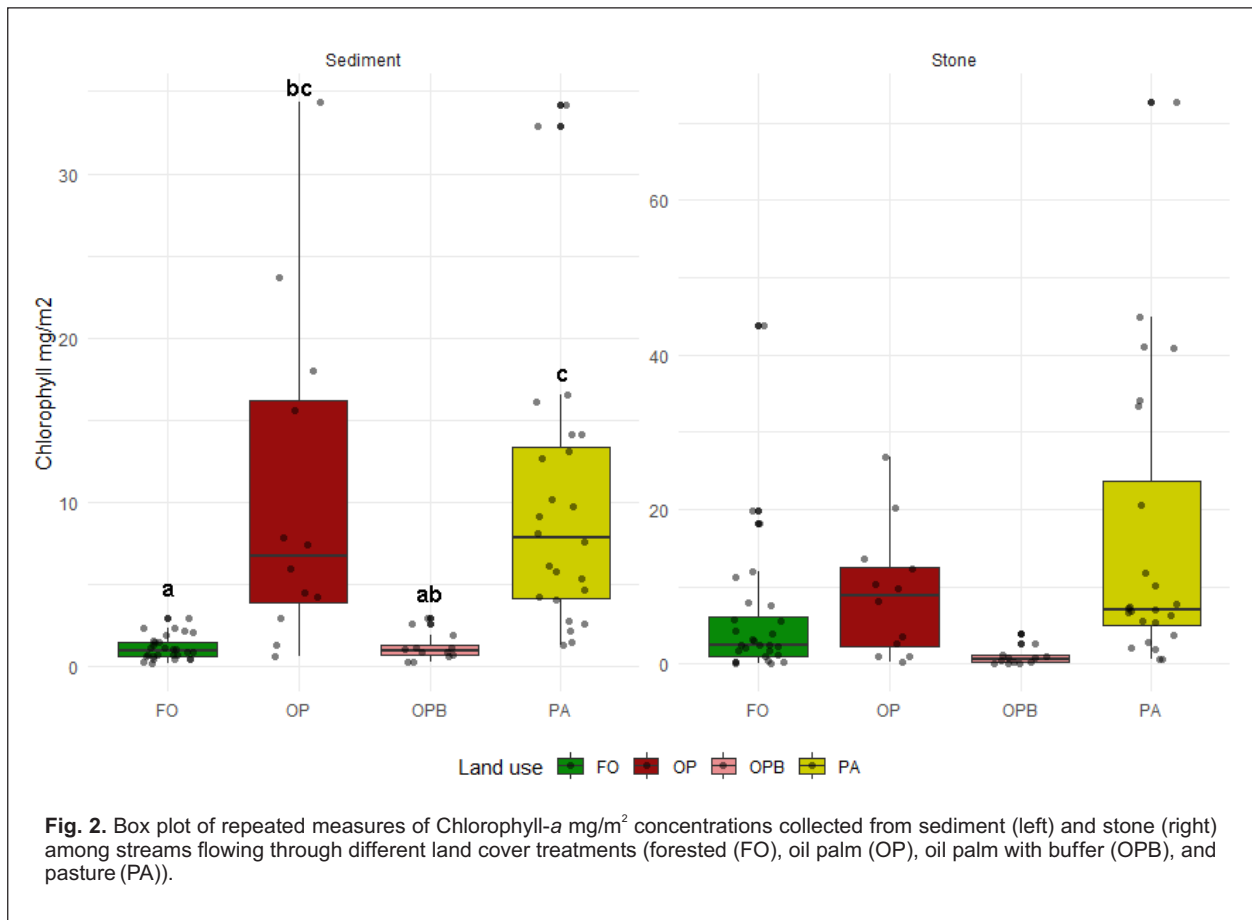
#### Periphyton diversity and community composition.

Collectively, among all of our sites, we documented a total of 69 taxa belonging to 42 genera, distributed in 5 phyla: 36% Bacillariophyta, 29% Charophyta, 17% Cyanophyta, 14% Chlorophyta, and 5% Rhodophyta (Supplementary material 2). Overall, we found greater taxa richness and diversity in pasture streams relative to the other land uses. Total taxa richness (Fig. 3) ranged from 11 to 34 and was significantly impacted by land cover (ANOVA;  $F = 5.7869$ ,  $p = 0.0078$ ). Taxa richness was higher in pasture (mean = 24.0; sd = 5.7) followed by unbuffered oil palm (mean = 19.1; sd = 6.0), buffered oil palm (mean = 17.7; sd = 4.5) and forest (mean = 17.4; sd = 3.4) streams. Post hoc Tukey test indicated that streams in pastures had the highest taxa richness compared to forest streams (Tukey,  $p < 0.001$ ) and oil palm with buffer strips (Tukey;  $p = 0.0174$ ). Richness did not vary between buffered and unbuffered oil palm (Tukey;  $p = 0.9447$ ; Supplementary material 3) and there was no significant difference between streams in either type of oil palm plantations and forest streams (Tukey for OP:  $p = 0.8551$  and OPB:  $p = 0.9990$ ; Supplementary material 3).

Diversity, measured with Simpson Index ranged from 0.507 to 0.942 and was also significantly influenced by land cover (ANOVA;  $F = 3.491$ ,  $p = 0.0423$ ; Fig. 3). The lowest diversity was found in

unbuffered (mean = 0.81; sd = 0.04) and buffered oil palm streams (mean = 0.82; sd = 0.12). The highest diversity was found in pasture (mean = 0.89; sd = 0.03) and forest streams (mean = 0.86; sd = 0.06). Post hoc Tukey test indicated that diversity in pasture streams was significantly higher compared to unbuffered oil palm streams (Tukey;  $p = 0.0317$ ). There was also a marginal relationship between pasture streams and buffered oil palm (Tukey;  $p = 0.0537$ ). Diversity did not vary between buffered and unbuffered oil palm (Tukey;  $p = 0.9983$ ) or between forested systems and either type of oil palm plantation (Tukey for OP:  $p = 0.3788$  and OPB:  $p = 0.4999$ ; Supplementary material 4). Pielou Evenness Index ranged from 0.444 to 0.942; however, it was not significantly influenced by land cover (ANOVA;  $F = 1.812$ ,  $p = 0.1883$ ).

The periphyton community composition was impacted by land cover (Permanova;  $F = 4.6856$ ,  $p = 0.001$ ). Pairwise Permanova showed that forest streams differed from pasture ( $p = 0.009$ ) and buffered and unbuffered oil palm streams ( $p = 0.006$ ). Pasture streams also differed from the buffered and unbuffered oil palm streams ( $p = 0.009$ ; Fig. 4). The nonmetric multidimensional scaling (NMDS; stress value = 0.166921) showed that the periphyton community composition in forest streams was influenced by canopy coverage ( $R^2 = 0.7518$ ,  $p = 0.001$ ) and dissolved oxygen ( $R^2 = 0.4132$ ,  $p$ -value 0.028). In the pasture streams the variables that showed a significant influence were light ( $R^2 = 0.7024$ ,  $p = 0.002$ ), water temperature ( $R^2 = 0.6119$ ,  $p = 0.004$ ), and NO<sub>3</sub> ( $R^2$



**Fig. 2.** Box plot of repeated measures of Chlorophyll-a mg/m<sup>2</sup> concentrations collected from sediment (left) and stone (right) among streams flowing through different land cover treatments (forested (FO), oil palm (OP), oil palm with buffer (OPB), and pasture (PA)).

0.4518,  $p = 0.008$ ). For the oil palm streams with and without a riparian buffer strip, turbidity ( $R^2 = 0.4687$ ,  $p = 0.012$ ) seemed to be the variable influencing the community composition (Fig. 4). The indicator species analysis showed that from the 69 taxa reported, 29 can describe communities in the different land cover (Table 3). 18 taxa were associated with pasture streams, 6 taxa were associated with forest streams, and 5 were taxa associated with oil palm streams with and without a riparian buffer strip.

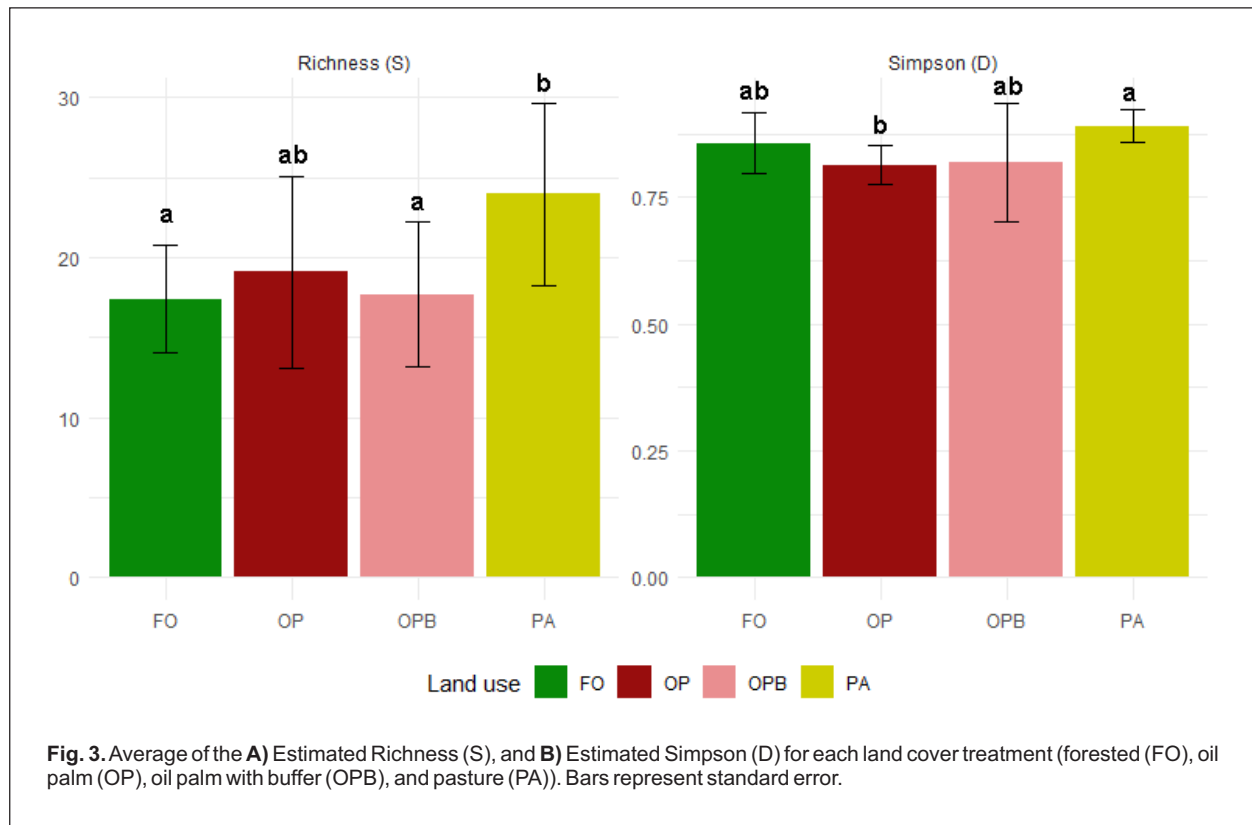
## DISCUSSION

This study demonstrated that land conversion influences algal biomass, periphyton taxa richness, and diversity compared to streams flowing through forested landscapes in Guatemalan streams. Specifically, we found significant differences in the structure and composition of periphyton in streams draining pasturelands relative to the other streams in our study. We found that benthic algal biomass was greater in landscapes dominated by that remove riparian buffers, such as pastures but also oil palm plantations without buffers. We found no significant differences in taxa richness and diversity between forested streams and those in oil palm planta-

tions. Similarly, there was no distinction in richness and diversity between palm streams with riparian buffers and those without. Yet, periphyton community composition was impacted by dominant land cover, and communities collected in streams impacted by oil palm cultivation were similar, containing more tolerant species, in buffered and unbuffered systems. Our results illustrate that land cover change can influence photosynthetic microorganisms, as previous studies show for before (Bere & Tundisi, 2011; Burgos-Caraballo et al., 2014; Quinn et al., 1997; Tromboni et al., 2019; Vázquez et al., 2011; Von Schiller et al., 2007). Notably, we anticipated differences between forest streams and oil palm plantations; however, our findings did not support this prediction. We also expected to find a difference between periphyton in oil palm with and without a buffer strip because of differences in riparian shading, contribution to allochthonous material inputs. However, the differences we documented were minimal.

### Benthic chlorophyll-a and periphyton richness and diversity.

Regardless of the dominant land cover, all streams in this study were classified as oligotrophic-mesotrophic based on chlorophyll concentrations



(mean 20 mg m<sup>-2</sup> and maximum limit 60 mg m<sup>-2</sup>; Dodds et al., 1998). However, pasture streams were where we observed the most significant differences in periphytic algae, including biomass, taxa richness, and diversity. This may have been attributed to increased light penetration in pasture streams, as taxon richness, diversity and benthic chlorophyll-*a* concentrations can be influenced by light (Quinn et al., 1997; Vázquez et al., 2011; Von Schiller et al., 2007; Tromboni et al., 2019). Buffer strips reduce the amount of light entering streams (Montgomery & Chazdon, 2001), consequently limiting the growth and reproduction of algae (Hill, 1996). In our systems, closed canopy streams (forest and oil palm with riparian buffer) had five times more canopy cover than pasture streams, and two times more canopy than oil palm streams without a riparian buffer (Rojas-Castillo et al., 2023).

We initially expected that streams associated with unbuffered oil palm plantations would exhibit similar conditions to those in pasture areas, as we predicted that both types of land conversion would similarly affect light penetration and nutrient inputs. However, our results did not support this prediction. To assess the shading effect of vegetation in the riparian zone, we measured the percentage of canopy cover. However, this metric did not account for the presence of shrubs or grasses along the stream edges within oil palm plantations, which can limit light entry. Similar observations have been reported in other plantation

studies (Chellaiah & Yule, 2018; Lucey et al., 2018). Additionally, the shade produced by the oil palms themselves may have influenced our results. Oil palms can grow up to 30 meters in height (Arias et al., 2009), and their canopies may have restricted the amount of light reaching the streams.

Ambient nutrient concentrations can also be impacted by land cover change within a watershed, and access to nitrogen or phosphorus can influence algal abundance (Mangadze et al., 2015; Tromboni et al., 2019) and biofilm diversity (Burgos-Caraballo et al., 2014). Nutrients can also interact with light to support greater biomass (Pacheco et al., 2022). Nutrient data in this study were exceptionally limited (i.e., one sample per stream reach); therefore, drawing conclusions about predicted changes in water chemistry and subsequent interactions with the periphyton community should be done with care. We did not document any statistically significant difference between N or P and predominant land cover in our study. However, this again could be due to extremely low P concentrations (all below the limit of detection) and the small number of samples we collected. Future work should examine interactions between nutrients and light in streams impacted by palm cultivation.

Turbidity in oil palm streams may have also influenced periphyton communities through light occlusion (Mori et al., 2018). Our data indicated that buffered and unbuffered oil palm streams had the highest water



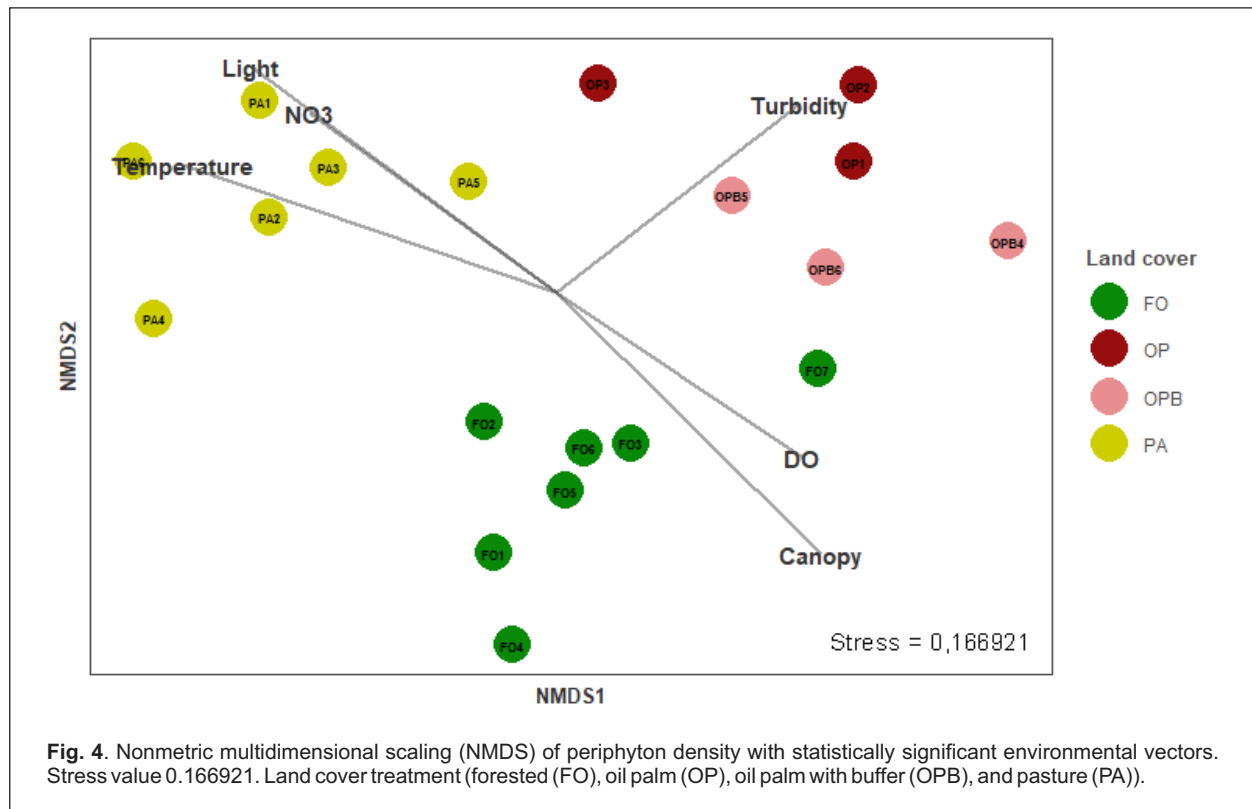
**Table 3.** Indicator taxa derived using the multipatt function in R. P-values are in parentheses.

Land cover	Indicator species	
Forest (7 taxa)	<b>Bacillariophyta</b>	<b>Cyanophyta</b>
	<i>Eunotia</i> sp 3 (0.008)	<i>Phormidium</i> sp 1 (0.009)
	<i>Frustulia</i> sp 1 (0.011)	<i>Stigonema</i> sp 1 (0.0013)
	<i>Amphipleura</i> sp 1 (0.043)	<b>Chlorophyta</b>
		Morpho genera 1 (0.025)
Pasture (16 taxa)	<b>Bacillariophyta</b>	<b>Charophyta</b>
	<i>Stauroneis</i> sp 1 (0.003)	<i>Closterium</i> sp 1 (0.001) sp 2 (0.001) sp 3 (0.043)
	<i>Gomphonema</i> sp 1 (0.002)	<i>Cosmarium</i> sp 2 (0.019) sp 3 (0.034)
	<i>Synedra</i> sp 1 (0.001)	<i>Netrium</i> sp 1 (0.004)
	<i>Cymbella</i> sp 1 (0.003)	<i>Gonatozygon</i> sp 1 (0.012) sp 2 (0.007)
	<b>Chlorophyta</b>	<i>Mougeotia</i> sp 1 (0.009)
	Morpho genera 5 (0.028) and	<i>Bulbochaete</i> sp 1 (0.041)
	Morpho genera 4 (0.048)	
Oil palm with and without buffer strip (5 taxa)	<b>Bacillariophyta</b>	
	<i>Gyrosigma</i> sp 3 (0.002)	
	<i>Navicula</i> sp 2 (0.006)	
	<i>Navicula</i> sp 1 (0.004)	
	Morpho genera 2 (0.005) and Morpho genera 3 (0.039)	

turbidity. Oil palm plantations can have greater water turbidity due to soil erosion (Comte et al., 2012; Sahat et al., 2016; Afandi et al., 2017) and this could have important effects from a button-up perspective. For instance, Chua et al., (2020) hypothesized that due to high turbidity, oil palm streams could have reduced autochthonous primary production and therefore result in changes in fish traits. This may be especially important for secondary consumers in unbuffered palm plantations because they receive less allochthonous material (Chua et al., 2020; Rojas-Castillo et al., 2023) and therefore there will be fewer food resources (Brett et al., 2017). The implementation of riparian buffers in oil palm plantations aimed to soil erosion might need to be improved in the plantation under study (Lucey et al., 2018). Streams adjacent to oil palm plantations lacking buffer strips are directly affected by agricultural activities, allowing sediments to enter the water, and consequently elevating turbidity levels (Mercer et al., 2014; Luiza-Andrade et al., 2017).

### Periphyton community composition

The periphyton communities we documented through this study grouped by the type of dominant land cover in the watershed. In forested streams, some of the taxa we documented, including diatoms such *Eunotia* sp. and *Frustulia* sp. are indicators of oligotrophic systems that are typically rich in oxygen, and poor in inorganic nitrogen inputs (Schneider et al., 2013; Van Dam et al., 1994). In Brazil, some *Eunotia* species, were reported in streams with high forest cover and low levels of BOD5 (Bere & Tundisi, 2011). Our work supports studies conducted in Mexican streams by Ramírez-Babativa & Vázquez, (2015) and Vázquez et al., (2011) who documented species of *Frustulia* in well-conserved forested streams in Mexico. In forested systems we also documented Cyanophyta taxa inhabiting forest streams, *Stigonema* sp. and *Phormidium* sp. Cyanobacteria can thrive in forest-associated streams due to the phycobilin complex capacity to utilize long wavelengths, enabling their



presence in low light conditions (Whitton, 2012; Komárek & Johansen, 2015). Members of the genus, *Stigonema* sp. have been reported in low nutrient concentration streams (Sagarra, 2017; Schneider et al., 2013). Additionally, we documented *Phormidium* that is a genus that has been widely observed in periphyton mats, forming dense masses on substrates such as rocks, plants or in sediments (Azim, 2009; Komárek & Johansen, 2015). Our results are similar to those of the species *P. autumnale* which was reported in oligotrophic streams with limited light entry (Sagarra, 2017) and low nutrients and temperature (Lindstrøm et al., 2004).

Our NMDS and subsequent analyses suggested that communities in pasture streams were affected by light, NO<sub>3</sub>, and temperature, and were characterized by 16 associated taxa. These streams had the greatest number of associated taxa, and, in previous work, most of these taxa have been associated with pollution or tolerance to high light conditions. For instance, certain genus of Zygnematophyceae, such as *Cosmarium*, *Closterium*, and *Gonatozygon*, have been associated with eutrophic waters with high benthic chlorophyll-*a* concentrations (da Silva et al., 2018) as in our study. Diatoms associated to pasture streams were *Gomphonema* sp., *Synedra* sp., and *Cymbella* sp. Many species reported in those genera are associated in streams with high organic matter concentrations (Salomoni et al., 2006; Day & Dhlomo, 2007; Daruich et

al., 2013). Some species of *Gomphonema* have been associated with pasture streams and coffee plantations (Vázquez et al., 2011). *Synedra* species were reported as primary indicators of anthropogenic eutrophication, in rivers with organic matter (Maishale & Ulavi, 2015). Regardless of the genus *Cymbella*, our results support those of Tromboni et. al., (2019) who linked the genus to high light tolerance, and those of Vázquez et al. (2011) who exclusively found *C. tumida* in pasture and coffee plantations streams.

In the streams running through palm oil plantations, indicator species, such as *Navicula* sp. and *Gyrosigma* sp. are associated with water turbidity. The genus *Navicula* includes species adapted to a variety of habitats (Van Dam et al., 1994), with some known for their tolerance to turbid conditions (Eloranta & Soininen, 2002). One species from the *Navicula* genus has been reported in urban streams with low oxygen levels and high biochemical oxygen demand (BOD<sub>5</sub>) (Bere & Tundisi, 2011). Additionally, *Navicula* have been identified as pollution-tolerant species (Salomoni et al., 2006). The genus *Gyrosigma* also contributed to the assemblages found in oil palm streams and is known for its tolerance to disturbed environments. For example, a *Gyrosigma* species was reported in agricultural streams experiencing eutrophic conditions, with elevated sediment loads and high concentrations of suspended solids and nutrients (Bona et al., 2007; Vázquez et al., 2011). Another *Gyrosigma* species has

been associated with streams containing elevated levels of total nitrogen, nitrate, and phosphorus (Mangadze et al., 2015).

Our work should be considered with the following limitations in mind. First, we were only able to include a small number of sites for comparing buffered and unbuffered oil palm. Additionally, we were limited in our capacity to collect repeated samples of physico-chemical data. Our findings may also have been influenced by the approach we used to identify algal taxa. In recent years, much of the research on photosynthetic microorganisms has employed genomic methods that permit a more robust species classification compared to traditional morphological identification (Pawlowski et al., 2022). Here, we used morphological identification similar to Bere & Tundisi (2011), Mangadze et al. (2015), Tromboni et al. (2019), and Vázquez et al. (2011). Errors in identification may come from the complexities of organism morphologies and cell structures, which can be challenging to distinguish without proper microscopes and specialized expertise in algae taxonomy (Manoylov, 2014). Another important limitation of our study was the variability in hard substrates in our research sites, particularly in pasture and oil palm streams. In many of these streams, stones were absent, requiring us to collect periphyton from woody substrates. We acknowledge that this choice could obscure the effects of land cover, as different algae species exhibit substrate-specific preferences and introduce bias into our interpretations (Allan & Castillo, 2021). Future work in oil palm plantations should employ artificial substrates, such as unglazed ceramic tiles, to standardize periphyton collection (Porter-Goff et al., 2010; Tromboni et al., 2019).

## CONCLUSION

This study aimed to elucidate the relationship between land use, benthic chlorophyll-*a* concentrations (algae biomass), as well as periphyton richness, evenness, and community composition. We found that streams with high canopy cover (forest and oil palm with buffer strip) had reduced algae biomass compared to open canopy streams (pasture and unbuffered oil palm streams). We found no substantial differences in periphyton taxa richness and diversity between forested areas, oil palm plantations, and oil palm plantations with or without buffer strips. Additionally, we found that pasture streams changed significantly periphyton taxa richness and diversity, exhibiting the highest number of associated taxa known from previous studies to be linked to pollution or tolerance to high light conditions. While we observed no substantial differences in taxa richness and diversity between forested streams and oil palm plantations, the composition of periphyton

communities was notably influenced by land cover. Specifically, streams affected by oil palm cultivation hosted more tolerant species, regardless of the presence of riparian buffers. These results underscore the complex interactions between agricultural practices and aquatic ecosystems, challenging our initial expectations regarding the differences between forested and oil palm-impacted streams. Ultimately, our study contributes to the understanding of how land use alterations affect photosynthetic microorganisms and emphasizes the need for further research into the ecological implications of oil palm cultivation and agricultural practices on freshwater biodiversity.

Biofilms, which include periphytic algae and decomposers, contribute to energy flow and biogeochemical cycling in rivers and often serve as the foundation of aquatic food webs (Brett et al., 2017; Guo et al., 2016; Hall & Meyer, 1998; March & Pringle, 2003; Marks, 2019). Our work documented that one of primary conservation measures used by plantation managers, the use of riparian buffers (Lucey et al., 2018), had limited influence on the composition of photosynthetic microorganisms, despite our small sample size. Further, research on leaf litter decomposition within oil palm plantations has yielded mixed results, with no consistent pattern emerging regarding the effectiveness of riparian zone conservation to maintain ecosystem processes (Chellaiah & Yule, 2018b; Rojas-Castillo et al., 2024b). This underscores the need for additional studies focused on both the autotrophic and decomposer components of biofilms in oil palm-affected streams. Such research would deepen our understanding of how palm plantations impact aquatic ecosystems, and could enhance conservation strategies aimed for mitigating the ecological impacts of continued palm expansion.

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