The use of epilithic diatom assemblages in assessing land use in Silago, Southern Leyte, Philippines

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ABSTRACT

Silago is a municipality in Southern Leyte in which logging for high-quality timber and land clearing for agricultural purposes threaten stream ecosystems. The objective of this study was to assess the response of diatom assemblages in relation to land use. Diatoms and water quality sampling was done at 27 sites on two sampling occasions (June and July 2014). Multiple diatom metrics were calculated to measure the response of diatoms to changes in land use. In all, 135 diatom species distributed to 48 genera were recorded. The results showed that diatom species and their attributes gave similar responses to those obtained in environmental variables. Pollution tolerance index classified all sampling sites as oligo-b-mesosaprobic. Meanwhile, *Cymbella* richness, percent motile taxa, and percent *Achnanthidium minutissimum* indicated good water quality in forested areas, distinguishing them from other land use types. Contrary to other studies, species richness was found to increase with greater degrees of disturbance, thus giving unreliable evaluation of water quality. Overall, the study suggests that epilithic diatoms can be applied in biomonitoring of freshwater bodies in the country.

KEYWORDS: *bioindicators, biomonitoring, community structures, diatom attributes, multimetric, water quality*

INTRODUCTION

The ecological integrity of running waters is influenced by the landscape through which they flow (Hynes 1975; Allan 2004; Broadbent et al. 2012; Pan et al. 2014), such that rivers or streams are degraded when natural forestlands are converted for agricultural and urbanization purposes. Over the years, land conversion has become widespread due to the increase in population and urbanization, and this resulted in sedimentation, water pollution, and most importantly, loss of biodiversity in most streams worldwide (Couceiro et al. 2007; Carlson et al. 2013; Pan et al. 2014). It has now become a persistent threat to running waters that aquatic ecologists and resource managers must address.

Land conversion alters stream morphology and water chemistry (Allan 2004; Deborde et al. 2016). One of the main causes of this is the clearing of the riparian zone along with forestland (Allan 2004). Riparian vegetation maintains the integrity of the stream by stabilizing banks, limiting erosion and sedimentation, absorbing nutrients in the water, and providing shade that decreases stream temperature (Wallace et al. 1997; Hill et al. 2004; Niyogi et al. 2007). Agricultural and urban lands, whose riparian zone was removed, thus have higher temperature, wider stream widths, and reduced flow rates due to unstable banks (Sabo et al. 2005; Couceiro et al. 2007; Deborde et al. 2016). Furthermore, deforested lands have high levels of electrical conductivity, pH, and nutrients (e.g. nitrogen and phosphorus) in streams due to increased runoffs (Couceiro et al. 2007; Girmay et al. 2009).

Alterations in hydromorphology and water chemistry affected by land conversion heavily impact aquatic communities as well. For example, general degradation in water quality influences macrophytes and fish, while organic pollution affect fish and macroinvertebrate communities (Hering et al. 2006; Mackay et al. 2010). Hydromorphological degradation changes macroinvertebrate, fish, and macrophyte assemblages (Hering et al. 2006). Meanwhile, nutrient loading from agricultural runoffs or waste water discharged from urban sewages changes macroinvertebrate, macrophyte, and

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diatom assemblages (Hering et al. 2006; Torrisi et al. 2010; Lock et al. 2011; Triest et al. 2012). As such, a number of mitigating measures and protocols were developed to evaluate the detrimental effects of land modification on stream ecology.

In many cases, physicochemical parameters together with bioindicator species are used for monitoring stream integrity (Taylor et al. 2007a; Uherek & Pinto Gouveia 2014). Most of these favored benthic macroinvertebrates as the bioindicator species because they respond to hydromorphological gradients and changes in water chemistry (Revenga et al. 2005; Hering et al. 2006; Lock et al. 2011; Deborde et al. 2016). However, other studies have shown that benthic macroinvertebrates cannot indicate pollution gradients in extremely degraded streams where dissolved oxygen is low and show less sensitivity to eutrophication, land use gradients, and organic pollution (Hering et al. 2006; Beyene et al. 2009; Torrisi et al. 2010). Meanwhile, the same studies posited that diatoms outperformed macroinvertebrates in these aspects. But despite the extensive use of diatoms in running waters worldwide, there are but few studies of diatoms as bioindicator species in the tropics (Fore & Grafe 2002; Walsh & Wepener 2009; Lobo et al. 2010; Marzin et al. 2012; Lavoie et al. 2014) including the Philippines (Magbanua et al 2017).

The municipality of Silago in Southern Leyte, Philippines is composed of forest, agricultural, and mixed forest and agricultural lands (Narisma et al. 2011). The vast forestlands in the municipality allowed for the production of high-quality timber and furniture. Because of the high demand for timber, land clearing has become rampant despite impositions of logging moratorium, and land conversion remains a major threat to rivers and streams within Silago (Mangaoang et al. 2005; Cedamon et al. 2011). Assessing the ecological status of running waters in the municipality is thus necessary in order to investigate the effects of land conversion on running waters and establish protocols for management. Recent study has shown that commonly used macroinvertebrate-based metrics and widely accepted biological scoring systems for evaluating stream condition were relatively the same across land use in Silago, Southern Leyte (Deborde et al. 2016).

Given this background, the objective of this study was to assess the response of epilithic diatom assemblages to changes in water quality associated with land use. As suggested by several papers it is expected that diatom community structures would change according to land use: more tolerant species will dominate in agricultural and mixed areas while sensitive species will be observed in forested areas, which have the least disturbance (Kutka & Richards 1996; Walsh & Wepener 2009; Urrea-Clos & Sabater 2012). The results of this study will serve as a baseline for further studies in assessing the influence of land use on aquatic community assemblages, especially in the tropics.

Materials and Methods

Study site. The study was carried out in Silago, which is a fourth-class municipality of Southern Leyte in Eastern Visayas located at 10⁰31’56” N and 125⁰9’56” E. It has a Type II climate with no distinct dry season: pronounced wet season lasts from November to February and less wet period lasts from June to October (Narisma et al. 2011).

Silago is mainly composed of forestland (58%) and agricultural land (39%), while small parts of the land are residential, commercial, or institutional areas (0.43%). The forestlands in the municipality supply timber for wood and furniture production (Mangaoang et al. 2005; Cedamon et al. 2011) and also provide hydrological services, giving Silago abundant freshwater supply (Narisma et al. 2011). Agriculture is thus one of the main sources of income, employing almost half of the working members of Silago. The main crop planted in agricultural lands is coconut, followed by rice. Other products include corn, sweet potato, cassava, taro, and assorted vegetables (Narisma et al. 2011).

Twenty-seven stream reaches were selected according to three types of land use and are grouped as agricultural (A), forested (F), and mixed agricultural and forested areas (M). They were sampled in June and July of 2014 in Silago

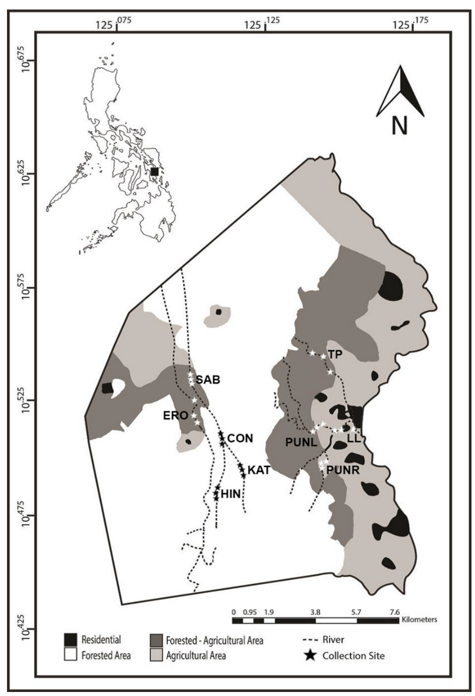

Figure 1. Map of sampling stations in Silago, Southern Leyte, Philippines. CON – Hinagimitan downstream; ERO – Pinunilan; HIN – Hinagimitan; KAT – Katigahan; LL – Maag; PUNL – Malabad; PUNR – Puntana; SAB – Sabang; TP – Tree Park.

(Figure 1).

Measurement of physicochemical parameters. The following physicochemical parameters were measured using a multi-parameter water quality meter (YSI Professional Plus; Yellow Spring Instruments, Ohio, USA): temperature (°C), conductivity (µS/cm), total dissolved solids (mg/L), salinity (ppt), dissolved oxygen (mg/L), and pH. Wetted width (m), bank full width (m), water depth (ft), and flow rate (ft/s) were also recorded.

Diatom sampling and laboratory processing. Epilithic diatoms were sampled from six cobble-sized rocks at randomly selected positions within the riffles along the stream reach. All samples were brought and processed at the Aquatic Biology Research Laboratory (ABRL) at the Institute of Biology, University of the Philippines Diliman, Quezon City. Epilithic diatoms were removed from the rocks with a toothbrush and rinsed with tap water. The composite algal samples were cleaned with hydrogen peroxide. Cleaned diatom frustules were mounted on a permanent slide. A minimum of 300 diatom frustules in each sampling site were counted at 1000x and identified to the lowest practical taxonomic level by taking series of images and measurements (frustule dimensions) necessary to distinguish the morphological features of individual diatom species. Samples were then compared based on the species description and nomenclature of Krammer & Lange-Bertalot (1997a; 1997b; 2000; 2007), Bellinger and Sigee (2010), and Diatoms of North America (https://diatoms.org). The nomenclature recorded in AlgaeBase (http://www.algaebase.org) was followed (Guiry et al. 2014).

Diatom assessment. Five commonly used metrics associated with land use patterns were computed: species and *Cymbella* C. Agardh richness, percent *Achnanthidium minutissimum* (Kützing) Czarnecki, percent motile taxa (also known as siltation index), and pollution tolerance index (PTI).

Statistical analyses. Principal Component Analysis (PCA) was used to identify the most influential environmental variables across land use type. Based on Detrended Correspondence Analysis (DCA; *DCA1 = 3.8557, DCA2 = 2.1204*), relationships between the diatom species assemblage and environmental variables across land use type were explored using Canonical Correspondence Analysis (CCA). Spearman correlation analysis was used to test relationships among diatom attributes and environmental variables. Lastly, non-metric multidimensional scaling (NMDS) through Bray-Curtis similarity matrix was used to determine the spatial patterns of diatom species assemblages across land use type and sampling time, which was confirmed by computing similarity percentages (SIMPER) and by analyses of similarity (ANOSIM) (Clarke 1993).

R Studio (version 3.2.3) was used to create PCA and CCA plots (Bellinger et al. 2006) and PRIMER 7.0 was used to run SIMPER and ANOSIM. The data was log-transformed, and SPSS Statistics 20 was used to run (1) multivariate ANOVA to test for significant difference in diversity, diatom metrics, and environmental variables across the land use types; and (2) Spearman correlation analysis.

Table 1. Mean (±standard error) values of environmental variables across land use types in Silago, Southern Leyte. Rankings for post hoc tests in cases with significant effects are given. *P*-values < 0.05 are in bold print. A – agricultural; F – forested; M – mixed.

Variable	Land use			*P*-value	Ranking
	Agricultural	Forested	Mixed		
Temperature (°C)	25.50 (0.63)	23.86 (0.09)	26.93 (0.31)	**<0.001**	F < A < M
Conductivity (µS/cm)	128.03 (17.69)	90.57 (3.32)	130.66 (2.90)	**0.010**	F < M
Total dissolved solids (mg/L)	83.08 (11.83)	61.09 (1.74)	81.73 (1.68)	**0.040**	F < M
Salinity (ppt)	7.40 (1.14)	5.25 (0.16)	7.19 (0.17)	**0.039**	F < M
Dissolved oxygen (mg/L)	5.74 (0.86)	4.97 (0.65)	7.13 (0.16)	0.161	
pH	6.69 (0.62)	7.24 (0.26)	7.11 (0.33)	0.537	
Wetted width (m)	10.32 (2.78)	4.76 (0.65)	18.63 (2.12)	**<0.001**	F < A < M
Bank full width (m)	18.59 (2.38)	11.59 (0.94)	31.25 (4.67)	**<0.001**	F < A < M
Water depth (ft)	0.18 (0.01)	0.14 (0.02)	0.17 (0.03)	0.242	
Flow rate (ft/s)	0.30 (0.03)	0.27 (0.04)	0.36 (0.05)	0.298	

RESULTS

Environmental variables. The values of the physical and chemical variables measured are shown in Table 1. Six out of the ten environmental variables differed significantly across land use types, wherein forested sites resulted in having the lowest values in all environmental variables but pH. Temperature, wetted width, and bank full width—all physical variables—significantly differentiated all three land use types. On the other hand, conductivity, total dissolved solids, and salinity—all chemical variables—distinguished only forested and mixed areas.

Only temperature, dissolved oxygen, and pH are included in the Water Quality Guidelines and General Effluent Standards of the Department of Environment and Natural Resources (DENR 2016). None of these parameters exceeded the Philippine standards.

Based on multivariate analysis, PCA Axes 1 and 2 accounted for 62.83% of the variance across sampling stations (Table 2). The highest contributing variables in the first axis were conductivity, salinity, total dissolved solids, bank full width, and flow rate (Table 3). Meanwhile, wetted width, bank full width, total dissolved solids, and salinity were the highest contributors in the second axis. The first two axes separated all sampling stations into three land use types (Figure 2). Agricultural sites showed correlation with chemical variables, while mixed sites were associated with physical properties.

Table 2. Summary of principal component analysis (PCA) of the first two ordination axes. λ = eigenvalue of each axis; S = % contribution of each axis.

Axis	λ	S
PCA 1	4.1072	41.0718
PCA 2	2.1759	21.7589

Diatom community assemblages

In all, 135 diatom taxa were observed across all sites belonging to 48 genera. Of these, only five species were considered the most abundant across all sites: *Geissleria schoenfeldii* (Hustedt) Lange-Bertalot & Metzeltin, *Epithemia adnata* (Kützing) Brébisson, *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow, *Nitzschia paleacea* (Grunow) Grunow, and *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot (Figure 3). As can be seen from Figure 3, agricultural and mixed sites shared common dominant taxa such as *C. placentula, Nitzschia palea* (Kützing) W. Smith, and *Gomphonema parvulum* (Kützing) Kützing. The diatom community at the forested sites was more structurally different, represented by *Adlafia minuscula* (Grunow) Lange-Bertalot, *Psammothidium hustedtii* (Krasske) S. Mayama, *Mayamaea fossalis* var. *obsidialis* (Hustedt) Lange-Bertalot, *Achnanthes crenulata* Grunow, and *Achnanthidium minutissimum* var. *jackii* (Rabenhorst) Lange-Bertalot. These species had much higher abundances in forested areas than

Table 3. Contributions of environmental variables for each principal component.

Variable	Principal component axis	
	PCA 1	PCA 2
Temperature	8.6771	8.1326
Conductivity	18.8979	8.4681
Total dissolved solids	17.2129	12.0228
Salinity	17.5210	11.4747
Dissolved oxygen	1.6453	5.9669
pH	1.5721	9.0879
Wetted width	7.8430	26.9219
Bank full width	13.7736	13.8867
Water depth	2.3440	3.5759
Flow rate	10.5129	0.4625

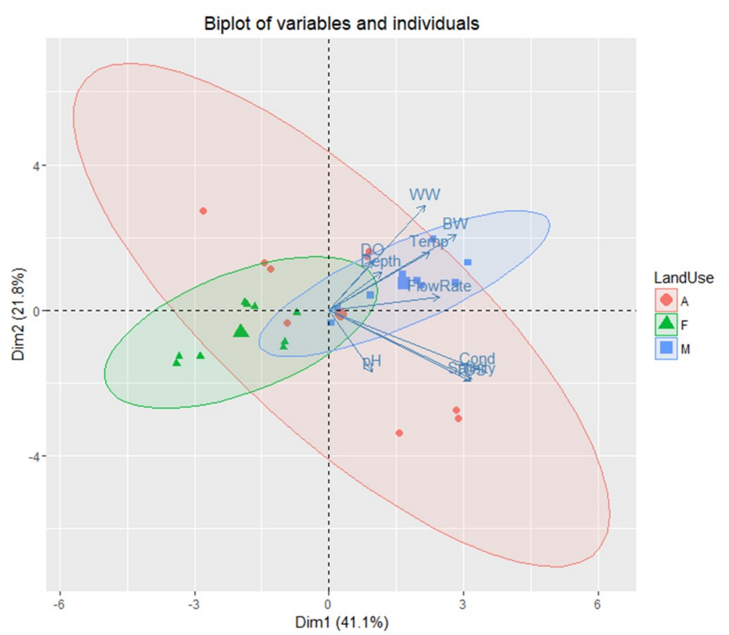

Figure 2. PCA plot of the stream reaches grouped according to land use. A – agricultural; F – forested; M – mixed.

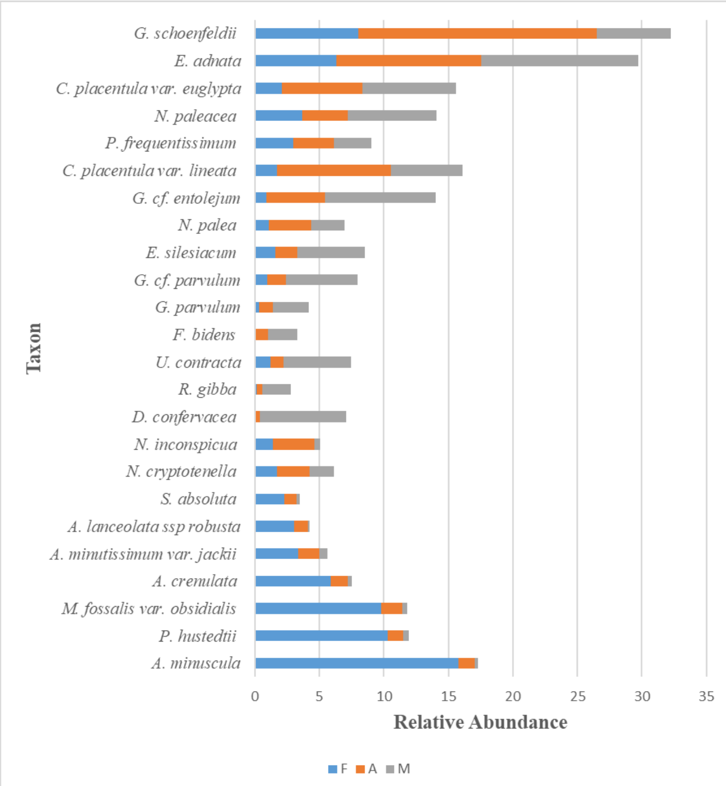

Figure 3. Proportional abundance of dominant taxa (≥100 individuals) across sites in Silago, Southern Leyte, Philippines. F – forested; A – agricultural; M – mixed

in agricultural or mixed areas.

Diatom community composition along environmental variables

The results of ordination analysis (CCA) are presented in Figure 4. The first two axes accounted for 45.45% of the variance in diatom communities due to the environmental variables (Table 4). The first two CCA axes separated all sampling stations according to land use type (Figure 4) similar to the PCA results shown earlier. Agricultural and mixed sites were clustered in the upper left quadrant, with forested sites on the lower left quadrant. Most diatom species were positively correlated to DO (Figure 4). Meanwhile, the diatom species *C. placentula*, *Encyonema silesiacum* (Bleisch) D.G.

Table 4. Summary of canonical correspondence analysis (CCA) of the first two constrained ordination axes. λ = eigenvalue of each axis; S = proportion explained by each axis.

Axis	λ	S
CCA 1	0.5726	0.2700
CCA 2	0.3911	0.1845

Mann, and *E. adnata* were positively correlated to high pH.

Spatial and seasonal pattern of epilithic diatoms

NMDS ordination showed no seasonal variation in diatom species assemblages across sampling stations (Figure 5) but

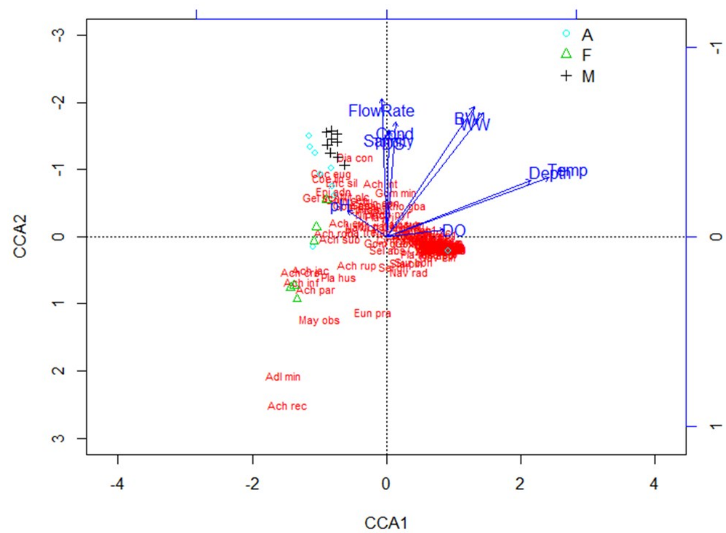

Figure 4. CCA plot showing the relationship between diatom taxa and environmental variables across land use. A – agricultural; F – forested; M – mixed

showed significant grouping according to land use type (Figure 6). Although there were many diatom species common among the three land use types, there were substantial differences between forested and mixed sites. These differences are highlighted in Table 5.

Diatom attributes

As seen in Table 6, only percent *A. minutissimum* significantly discriminated sampling stations according to land use type. Only pollution tolerance index characterized all sampling stations as oligo-b-mesosaprobic or having low to moderately nutrient-rich waters. Nonetheless, almost all diatom metrics met the predicted responses to different land

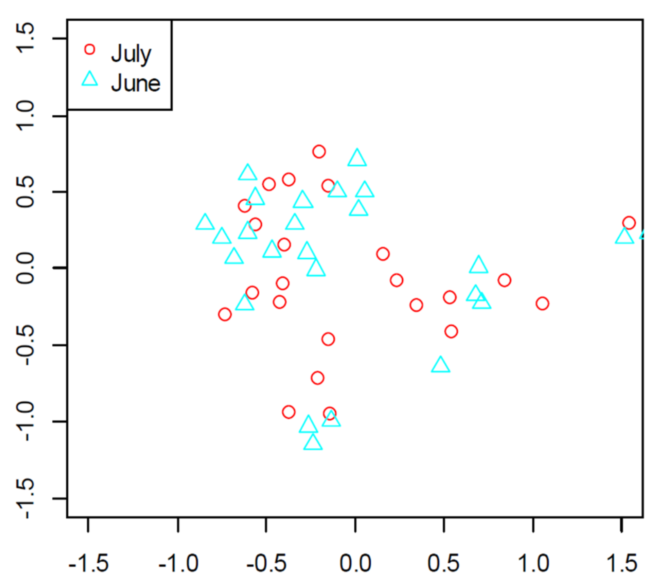

Figure 5. NMDS plot of sites grouped by sampling time through Bray-Curtis. Stress value = 0.12.

Table 5. Percentage breakdown of average dissimilarity between land use types according to the diatom assemblages using SIMPER analysis. For the presentation of the average dissimilarity (%) the following abbreviations have been used: A – agricultural; F – forested; M – mixed. Statistical and global R values for the pair-wise analysis of similarity (ANOSIM) tests are presented.

Factor	Groups	Average similarity (%)	Average dissimilarity (%)	Statistical *R*	Global *R*
Land Use	Agricultural	45.01	A/F = 63.38	*R* = 0.363, *P* = 0.001	Global *R* = 0.498, *P* = 0.001
	Forested	47.73	A/M = 55.63	*R* = 0.367, *P* = 0.001	
	Mixed	57.99	M/F = 67.07	*R* = 0.766, *P* = 0.001	

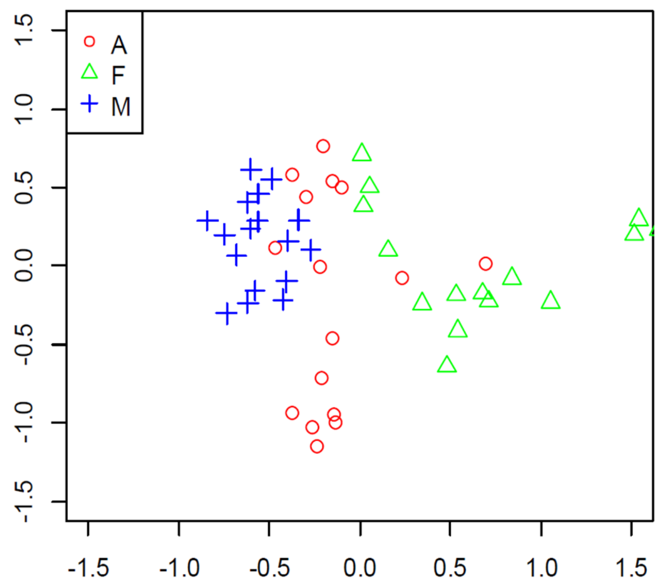

Figure 5. NMDS plot of sites grouped by sampling time through Bray-Curtis. Stress value = 0.12.

use types.

Discussion

Environmental variables

Across land use types, forested streams had the lowest values for temperature, conductivity, TDS, salinity, wetted width and bank full width. Natural riparian vegetation plays an important role in maintaining stream integrity and good water quality. The flora in the riparian area decreases solar heating, limit erosion and runoff, absorb excess nutrients in the soil, and keep stream banks intact (Wallace et al. 1997; Hill et al. 2004; Niyogi et al. 2007; Bere & Mangadze 2014). When forestlands are converted into agricultural or urban lands, riparian vegetation are cleared together with the forests, changing the hydromorphology and water chemistry of streams (Allan 2004; Couceiro et al. 2007; Girmay et al. 2009). Changes in physical and chemical properties of streams in response to changes in land use were apparent in this study: agricultural and mixed areas had higher measures in almost all variables than forested areas. This observation is expected of deforested lands and supported by other studies (Jüttner et al. 2003; Bellinger et al. 2006; Couceiro et al. 2007; Moonsin et al. 2013; Mangadze et al. 2015). It is also worthy to note that the salinity level in all sites are not typical of freshwater bodies (Schwartz 2005). This provides important insights into the effect of altered land use in the area.

Diatom community composition along environmental variables

Agricultural and mixed sites shared common dominant taxa while the diatom community at the forested sites was more structurally different. Many studies suggest that diatoms are more commonly structured by chemical than physical variables (Leland & Porter 2000; Pan et al. 2014; Mangadze

Table 6. Mean (±standard error) values of diatom metrics across land use types in Silago, Southern Leyte. Rankings for post hoc tests in cases with significant effects are given. *P*-values < 0.05 are in bold print. A – agricultural; F – forested; M – mixed.

Metric	Land Use			*P*-value	Ranking
	Agricultural	Forested	Mixed		
Species richness	31.94 (2.27)	25.22 (3.66)	33.33 (1.25)	0.150	
Cymbella richness	1.13 (0.32)	2.50 (1.57)	1.39 (0.20)	0.726	
Percent motile taxa	18.74 (2.04)	11.48 (3.15)	16.15 (2.37)	0.057	
Percent *A. minutissimum*	1.65 (0.36)	3.80 (0.87)	0.65 (0.22)	**0.001**	F > M
Pollution tolerance index	2.24 (0.03)	2.15 (0.10)	2.39 (0.03)	0.059	

et al. 2015). These support the association of diatom assemblages with the chemical variables pH and dissolved oxygen observed in this study. So far, however, indication of water quality is insufficient. Perhaps the most possible reason is that other studies often included water quality analysis based on the land use of the watershed. For instance, for agricultural, forest, and croplands, nutrient concentrations, total suspended solids, and turbidity are common discriminatory factors (Jüttner et al. 2003; Walsh & Wepener 2009; Triest et al. 2012).

Spatial pattern of epilithic diatoms

Diatom species assemblages showed significant clustering according to land use. Generally, species richness decreased in response to stress or disturbance (Hill et al. 2000a,b). However, this did not appear to be the case because species richness did not differ significantly across land use and instead altered the species composition. Although this result was not expected, this pattern was consistent with those observed in other studies (Townsend et al. 1997; Hill et al. 2003). Chessman et al. (1999) showed an increase in the diatom genera in response to land use (e.g. agricultural, cropland, urban, industrial, mining, and sewage treatment plants) in New South Wales and Victoria, Australia. Lavoie and colleagues (2008) also found strong correlation between diatom taxa and trophic status. Accordingly, as trophic status (e.g. total phosphorus) increases, so do the diversity measures. At this stage, these observed patterns can only be assumed as consequences of changes in the physical and chemical properties of the water bodies resulted from intense pressure in land use changes. Pollution-sensitive diatom species (*A. minuscula, Achnanthes crenulata* Grunow, *A. minutissimum, P. hustedtii,* and *E. silesiacum*) were much more abundant in the forested areas. Presence of these taxa indicates low conductivity, high percentage of riparian shading, and low nutrient concentrations (Van Dam et al. 1994; Toyoda et al. 2006; Yu & Lin 2009; Urrea-Clos & Sabater 2012). The relative high counts of *A. minutissimum* in forested areas, the low counts of pollution-tolerant taxon *N. palea*, and the absence of *Nitzschia nana* in the land use type confirm the observations of Lavoie et al. (2004) and Lobo et al. (2010) in reference sites.

The relative abundance of the algal taxa in the agricultural and mixed areas were structurally similar. These altered land use types were characterized by diatom taxa (*E. adnata, C. placentula, G.* cf. *entolejum, G. schoenfeldii, G. parvulum, N. palea,* and *N. paleacea*) with high affinities to moderate to high conductivity and pollution levels (Taylor et al. 2007b; Van Dam et al. 1994). Motile diatoms such as *Nitzschia* spp. and *Navicula* spp. were also greatest at these sites (Bahls 1993; Kelly et al. 2001; Niyogi et al. 2007). Predominance of *Diadesmis confervacea* Kützing in the mixed areas confirms further their water quality status, as *D. confervacea* is a cosmopolitan species that favors eutrophic waters (Taylor et al. 2007b).

Diatom attributes

A. minutissimum significantly discriminated sampling stations according to land use types and pollution tolerance index characterized all sampling stations as oligo-b-mesosaprobic or having low to moderately nutrient-rich waters. The diatom indices gave similar responses to those obtained in environmental variables and diatom assemblages. Species richness was recorded highest in the mixed and agricultural areas than the forested ones. This metric, however, failed to provide the most reliable evaluation of pollution in the sampled streams (Bellinger et al. 2006; Hill et al. 2003; Boonsoong et al. 2009; Kalyoncu & Zeybek 2011). Instead, classification based on the relative abundances of each taxa in the assemblages (e.g. taxonomic composition measures) performed better in discriminating water quality impairment (Bellinger et al. 2006; Kalyoncu et al. 2009). Therefore, in this study, as seen in the literature, diversity measures should be used together with other metrics (e.g. pollution tolerance index, percent motile taxa, and percent *A. minutissimum*) to be able to successfully identify water quality change due to land use activities. Based on pollution tolerance index, all sites (agricultural, forested, and mixed) were classified as oligo-b-mesosaprobic. This shows that all sites are already experiencing nutrient stress (oligo-mesotrophic) from land use changes. On the other hand, better results were obtained according to *Cymbella* richness, percent motile taxa, and percent *A. minutissimum*. These metrics support each other in describing the good water quality class of forested sites (Hill et al. 2001; Musico 2002a, b; Wang et al. 2005; Walsh & Wepener 2009). Several other studies have also demonstrated *Cymbella* richness as better indicator of good water quality, in support of the present study (Musico 2002a, b; Shen et al. 2018; Stevenson et al. 2010; Wang et al. 2005; Wang et al. 2014). The high mean values of percent motile taxa in the agricultural and mixed areas only indicate the presence of some degree of sedimentation and higher amounts of nutrients from erosion and possible runoffs (Jüttner et al. 1996; Bellinger et al. 2006). As described in previous studies, motile taxa represent species of *Nitzschia, Navicula, Rhopalodia*, *Sellaphora,* and *Stauroneis* that are capable of moving in unstable substrates with high resistance to low irradiance and increased concentration of suspended solids (Riato et al. 2017; Fore & Grafe 2002).

Conclusion and Recommendations

This study supports the view that diatom communities are excellent indicators of water quality impairment. Diatoms are spatially structured according to land use settings but showed weak correlation with the physical and chemical variables. As such, inclusion of environmental parameters for river health assessment must be based on the land use type of the watershed. Furthermore, integration of several diatom attributes instead of just one should be considered for better assessment. More research needs to be carried out in order to examine more closely the links between diatoms and pollution levels.

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