Denitrification within the sediments and epiphyton of tropical macrophyte stands

Denitrification within the sediments and epiphyton of tropical macrophyte stands

Adame, MF[1*], NJ Waltham[2], N Iram[1], B Shahrabi Farahani[1], C Salinas[3], M Burford[1], M Ronan[4]

[1]*Australian Rivers Institute, Griffith University, Nathan, QLD, Australia*

[2]*TropWATER (Centre for Tropical Water and Aquatic Ecosystem Research), College of Science and Engineering, James Cook University, QLD, Australia*

[3]*School of Sciences & Centre for Marine Ecosystems Research, Edith Cowan University, Joondalup Drive, Joondalup, W Australia.*

[4]*Wetlands Team, Department of the Environment and Science, Queensland Government, Brisbane, QLD, Australia*

*corresponding author: f.adame@griffith.edu.au

Abstract

Excess nitrogen (N) is one of the most widespread and serious pollutants in the environment, but wetlands can reduce N loads ameliorating its damaging effects downstream. Tropical wetlands are highly productive and experience high temperatures year-round, resulting in potentially high denitrification rates. However, few measurements of denitrification have been reported for tropical wetlands. In this study, we measured denitrification within stands of macrophytes at the edge of a tropical lake in Australia. We compared denitrification rates among sediments underneath aquatic grass (giant bulrush, *Actinoscirpus grossus*), sediments underneath waterlilies (*Nymphae* spp), and sediments without macrophytes. We also measured the denitrification and primary productivity of the epiphyton on macrophytes and compared the rates with those from the sediment. Denitrification in the sediment was higher (D_t =3.3 - 52 mg m^{-2} h^{-1}) than denitrification of the epiphyton (D_t= 1.9 -3.6 mg m^{-2} h^{-1}) and was mostly coupled with nitrification (D_n). Denitrification was highest in sediments rich in organic carbon (32.3%) and N (1.4%), and in times of the year when nitrate (NO_x^--N) concentrations were relatively high (> 0.10 mg L^{-1}). Denitrification was lowest in sediments with no macrophytes, which comprised most of the area of the lake. Denitrification rates of sediments underneath these macrophytes stands were within the highest values measured for natural wetlands and highlight the potential role of this process in ameliorating N pollution in tropical catchments.

Keywords: biofilm; nitrate; nitrogen; productivity; water quality; weeds

Introduction

Excess nitrogen (N) is one of the most widespread and severe pollutants affecting waterways. The addition of N by agricultural, urban, and industrial activities has increased the amount of reactive N globally by a factor of nine from pre-industrial times (Galloway and Cowling 2002; Kulkarni et al. 2008). The excess N is added to the soils and waterways, causing eutrophication and, ultimately, the degradation of these ecosystems (Galloway et al. 2003). Wetlands are known for effectively removing N from waterways, thus, ameliorating its damaging effects (Land et al. 2016; Adame et al. 2019b). Therefore, many restoration projects have focused on wetlands as a complementary strategy to improve water quality (Zedler 2000).

Tropical wetlands are some of the most productive ecosystems on the planet. Just one hectare of macrophytes can fix up to four kg of carbon every hour (Junk and Furch 1993; Adame et al. 2017). Their high productivity, carbon-rich soils, high temperatures, and anoxic conditions have the potential to result in high N removal (Lewis 1996). The primary process responsible for the removal of N is denitrification, which is the microbial reduction of nitrate (NO_3^-) to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) and finally to gaseous N (N_2). Denitrification is higher under macrophytes grow, as these provide carbon to the soil and create sub-surface spaces where coupled nitrification-denitrification can occur (Risgaard-Petersen and Jensen 1997). However, very few denitrification measurements have been conducted in natural tropical wetlands (Enrich-Prast et al. 2016; Adame et al. 2019a), although they account for 30% of all wetlands in the world (Mitsch et al. 2010). Thus, the potential benefit for N processing of tropical wetlands tends to be largely extrapolated from temperate locations, despite vast climatic differences (Piña-Ochoa and Álvarez-Cobelas 2006).

Denitrification can occur in the sediment-water interface or within the epiphyton, which is the bacteria and algal communities attached to the submerged portions of the macrophytes. These two microhabitats have distinctly different nitrifying-denitrifying bacterial communities (Pang et al. 2016), carbon and nutrient availability, and oxygen conditions, all of which affect denitrification (Eriksson 2001). Additionally, differences in macrophyte species and structures (e.g. floating water lilies versus emerging grasses) within tropical wetlands could also influence the amount and type of epiphytic communities growing on them, and thus, their denitrification potential (Bastviken et al. 2005; Zhang et al. 2016; Adame et al. 2017).

Incentives for wetland restoration are high in tropical countries, where wetlands are considered a key component for meeting The Global Goals for Sustainable Development, such as the universal provision of clean water (Jaramillo et al. 2019). Wetland restoration could help achieve this goal by conducting projects with clear goals, objectives, and indicators to demonstrate success for investment (Waltham et al. 2019). For instance, removing non-native macrophytes from wetlands is a common practice in restoration projects regardless of whether the restoration objective is to improve water quality or increase biodiversity (Zedler 2000). Non-native macrophyte removal could be important if biodiversity outcomes are expected, as dense macrophytes reduce oxygen availability in the water column, which reduces fish habitat (Perna et al. 2012; Waltham and Fixler 2017). However, macrophyte removal efforts might be conducted differently (e.g. removal from certain locations, reduced removal effort) if the sole goal is to improve water quality, as denitrification could decrease if macrophytes are removed (Alldred et al. 2016). Finally, in some wetlands, epiphyton, and not macrophytes, are responsible for most

of the N removal, and thus, they need to be managed accordingly (Vymazal 1988; Soana et al. 2018).

This study measured denitrification rates within macrophytes in a tropical floodplain lake surrounded by sugar cane plantations in Australia (Fig. 1). We compared denitrification rates among sediments underneath native aquatic grass (giant bulrush, *Actinoscirpus grossus*), sediments underneath native waterlilies (*Nymphae* spp), and sediments without macrophytes. We also measured the denitrification and primary productivity of the epiphyton on macrophytes and compared the rates with those from the sediment. The practical application of this study was to assess whether macrophyte management could enhance N removal if the goal of wetland restoration/creation is to improve water quality. We predict that denitrification rates are highest where macrophytes and epiphyton are abundant. We also expect that high denitrification rates would be associated with high carbon and nitrate (NO_x^--N) availability.

Material and methods

Study sites

Barrett's Lagoon is in the Wet Tropics of northeast Australia within the Tully-Murray catchment (Fig. 1A). The region has a tropical climate with monthly mean temperatures ranging from 22 to 34 ˚C (Australian Bureau of Meteorology, ABM 2018; 1907-2018), and a total mean annual rainfall of 2,700 mm, mostly falling within the wet season (January to April, ABM 2018; 1871-2018). Barrett's Lagoon is classified as a coastal floodplain lake (Department of Environment and Sciences, DES, Wetlandi*nfo*, 2013). The lake is about 900 m long, 46 m wide, a mean depth of

4 m, with 8 m at its deepest part, and less than 2 m deep at the edges, where macrophytes grow. The lake is surrounded by sugar cane farms (Fig. 1B, C), which cover 12% of the catchment (DES, Wetland*Info*, 2018). This lake is a remnant of a series of floodplain lakes that have been drained for agriculture expansion, particularly before 1997 (Accad et al. 2019). A decade ago, the lake was covered by invasive, non-native macrophytes, primarily *Hymenachnea amplexicaulis* (Fig. S1), but has been recently managed to restore the natural and cultural values through both manual removal of the weeds and herbicide application, common strategies used in the region (Waltham and Fixler 2017). After the management interventions, native macrophytes have grown along the edges of the lake, including *Nymphae* spp (henceforth, "waterlilies") and giant bulrush (*Actinoscirpus grossus*, henceforth, "grass", Fig. 1B). These recovered vegetation habitats provide habitat for native fish, reptiles and birds (Adame and Waltham, pers. obs.).

Methods

Sampling was conducted during the early wet season (January 2018) and the post-wet season (June 2018, and June 2019). We measured surface water characteristics, dissolved nutrient concentrations, sediment characteristics, and denitrification rates in the sediments. We also conducted experiments on denitrification and primary productivity of the epiphyton.

Surface water characteristics

During each sampling trip, we measured temperature, electrical conductivity, pH and dissolved oxygen concentrations at 20 cm from the water surface (ProPlus, YSI meter, OH, USA). During January 2018, we sampled dissolved nutrients (NO_x^--N,

NH_4^+-N, and PO_4^--P) in thirteen points within the lake: four at the edge of the lake, which is the shallowest section (< 1 m) and is covered by aquatic grass, three in deeper areas of the lake with waterlily patches (< 2 m), and six where there are no macrophytes, at deeper sections of the lake (< 4 m, Fig. 1c). At each site, surface water samples (n = 26) were collected in duplicates that were filtered through a 0.45 µm membrane filter and stored frozen before being analysed for nutrients within the next week (colorimetric analyses based on APHA/AWWA/WPCF, 2012; Chemistry Centre, Department of Science Information Technology and Innovation, Brisbane, Australia). Detection limits for nutrient concentrations (mg L^{-1}) were: 0.002 for NH_4^+-N and 0.001 for NO_x^--N and PO_4^--P.

Sediment characteristics

Within the immediate area where water samples were collected (n = 9, Fig. 1c), benthic sediment was sampled using a Van Veen grab sampler. Samples were taken in duplicates (n = 18) from the grab with 60 mL-cores, oven-dried at 60˚C for 48 h, ground, and analysed for N, and organic carbon (%) with an elemental analyser (EA-IRMS, Serco System, Griffith University). Soil samples were tested for inorganic carbon through the addition of 1M HCl, but none of them displayed bubbling, indicating little or no presence of carbonates. Bulk density was estimated from the dry weight of the sample and its volume (g cm^{-3}).

Denitrification

We conducted three sets of denitrification experiments: 1) comparison between denitrification of the sediment underneath grass and waterlilies during the early wet season (January 2018), 2) comparison among denitrification of the sediment

underneath grass and waterlilies, and a site with no macrophytes during the end of the wet season (June 2018), and 3) comparison of denitrification between macrophytes with and without epiphyton during the end of the wet season (June 2019). Denitrification was measured with the isotope pairing technique (Nielsen 1992; Steingruber et al. 2001), which involves adding enriched $^{15}N\text{-}NO_3^-$ to water on sediments or macrophytes and estimating $^{15}N\text{-}N_2$ gas production. We targeted a final $NOx^-\text{-}N$ concentration of 0.10 mg L^{-1}, because it's a concentration commonly found in this lake, and because previous experiments in wetlands in the region (Adame et al. 2019a,b) have shown that denitrification tends to peak at concentrations around 0.5 mg L^{-1}.

The isotope pairing technique has been previously been successfully used in similar wetlands in the region (Adame et al. 2019b). However, it has some caveats, such as the overestimation of denitrification because N_2 can be produced simultaneously through anaerobic ammonium oxidation (anammox, Robertson et al. 2019). Although the contribution of N_2 generated by anammox is generally low with less than < 6 % of that produced by denitrification (Risgaard-Petersen et al. 2003). This technique can artificially reduce oxygen concentrations in the enclosed chambers (Robertson et al. 2019); thus, we maintained short incubations periods (< 6 h), as we know from previous studies that within this time frame, N_2 production is linear and oxygen conditions do not change considerably (Adame et al. 2019a). We maintained continuous water movement within each core with a stirrer bar suspended ~3 cm above the sediment, to avoid water stratification, which could artificially decrease oxygen concentrations. The third caveat of our methodology was that denitrification was only measured during the day in light conditions, so our values of denitrification are likely to be underestimated. During the day, photosynthesis increases oxygen in

the sediment, thus increases the depth at which nitrate from the water column needs to penetrate to be denitrified (Robertson et al. 2019).

For the experiments on sediments, we collected 6 to 9 cores for each treatment (grass/ waterlilies/ no macrophytes) with Perspex® acrylic tubes (4.8 cm internal diameter × 30 cm long) capped with a rubber bung. The sediment cores were filled with water collected from the site and left to equilibrate overnight. For the experiments on epiphyton, we compared waterlilies and grass, which were collected with a net and kept overnight in aerated buckets with water from the site. For the waterlilies, half of the cores were filled with a piece of a leaf (2 × 10 cm) and a stem (15 cm long) of known weight; the other half was filled with similar plant material without epiphyton, which was previously scrubbed with a soft brush until clean. For the grass, half the cores were filled with a piece (15 cm long) of green emergent grass, which had no epiphyton; the other half was filled with a piece of the submerged portions of the grass, which had epiphyton attached. The dry weight of the incubated plants was obtained by oven-drying at 60 ˚C. The day after collection, the experiments were run at the nearby town of Cardwell, with similar ambient light and air temperature conditions as in the field.

All cores were set in large plastic containers (1030 × 510 × 495 mm) filled with water to keep a constant temperature throughout the experiment (ProPlus, YSI meter, OH, USA). At the beginning of the experiment, $^{15}N\text{-}NO_3$ was added to each core, which was capped to minimise headspace. After approximately 20 min (T0), 2 to 3 cores from each batch were sacrificed by adding 1 ml of 50% w/v zinc chloride ($ZnCl_2$) and mixing it throughout the sediment and overlying water to stop the microbial activity. Triplicate 10 mL-water samples from each core were collected with a syringe and placed in a 12.5-ml Exetainer vial (Labco, High Wycombe, UK)

which had 250 µL of 50% w/v $ZnCl_2$. Sampling was conducted at 2 h and then at 5 h after initial sampling (T2 and T5).

The headspace gas was analysed by continuous-flow mass spectrometry for $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$-gas (elemental-analyser isotope ratio mass spectrometer, EA-IRMS, Serco System at Griffith University). Denitrification rates are reported for ambient light conditions in mg m^{-2} h^{-1} and mg h^{-1} g $macrophyte^{-1}$ as the mean ± standard error, with two significant digits. The detection limit for the denitrification rate was 0.01 mg m^{-2} h^{-1}. We calculated total denitrification (D_t), total denitrification of NO_3^- from the water column (D_w^{tot}), denitrification from the water column corrected for tracer addition (D_w), and coupled nitrification-denitrification (D_n) with equations by (Steingruber et al. 2001):

D_{15}: denitrification from labelled $^{15}NO_3$ as from the production rate of $^{29}N_2$ and $^{30}N_2$:

$$D_{15} = r_{29} + 2r_{30} \tag{1}$$

where r_{29} and r_{30} are the production rates of $^{29}N_2$ and $^{30}N_2$, respectively,

D_{14}: denitrification from unlabeled $^{14}NO_3$:

$$D_{14} = D_{15} \cdot \frac{r_{29}}{2r_{30}} \quad , \tag{2}$$

D_t = total denitrification or potential denitrification:

$$D_t = D_{15} + D_{14} \quad , \tag{3}$$

D_w^{tot}: total denitrification of NO_3 diffusing from the water column into the sediment:

$$D_w^{tot} = \frac{D_{15}}{\varepsilon}$$

(4)

where ε is NO_3 enrichment during incubation from $^{15}NO_3$ additions:

$$\varepsilon = \frac{[NO_3^-]_a - [NO_3^-]_b}{[NO_3^-]_a} ,$$

(5)

where *a* and *b* are NO_3 concentrations after and before $^{15}NO_3^-$ addition,

D_w: denitrification from NO_3 diffusing from the water column and corrected for tracer addition:

$$D_w = D_w^{tot}(1-\varepsilon) ,$$

(6)

D_n: Coupled nitrification-denitrification:

$$D_n = D^{tot} - D_w^{tot} .$$

(7)

We estimated epiphyton aerial denitrification rates with the biomass of epiphyton measured in this study, along with the depth of the euphotic zone (> 10% of surface light). We considered that epiphyton grew only in the euphotic zone which has been estimated in similar tropical lakes in Australia as ~80 cm for water lilies and ~36 cm for aquatic grasses (Pettit et al. 2016).

Epiphyton primary production

The primary production was measured with the ^{13}C uptake technique adapted for epiphyton (Adame et al. 2017). A known weight of macrophyte (two segments of 13 × 1.5 cm of waterlily leaf and two segments of 13.5 × 3 cm of grass) were set in polycarbonate bottles (500 mL) filled with water from the site. The bottles were

placed inside large containers with water at constant temperature at either light, dark, or 30% light conditions (covered with a 70% shade cloth). The incubations were run during a cloudy day from 10:00 h to 13:00 h.

At the beginning of the experiment, 600 µL ^{13}C-bicarbonate (^{13}C 99%; Cambridge Isotope Laboratories, Tewksbury, Massachusetts, USA) was added to each bottle. The exact time of addition was recorded; the bottles were incubated for three hours. The temperature was recorded throughout the experiment (range from 22 to 23 °C). The bottles were placed in the dark at 4 °C to stop the incubation. The epiphyton was filtered onto pre-weighed, pre-combusted filters (Whatman GF/F) that were kept frozen in the dark. In the laboratory, macrophytes were dried at 60 °C and weighed to measure dry weight. Epiphyton biomass was measured as chlorophyll *a* (Chl *a*), which was extracted with 90% acetone using a sonicator and read in a spectrometer following standard methodology (Lorenzen 1967; Jeffrey and Humphrey 1975). The rate of carbon uptake was analysed from the ^{13}C/^{12}C of epiphyton (EA-IRMS, Sercon System, Griffith University). Productivity is expressed as mg dw g h^{-1} from the amount of ^{13}C-bicarbonate uptake following equations in Adame et al. (2017).

Data analyses

Differences among sites (grass/ waterlily/ no macrophyte) were assessed with a one-way ANOVA, where NH_4^+-N and NO_x^--N, sediment organic carbon, N and bulk density were the dependent factors, and macrophyte presence was the fixed factors of the model. Normality was assessed through Shapiro-Wilk tests; when data did not comply with normality, it was transformed (log10 [NO_x^--N] and 1/ [NH_4^+-N], log [bulk density]). All data were analysed with SPSS Statistics (v.24 IBM, NY, USA).

Results

Surface water characteristics

Surface water (0-20 cm) characteristics ranged between electrical conductivity of 0.05 to 0.06 mS, pH between 5.3 and 5.9, and temperature between 23 and 28 °C. Surface dissolved oxygen concentrations were around 2.5 mg L^{-1}. During the early wet season of 2018, dissolved inorganic N concentrations (0.01 ± 0.001 NH_4^+-N, 0.008 ± 0.001 NO_x^--N) were lower than during the post-wet season of 2018 and 2019 (0.02 ± 0.001 NH_4^+-N, 0.18 ± 0.028 NO_x^--N mg L^{-1}). The PO_4^--P concentrations were similar during all sampling occasions with most samples (92%) being below detection limits (< 0.001 mg L^{-1}).

Nutrient concentrations were lower in the sites with macrophytes compared to sites without them; with 8.6 % and 19% lower NH_4^+-N concentrations, and 19 and 24% lower NO_x^--N concentrations in waterlilies and grass, respectively. However, these differences were not significant ($F_{1,24}$ = 1.693, p = 0.206 and $F_{1,24}$ = 1.233, p = 0.278, n = 26; Fig. 2a).

Sediment characteristics

Sediment characteristics differed across the lake. Highest organic carbon and N were found in sediments under grass, followed by sediments under waterlilies, and lowest in open water sites without macrophytes ($F_{2,4}$ = 61.76, p =0.01 for N, and $F_{2,4}$ = 50.88, p =0.01 for C, n = 18). On the contrary, bulk density was lowest in the grass and highest in the sediment without macrophytes ($F_{2,4}$ = 27.75, p =0.005, n = 19; Table 1).

Denitrification

Denitrification rates on the sediment ranged between 3.3 to 51.5 mg m^{-2} h^{-1} (Table 2). Following a similar pattern as carbon and N, denitrification was highest in sediments under grass, followed by sediments under waterlilies, and lowest in sites without macrophytes (Table 2, Fig. 2b). Most of the denitrification was coupled with nitrification (D_n; Fig. 2b) and was highest during the post-wet season when NO_x^--N concentrations were highest.

Denitrification rates were higher when macrophytes were incubated with the epiphyton attached to them (Table 3). Denitrification of water lilies as aerial rates (3.7 ± 1.4 mg m^{-2} hr^{-1}) and per macrophyte of biomass (159 ± 62 mg kg $macrophyte^{-1}$ h^{-1}) were higher than for grass (1.9 ± 0.6 mg m^{-2} hr^{-1}, 3.6 ± 1.1 mg kg $macrophyte^{-1}$ h^{-1}, respectively).

Epiphyton primary production

Epiphyton biomass in waterlilies (584 ±129 µg Chl g $macrophyte^{-1}$) was higher compared to epiphyton on grass (42 ± 7.8 µg Chl g $macrophyte^{-1}$). The C uptake by epiphyton was similar in both conditions of full light (0.51 ± 0.04 and 0.35 ± 0.19 mg g $epiphyte^{-1}$ h^{-1}, for waterlilies and grass, respectively), and 30% of light (0.44 ± 0.06 and 0.84 ± 0.12 mg g $epiphyte^{-1}$ h^{-1}, respectively). These productivity rates correspond to 35 g per macrophyte biomass (g h^{-1}) for the grass and 257 g per macrophyte biomass (g h^{-1}) for the waterlilies.

Discussion

We measured high denitrification rates within tropical macrophytes, especially when the plants were covered with epiphyton and during times of the year when NO_x^--N concentrations were relatively high (> 0.10 mg L^{-1}). Denitrification in the sediment

was highest where carbon (32.3%, 22.6 mg cm^{-3}) and N (1.4%, 0.98 mg cm^{-3}) were high, and bulk density was low. Denitrification rates in the sediment (3.3 - 51.5 mg m^{-2} h^{-1}) were higher than on the epiphyton (1.9 - 3.7 mg m^{-2} h^{-1}).

Studies on denitrification of macrophytes within tropical wetlands are very scarce, but our values fall between the global rates for temperate lakes and rivers (5.6 - 5.9 mg m^{-2} h^{-1}) and agricultural streams (10 - 118 mg m^{-2} h^{-1}, reviewed by Piña-Ochoa and Álvarez-Cobelas 2006). The rates measured in this study were high, even though NO_x^--N concentrations were relatively low (< 0.2 mg L^{-1}). For instance, in a temperate lake in the Mississippi catchment, denitrification rates of 12.2 mg m^{-2} at NO_3^- concentrations of 5 mg L^{-1} were measured (Kreiling et al. 2011), a similar denitrification rate but with a nutrient concentration 50 times higher than that measured at our site. This suggests, that as we initially predicted, the high temperatures and increased carbon availability within tropical macrophytes support high denitrification rates, even at low NO_3^- concentrations. At higher concentrations, N removal through denitrification could be even higher. For instance, N pollution within the catchment of Barrett's Lagoon is mainly derived from fertilisers used for the cultivation of bananas and sugarcane. These agricultural activities are associated with nutrient concentrations of 0.1 to 1.5 mg L^{-1} in nearby streams (Mitchel et al. 2009). According to this study, a shallow pond with macrophytes could be highly efficient at removing some of this NO_3^-. Although denitrifications rates are likely to peak at the high end of these concentrations, due to rapid depletion of oxygen in tropical wetlands, where NH_4^+ would become the dominant form of N, thus limiting NO_3^- available for denitrification (Nahlik and Mitsch 2006).

Tropical macrophytes have not only high denitrification rates but also high levels of primary productivity, the former process uses carbon and N in low oxygen

conditions, while the latter consumes N but produces carbon and oxygen. This creates a series of complex relationships between carbon, N, and oxygen within lakes (Mulholland et al. 2008; Song et al. 2014). In the first instance, productivity competes with denitrification for N, especially when concentrations are low. The high N uptake through primary production and denitrification in these macrophytes might explain the lower N concentrations in the water column above the macrophytes compared to sites with no vegetation (Fig. 2a). Second, the high productivity might fuel denitrification but will also cause bacterial respiration, which rapidly depletes the oxygen in the lake sediments (Lewis 1996). For instance, in tropical Costa Rica, free-floating macrophytes decreased N substantially from banana and dairy farms but also sharply decreased water dissolved oxygen concentrations (Nahlik and Mitsch 2006).

The potential for denitrification was lowest in sites without macrophytes, followed by sites with water lilies and highest in soils underneath aquatic grass. Many studies in temperate lakes have found similar results, with higher denitrification rates underneath macrophytes, and variability of denitrification with different macrophyte species and structures (e.g. Veraart et al. 2011; Holmroos et al. 2015). Higher rates of denitrification are associated with macrophytes as they provide carbon to the soil through litter deposition and root growth. Thus, in sites where there are no macrophytes, such as in the deep sections of the lake, denitrification may be carbon-limited (Hernandez and Mitsch 2007). Macrophytes also favour coupled nitrification-denitrification because oxygen transported from the roots to the sediment create oxic spaces for nitrification to occur, generating NO_3^- that can be denitrified in nearby anoxic portions of the sediment (Risgaard-Petersen and Jensen 1997). As a result, most of the denitrification that we measured within the sediments with macrophytes was coupled with nitrification.

To understand the role of this entire floodplain tropical lake at the landscape scale, we can broadly calculate its potential for denitrification at one given day. Let us assume a volume of 86,700 m^3 or 86,700,000 L (calculated as half an ellipsoid with a length of 900 m, a width of 46 m and a mean depth of 4 m) and NO_x^--N concentrations of 0.10 mg L^{-1}, which are common throughout the year. This will equate to 8.7 kg of NO_x^--N within the lake on a given day. If NO_x^--N is homogeneously distributed within the water column, about 4.3 kg of NO_x^--N would be in contact with the bottom of the lake (1 m depth), but only 0.4 kg would be in touch with the sediment underneath macrophytes at the edge of the lake (which we estimated during surveys as about 10 % of its surface, Fig. 1c). The sediment and the epiphyton within these edges have the potential to denitrify 2.1 kg of NO_x^--N every day at these concentrations (rate of 22 mg m^{-2} hr^{-1} for denitrification in the sediment-water interface plus 1.5 mg m^{-2} hr^{-1} for the epiphyton). Thus, assuming that water is slow-moving, the NO_x^--N in contact with the macrophytes would be quickly depleted. This estimation is in accordance with previous studies (Lewis 1996; Lewis et al. 2000), who predicted that tropical lakes could denitrify all the NO_x^--N in less than two days once hypoxia in the hypolimnion was reached. However, in the water column (> 90%) that is not in contact with either sediment or macrophytes, the effects of denitrification would be much lower. This result is likely to explain the small differences in NO_3^- concentrations as the water moves through some nearby lakes (Mcjannet et al. 2012) despite the high denitrification rates measured in this study. Overall, the edge of the lake, where macrophytes are prevalent, can remove 12 % of the available NO_x^--N for a given day. This N removal could significantly contribute to the Queensland Government to reduce by 60% the anthropogenic-derived dissolved

inorganic N entering the Great Barrier Reef (Australian and Queensland Government, 2018). This broad calculation still needs to consider daily and seasonal fluctuations in productivity, dissolved oxygen, and NO_3^- flows. However, this initial study provides crucial information and a cohesive story on how denitrification in shallow ponds with high macrophyte cover could ameliorate N pollution in tropical regions.

Wetland restoration needs to have measurable and specific goals (Waltham et al. 2019; DES, 2018). If the restoration goal is to reduce NO_3^- concentrations, macrophytes are required (Alldred et al. 2016), and redox potential should be regularly monitored to ensure that it stays within the denitrification range of -100 to 200 mV (Mitsch and Gosselink 2015). Restoring wetlands to reduce N, should prioritise areas with intensive land use, high nutrient concentrations (Bruesewitz et al. 2011) and low biodiversity values. High productivity in N-enriched wetlands will not only result in high denitrification rates, but could result in low oxygen availability, and low rates of biodiversity recovery (Zedler 2000; Waltham et al. 2019). Suppose the restoration objective is to improve biodiversity or the cultural values, such as in Barrett's Lagoon. In that case, it may be essential to remove the excessive accumulation of invasive non-native macrophytes. The newly emerged native macrophytes provide not only biodiversity values, but also food provision (Perna et al. 2012; Davis and Moore 2015), habitat for aquatic invertebrates (Pearson et al. 2015), and thermal refugia for fish (Waltham and Sheaves 2017). Barrett's Lagoon represents a remnant floodplain lake with biodiversity values for fish, reptile, and birds. It should benefit from the continuing removal of invasive non-native macrophytes, with native macrophytes at its edges still providing some N removal.

Conclusion

High denitrification rates were measured in shallow areas of a tropical lake with dense macrophytes and abundant epiphyton. The high denitrification rates were associated with high soil carbon, high soil N, low bulk density, and high NO_x^--N concentrations in the water column. Denitrification was highest in sediment under grasses and waterlilies and macrophytes with dense epiphyton cover, and lowest in sediments with no macrophytes. Macrophytes play an essential role in the N removal potential of tropical wetlands; they sustain epiphyton communities and contribute to the formation of sediment rich in carbon and N, which fuels high levels of coupled nitrification-denitrification. Shallow ponds with macrophytes could be a complementary and sustainable option for reducing N pollution in tropical catchments.

Acknowledgements

We acknowledge the Traditional Owners of the land in which the field study was conducted, especially the Girramay and Gulnay people. We thank Santo Silvestro from Barrett's Lagoon for allowing us to work in his property and for sharing his knowledge on wetlands. Thanks to Bianca Molinari, Carolyn Trewin, and Emad Kavehi for their help in the field.

Funding details

This project was funded by the Queensland Government through the Advance Queensland Industry Engagement Program granted to MF Adame, and the Australian Government National Environmental Sciences Program (Tropical Water Quality Hub, Project 3.3.2) granted to NJ Waltham and MF Adame. We thank the Wetlands Team and Reef Water Quality Program from the Queensland Government for financial and logistic support.

Disclosure statement.

We declare no conflict of interest

References

Adame MF, Franklin H, Waltham N, Rodriguez S, Kavehei E, Turschwell M, Balcombe S, Kaniweska P, Burford M, Ronan M. 2019a. Nitrogen removal by tropical forested wetlands through denitrification. Mar Freshw Res 70:1513–21.

Adame MF, Roberts ME, Hamilton DP, Ndehedehe CE, Reis V, Lu J, Griffiths M, Curwen G, Ronan M. 2019b. Tropical coastal wetlands ameliorate nitrogen export during floods. Front Mar Sci doi:10.3389/fmars.2019.00671.

Adame M, Pettit N, Valdez D, Ward D, Burford M, Bunn S. 2017. The contribution of epiphyton to the primary production of tropical floodplain wetlands. Biotropica 49:461–71.

Alldred M, Baines SB, Findlay S. 2016. Effects of invasive-plant management on nitrogen-removal services in freshwater tidal marshes. PLoS One 11:1–15.

Australian and Queensland Government. 2018. Reef 2050. Water Quality Improvement Plan 2017-2022. Brisbane, Australia, 56 pp.

Bastviken SK, Eriksson PG, Premrov A, Tonderski K. 2005. Potential denitrification in wetland sediments with different plant species detritus. Ecol Eng 25:183–90.

Bruesewitz DA, Hamilton DP, Schipper LA. 2011. Denitrification potential in lake sediment increases across a gradient of catchment agriculture. Ecosystems 14:341–52.

Davis AM, Moore AR. 2015. Conservation potential of artificial water bodies for fish communities on a heavily modified agricultural floodplain. Aquat Conserv Mar Freshw Ecosyst 26:1184–96.

Department of Environment and Science, Queensland. 2013. Lacustrine ecology, WetlandInfo website. Viewed January, 2021. Available at: https://wetlandinfo.des.qld.gov.au/wetlands/ecology/aquatic-ecosystems-

natural/lacustrine/

Department of Environment and Science, Queensland. 2013. Tully drainage basin — facts and maps. Viewed January 2021. Available at: https://wetlandinfo.des.qld.gov.au/wetlands/facts-maps/basin-tully/

Department of Environment and Science, Queensland. 2018. Wetland site management and rehabilitation. Viewed January 2021. Available at: www.wetlandinfo.des.qld.gov.au/wetlands/management/rehabilitation

Enrich-Prast A, Figueiredo V, De Esteves FA, Nielsen LP. 2016. Controls of sediment nitrogen dynamics in tropical coastal lagoons. PLoS One 11:1–17.

Eriksson PG. 2001. Interaction effects of flow velocity and oxygen metabolism on nitrification and denitrification in biofilms on submersed macrophytes. Biogeochemistry 55:29–44.

Galloway J, Aber J, Erisman J, Seitzinger S, Howarth R, Cowling E, Cosby B. 2003. The Nitrogen Cascade. Bioscience 53:341.

Galloway JN, Cowling EB. 2002. Reactive nitrogen and the world: 200 years of change. AMBIO A J Hum Environ 31:64–71.

Hernandez ME, Mitsch WJ. 2007. Denitrification in created riverine wetlands: Influence of hydrology and season. Ecol Eng 30:78–88.

Holmroos H, Horppila J, Niemistö J, Nurminen L, Hietanen S. 2015. Dynamics of dissolved nutrients among different macrophyte stands in a shallow lake. Limnology 16:31–9.

Jaramillo F, Desormeaux A, Hedlund J, Jawitz JW, Clerici N, Piemontese L, Rodríguez-Rodriguez JA, Anaya JA, Blanco-Libreros JF, Borja S, et al. 2019. Priorities and interactions of Sustainable Development Goals (SDGs) with focus on wetlands. Water (Switzerland). 11.

Jeffrey SW, Humphrey G. 1975. New spectrophotometric equation for determining chlorophylls a, b, c1 and c2 in high plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191–4.

Junk WJ, Furch K. 1993. A general review of tropical South American floodplains. Wetl Ecol Manag 2:231–8.

Kreiling RM, Richardson WB, Cavanaugh JC, Bartsch LA. 2011. Summer nitrate uptake and denitrification in an upper Mississippi River backwater lake: The role of rooted aquatic vegetation. Biogeochemistry 104:309–24.

Kulkarni M V., Groffman PM, Yavitt JB. 2008. Solving the global nitrogen problem: It's a gas! Front Ecol Environ 6:199–206.

Land M, Granéli W, Grimvall A, Hoffmann CC, Mitsch WJ, Tonderski KS, Verhoeven JTA. 2016. How effective are created or restored freshwater wetlands for nitrogen and phosphorus removal ? A systematic review. Environ Evid:1–26.

Lewis W., Hamilton S, Rodríguez L, Saunders JFI. 2000. Ecological Determinism on the Orinoco Floodplain. Bioscience 50:681–92.

Lewis WM. 1996. Tropical lakes : how latitude makes a difference. (Schiemer F, Boland K, editors.). Amsterdam, The Netherlands: SPB Academic Publishing

Lorenzen CJ. 1967. Determination of chlorophyll and pheopigments: spectro-photometric equations. Limnol Oceanogr 12:343–6.

Mcjannet D, Wallace J, Keen R, Hawdon A, Kemei J. 2012. The filtering capacity of a tropical riverine wetland : II. Sediment and nutrient balances. 72:53–72.

Mitchell A, Reghenzan J, Faithful J, Furnas M, Brodie J. 2009. Relationships between land use and nutrient concentrations in streams draining a "wet-tropics" catchment in northern Australia. Mar Fresh Res 60: 1097–1108.

Mitsch W, Nahlik A, Wolski P, Bernal B, Zhang L, Ramberg L. 2010. Tropical

wetlands: seasonal hydrologic pulsing, carbon sequestration, and methane emissions. Wetl Ecol Manag 18:573–86.

Mitsch WJ, Gosselink J. 2015. Wetlands. 5th ed. New Jersey, USA: Wiley

Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SEG, Gregory S V., Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Niederlehner BR, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202–5.

Nahlik AM, Mitsch WJ. 2006. Tropical treatment wetlands dominated by free-floating macrophytes for water quality improvement in Costa Rica. Ecol Eng 8:246–57.

Nielsen L. 1992. Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microbiol Ecol 86.

Pang S, Zhang S, Lv XY, Han B, Liu K, Qiu C, Wang C, Wang P, Toland H, He Z. 2016. Characterisation of bacterial community in biofilm and sediments of wetlands dominated by aquatic macrophytes. Ecol Eng 97:242–50.

Pearson RG, Connolly NM, Boyero L. 2015. Ecology of streams in a biogeographic isolate — the Queensland Wet Tropics, Australia. Freshw Biol 34:797–819.

Perna C, Cappo M, Pusey BJ, Burrows D, Pearson RG. 2012. Removal of aquatic weeds greatly enhances fish community richness and diversity: An example from the Burdekin River Floodplain, tropical Australia. River Res Appl 28:1093–104.

Pettit NE, Ward DP, Adame MF, Valdez D, Bunn SE. 2016. Influence of aquatic plant architecture on epiphyte biomass on a tropical river floodplain. Aquat Bot

129:35–43.

Piña-Ochoa E, Álvarez-Cobelas M. 2006. Denitrification in aquatic environments: A cross-system analysis. Biogeochemistry 81:111–30.

Risgaard-Petersen N, Jensen K. 1997. Nitrification and denitrification in the rhizosphere of the aquatic macrophyte *Lobelia dortmanna* L. Limnol Oceanogr 42:529–37.

Risgaard-Petersen N, Nielsen LP, Rysgaard S, Dalsgaard T, Meyer RL. 2003. Application of the isotope pairing technique in sediments where anammox and denitrification coexist. Limnol Oceanogr Methods 1:63–73.

Robertson EK, Bartoli M, Brüchert V, Dalsgaard T, Hall POJ, Hellemann D, Hietanen S, Zilius M, Conley DJ. 2019. Application of the isotope pairing technique in sediments: Use, challenges, and new directions. Limnol Oceanogr Methods 17:112–36.

Soana E, Gavioli A, Tamburini E, Fano EA, Castaldelli G. 2018. To mow or not to mow: reed biofilms as denitrification hotspots in drainage canals. Ecol Eng 113:1–10.

Song K, Hernandez ME, Batson JA, Mitsch WJ. 2014. Long-term denitrification rates in created riverine wetlands and their relationship with environmental factors. Ecol Eng 72:40–6.

Steingruber S, Friedrich J, Gachter R, Wehrli B. 2001. Measurement of denitrification in sediments with the 15N isotope pairing technique. Appl Environ Microbiol 67:3771–8.

Veraart AJ, de Bruijne WJJ, de Klein JJM, Peeters ETHM, Scheffer M. 2011. Effects of aquatic vegetation type on denitrification. Biogeochemistry 104:267–74.

Vymazal J. 1988. The use of periphyton communities for nutrient removal from

polluted streams. Hydrobiologia 166:225–37.

Waltham N, Sheaves M. 2017. Acute thermal tolerance of tropical estuarine fish occupying a man-made tidal lake, and increased exposure risk with climate change. Estuar Coast Shelf Sci 196:173–81.

Waltham NJ, Burrows D, Wegscheidl C, Buelow C, Ronan M, Connolly N, Groves P, Marie-Audas D, Creighton C, Sheaves M. 2019. Lost floodplain wetland environments and efforts to restore connectivity, habitat, and water quality settings on the Great Barrier Reef. Front Mar Sci 6:1–14.

Waltham NJ, Fixler S. 2017. Aerial herbicide spray to control invasive water Hyacinth (*Eichhornia crassipes*): Water quality concerns fronting fish occupying a tropical floodplain wetland. Trop Conserv Sci 10:1–10.

Zedler JB. 2000. Progress in wetland restoration ecology. Trends Ecol Evol 15:402–7.

Zhang S, Pang S, Wang P, Wang C, Guo C, Addo FG, Li Y. 2016. Responses of bacterial community structure and denitrifying bacteria in biofilm to submerged macrophytes and nitrate. Sci Rep 6:1–10.

Tables

Table 1. Sediment characteristics underneath aquatic grass, waterlilies, and sites without macrophytes. Values are mean ± standard error for triplicate sites for each treatment (n = 18); C = organic carbon, N = nitrogen, C:N = atomic ratio

	%C	%N	C:N	Bulk density ($g\ cm^{-3}$)
Grass	32.3 ± 1.6	1.4 ± 0.1	28 ± 2	0.07 ± 0.01
Waterlilies	13.3 ± 3.4	0.8 ± 0.2	20 ± 3	0.06 ± 0.01
No macrophytes	2.0 ± 0.9	0.1 ± 0.0	22 ± 2	0.94 ± 0.16

Table 2. Denitrification rates (mg m^{-2} h^{-1}) of sediment underneath A) aquatic grass, B) waterlilies, C) and open water, where there were no macrophytes. D_t = total or potential denitrification during the experiment; D_w^{tot} = total denitrification of labelled plus unlabelled NO_3^- from water column; D_w = natural denitrification rate without tracer addition; D_n = coupled nitrification-denitrification (Nielsen 1992); NO_x^--N is the concentration during the experiment (mg L^{-1}).

Season	Structure	D_{tot}	D_w^{tot}	D_w	D_n	NO_x^--N
Early-wet	Grass	8.5 ± 2.4	6.0 ± 3.0	1.0 ± 0.5	1.4 ± 0.4	0.08 ± 0.03
	Waterlily	3.3 ± 1.7	1.9 ± 0.9	1.4 ± 0.7	0.8 ± 0.4	0.07 ± 0.03
Post-wet	Grass	51.5 ± 4.4	3.0 ± 0.3	2.8 ± 0.2	48.5 ± 4.1	0.15 ± 0.02
	Waterlily	23.5 ± 4.5	8.8 ± 1.7	8.3 ± 1.6	14.7 ± 2.8	0.12 ± 0.00
	No macrophytes	10.8 ± 3.5	4.2 ± 1.4	4.0 ± 1.3	6.6 ± 2.1	0.13 0.02

Table 3. Denitrification rates (mg m^{-2} h^{-1}) of macrophytes (grass and waterlilies) with and without epiphyton; D_t = total or potential denitrification during the experiment; D_w^{tot} = total denitrification of labelled plus unlabelled NO_3^- from water column; D_w = natural denitrification rate without tracer addition; D_n = coupled nitrification-denitrification; nd = not detected; NO_x^--N are the concentrations during the experiment (mg L^{-1})

	D_{tot}	D_w^{tot}	D_w	D_n	NO_x^--N
Submerged grass + epiphyton	1.6 ± 0.5	1.6 ± 0.4	1.4 ± 0.5	nd	0.15
Emergent grass	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	nd	0.15
Waterlilies + epiphyton	1.4 ± 0.5	1.4 ± 0.5	1.1 ± 0.4	nd	0.18
Waterlilies	1.2 ± 0.2	1.2 ± 0.2	0.7 ± 0.1	nd	0.08

Figure captions

Figure 1. (a) Location of Barrett's Lagoon within the tropical catchment of the Tully River, Australia, (b) different structures of macrophytes grow at the edges of the lake, including *Nymphae* spp (waterlily) giant bulrush (*Actinoscirpus grossus*), (c) aerial view of the lake surrounded by sugar cane farms, and sampling sites: waterlilies (triangles), grass (squares) and open water with no macrophytes (circles).

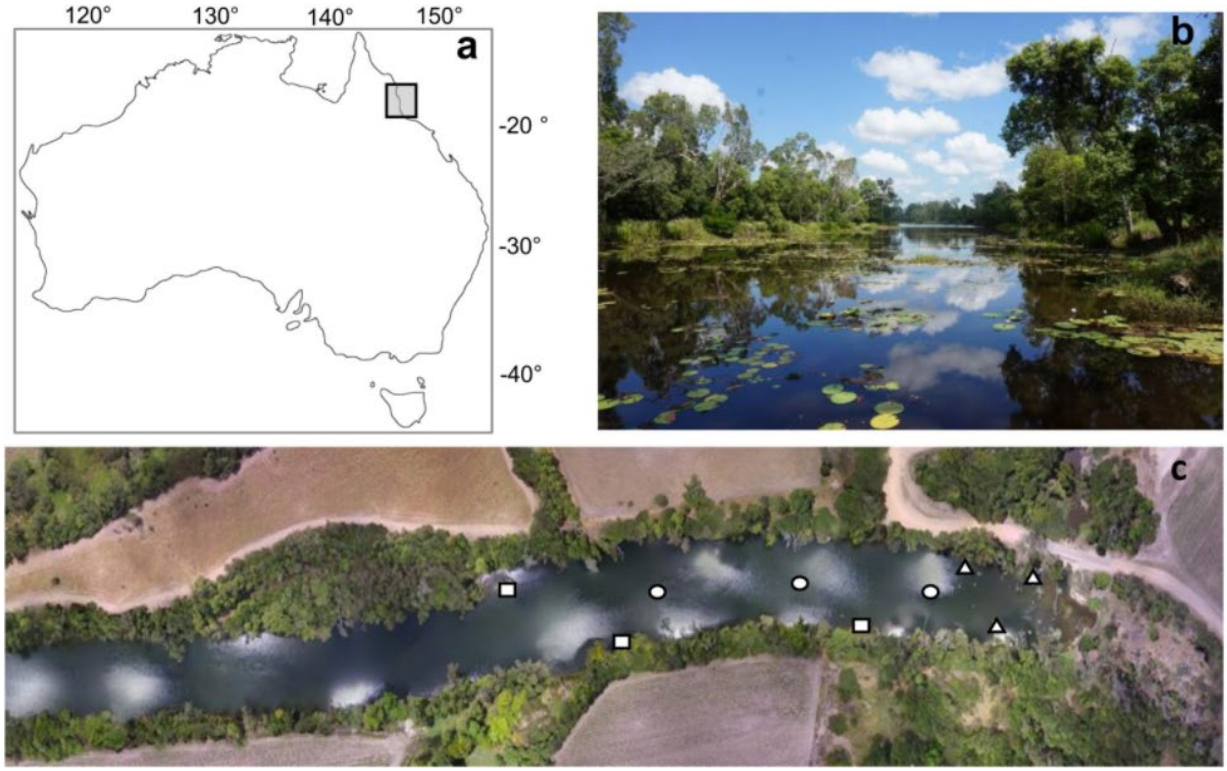

Figure 2. Dissolved inorganic nitrogen (DIN = NH_4^+-N and NO_x^--N) from the surface water (20 cm deep) and denitrification (D_n = coupled nitrification-denitrification; D_w = denitrification of NO_3^- from the water column) for sediment underneath aquatic grass, waterlilies, and in the open water, where there were no macrophytes.

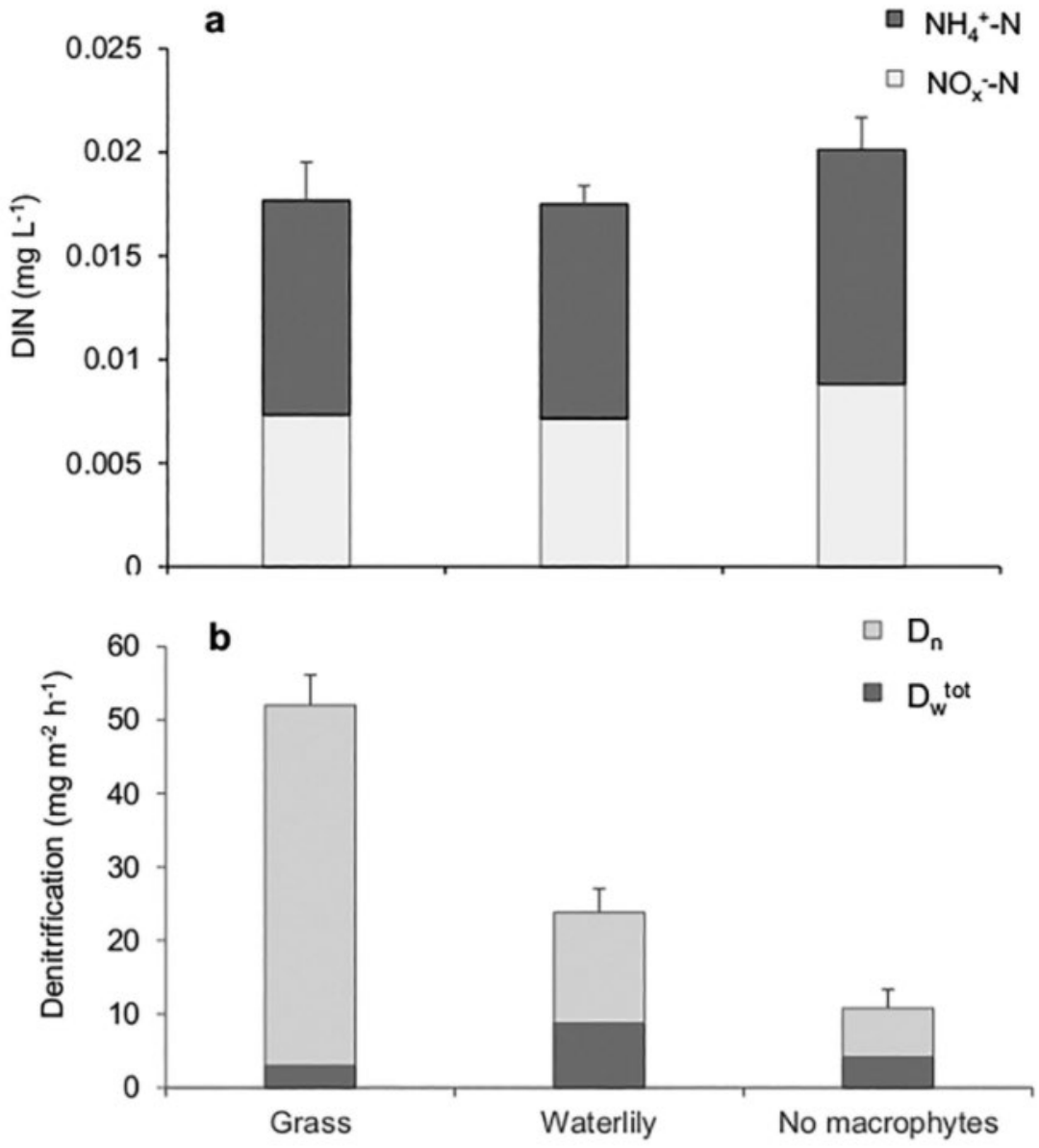

Figure 3. Schematic diagram of the capacity of tropical macrophytes to remove N-NO_3^- through denitrification (Dt) within sediment underneath them and from the epiphyton attached. The macrophytes sustain the epiphytes, consume NO_3^- and produce carbon that fuels denitrification, reducing NO_3^- concentrations in the water column, shown as smaller font size.