Nitrogen fixation by epiphytic and epibenthic diazotrophs associated with seagrass meadows along the Tanzanian coast, Western Indian Ocean

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ABSTRACT: Seasonal, diurnal, and age-dependent variations in nitrogen fixation (nitrogenase activity) by epiphytic diazotrophs colonizing the seagrasses *Halodule uninervis*, *Cymodocea rotundata*, *Thalassodendron ciliatum*, and *Thalassia hemprichii*, and by epibenthic diazotrophs associated with seagrass-vegetated and nonvegetated sediments, were estimated at 2 sites along the Tanzanian coast, Western Indian Ocean. Acetylene reduction-gas chromatography showed that nitrogenase activity values were significantly higher (p = 0.0004) at the site with low nutrient levels (Mjimwema) than at the site with higher nutrient levels (Ocean Road). The nitrogenase activity ranged from 10 to 192 nmol N g^{-1} h^{-1} for *H. uninervis*, 7 to 80 nmol N g^{-1} h^{-1} for *C. rotundata*, 10 to 75 nmol N g^{-1} h^{-1} for *Thalassia hemprichii*, and from 4 to 61 nmol N g^{-1} h^{-1} for *Thalassodendron ciliatum*. Nitrogenase activity values in sediments covered by seagrasses were significantly higher than in surrounding nonvegetated sediments (t = 4.021, p = 0.0005). Significant variations in nitrogenase activity were apparent depending on leaf age and season, with highest activity being found in mid-aged leaves during the northeastern monsoon (NEM), and in older leaves during the southeastern monsoon (SEM). Daytime nitrogenase activity was appreciable on above-ground seagrass parts, while rhizosphere activity peaked at night-time. Collectively our data show that diazotrophs (cyanobacteria and other bacteria) are associated with seagrasses (leaves and roots), and potentially constitute an integral part of the ecosystem. They show highly dynamic nitrogenase activity and a succession in seagrass colonization, and we concluded that their presence may contribute to the productivity of the seagrass beds.

KEY WORDS: Nitrogen fixation · Diazotrophs · Epibenthic · Epiphytes · Seagrass · Western Indian Ocean

INTRODUCTION

Seagrass ecosystems are known to be highly productive and important in nutrient cycling in coastal habitats of oceans worldwide. The high productivity in such ecosystems is the product of the physiological activities of the seagrasses and potentially of associated epiphytic micro-organisms (Hemminga & Duarte 2000). The epiphytic productivity has been shown to contribute 8 to 56 % of the total production in some seagrass beds (Borum et al. 1984, Morgan & Kitting 1984). Epiphytic micro-algae are also known to be responsible for 46 % of the total autotrophic production in seagrass beds in the Mississippi Sound (USA) (Moncreiff et al. 1992).

Prokaryotic epiphytes may also contribute to the nitrogen input in seagrass ecosystems: e.g. diazotrophs in the seagrass-associated sediments (Thursby & Harlin 1982) and diazotrophs colonizing the seagrasses, or via recycling of the fixed nitrogen from older to younger seagrass leaves (Johnson & Johnstone 1995). It has been shown that in some tropical and subtropical seagrass meadows, >50 % of the nitrogen used may originate from nitrogen fixation (Patriquin & Knowles 1972, Capone & Taylor 1980, Capone 1988). Moreover, O′ Donohue et al. (1991) estimated that 30 to 50 % of the

nitrogen fixed was contributed to the seagrass *Zostera capricornia* in the Gulf of Carpentaria. In temperate seagrass ecosystems, nitrogen-fixing epiphytes apparently contribute less. About 6.3 to 12% of the nitrogen used by *Z. noltii* in the Bassin d'Arcachon (France) (Welsh et al. 1996, 2000) and <5% by *Z. marina* in Limfjord (Denmark) (McGlathery et al. 1998) originated from activities of associated epiphytes. Higher nitrogenase activity values have been reported in seagrass rhizosphere sediments than in nonvegetated sediments (Moriarty 1980, McComb et al. 1989, Welsh et al. 1996, 2000, McGlathery et al. 1998, Hansen et al. 2000).

Colonization of seagrass plants and associated surface sediments by cyanobacteria has previously been demonstrated in the Western Indian Ocean (Lugomela 2002, Hamisi et al. 2004, Uku et al. 2007). A variety of nitrogen-fixing cyanobacterial morphotypes were discovered, such as filamentous heterocystous (*Anabaena* spp., *Nodularia* spp., and *Calothrix* spp.), filamentous non-heterocystous (*Lyngbya* spp. and *Oscillatoria* spp.), and some unicellular (*Gloeocapsa* spp.) morphotypes. Hamisi et al. (2004) were the first to report on the seasonal cyanobacterial species composition and abundance in seagrass meadows subject to different environmental conditions in the Western Indian Ocean (Tanzanian coast). More recently, the genetic identity and variations in prokaryotic epiphytes colonizing sea-

grasses along the Kenyan coast was determined (Uku et al. 2007).

However, there is still no information on nitrogenase activity in the ecologically important seagrass ecosystems of the Western Indian Ocean, while appreciable nitrogenase activity has been documented in open waters, intertidal sediments and for mangrove pneumatophores (Lugomela et al. 2001, Lugomela & Bergman 2002, Kyaruzi et al. 2003, Lyimo & Lugomela 2006, Bauer et al. 2008). To gain deeper understanding of the significance of diazotrophs associated with seagrass meadows, and as a follow-up of a previous study (Hamisi et al. 2004), a detailed survey on nitrogenase activity by diazotrophs associated with seagrass meadows along the Western Indian Ocean coast were determined. A year-round nitrogenase activity survey was conducted at 2 sites subjected to different anthropogenic impacts.

MATERIALS AND METHODS

Study sites and sampling strategies. Sampling and fieldwork were conducted at 2 sites, Mjimwema (MJ) and Ocean Road (OR), located on the coast of Dar es Salaam, Tanzania (Fig. 1). OR is characterized as being a more nutrient-rich site, being close to the harbor and
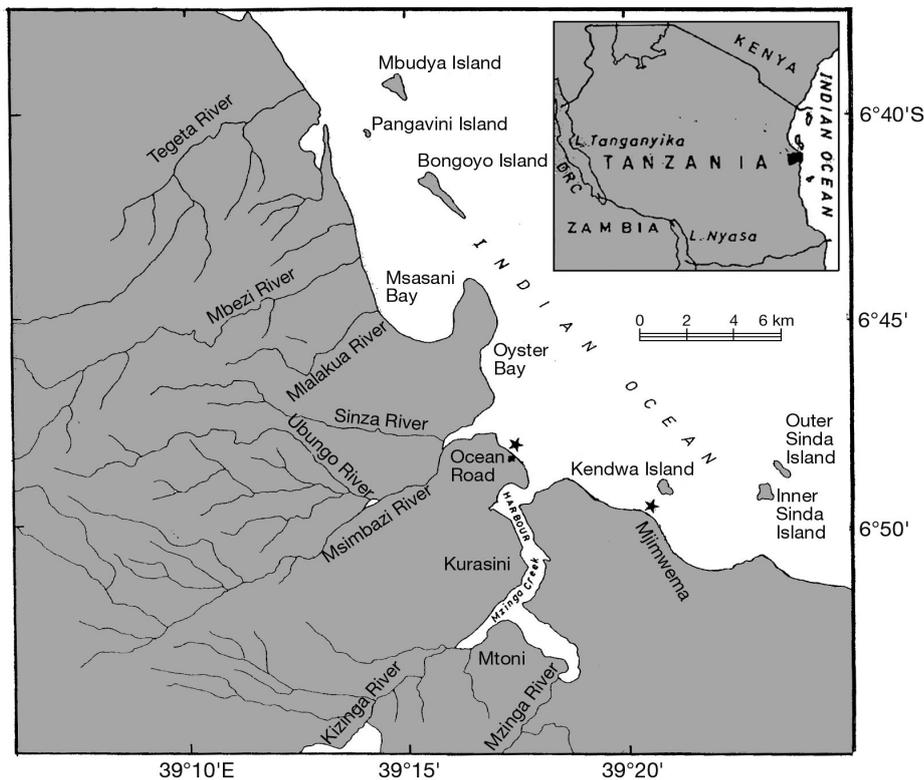

Fig. 1. Dar es Salaam coastal area in Tanzania, with study sites at Mjimwema and Ocean Road (★). Inset**:** Tanzania and its extended Western Indian Ocean coast

to fish landing sites, and it receives major inflows and the sewer pipeline that drains the city of Dar es Salaam (Fig. 1). Sampling for the determination of nitrogenase activity and the influence of seasonal variation was done in 4 selected seagrass species (*Halodule uninervis*, *Cymodocea rotundata*, *Thalassia hemprichii*, and *Thalassodendron ciliatum*) and associated seagrass vegetated and unvegetated sediments. The sampling was conducted during ebb tide at monthly intervals from September 2002 to August 2003 at both sites. Intermittently, in 2004 to 2006 measurements of nitrogen fixation in various parts of the seagrass plants (below and above ground) as well as in the leaves of various ages for *C. rotundata, Thalassia hemprichii*, and *Thalassodendron ciliatum* were performed at Site MJ. In addition, the nitrogen-fixation activity in seagrasses and the surrounding sediments was assayed from 10:00 to 13:00 h (daytime) and 20:00 to 24:00 h (night-time).

Mean seagrass biomass of the 4 seagrass species selected was estimated within 0.25 m × 0.25 m quadrants during November 2002 and March 2003. Physicochemical parameters were determined during each sampling period and included salinity, temperature, and dissolved inorganic nutrients (nitrate, nitrite, and phosphate). Salinity and temperature were measured using a salt refractometer (300011 SPER Scientific) and a mercury thermometer respectively, while dissolved inorganic nutrients were determined as in Parsons et al. (1989).

Determination of nitrogenase activity using acetylene reduction assay. Seagrass samples were collected in triplicate by removing whole seagrass plants. Sediments were collected from within seagrass meadows and in unvegetated open areas (away from the seagrasses) by a 6 cm corer inserted to the sediment to a depth of about 1 cm. Sediments were gently collected using a spatula to minimize destruction of the roots. Samples (seagrass and sediments) were individually placed in incubation vessels (250 ml serum bottles) filled to about one-third of the bottle's volume. To determine nitrogenase activity in the various aged seagrass leaves, 3 to 5 shoots of the same species were selected, and leaves were divided into young, mid-aged, and older starting from the leaves on top of the plant shoot (young) to the bottom (old). Leaves of the same age were then placed in a 10 ml assay bottle for analysis. The bottles filled with samples (seagrass or sediments) were sealed tightly with a rubber stopper and capped with aluminum caps. The samples were analyzed for nitrogenase activity using an acetylene reduction assay as described by Capone (1993). Incubation started after replacing 10% of the air in the bottles with acetylene gas using an airtight syringe, and the samples were incubated *in situ* for 2 h.

After incubation, 1.0 ml of the gas phase was withdrawn using syringes inserted in a rubber block during transportation. In the laboratory, ethylene concentration was analyzed using a flame ionization detector gas chromatograph (Hewlett Packard 5890 II), fitted with a column packed with porapack N (80/100-mesh size). Nitrogen gas was used as a carrier gas at a flow rate of 35 ml min^{-1}. Hydrogen and air-flow rates were set at 20 and 200 ml min^{-1}, respectively. Oven, detector, and injection temperatures were set at 80, 170, and 200°C, respectively. A known concentration of ethylene gas was used as a standard for calculating ethylene production. The amount of nitrogen fixed per gram of seagrass was calculated based on the dry weight of the samples, while for sediments, activity was expressed based on sample area. The acetylene reduced (ethylene produced) to nitrogen fixed ratio we used was the theoretical value of 4:1 (Capone 1993).

Statistical analysis. Data were analyzed using either a parametric (if a normality test was passed) or a nonparametric test (if the values did not show normal distributions). When comparing 2 variables, the *t*-test was used for normally distributed data, and the Mann-Whitney *U* test (unpaired) or Wilcoxon test (paired) for data lacking normal distribution. For multiple comparisons, the parametric ordinary ANOVA or the nonparametric test (Kruskal-Wallis) with a post hoc test was used. For instance, the ordinary ANOVA test was used to determine any significant differences among the seagrass species. The Mann-Whitney nonparametric test was used to determine significant variations in nitrogen fixation rates between the 2 sites. The Wilcoxon and Kruskal-Wallis tests with Dunn's post hoc test were used to test differences in activities between day and night and in leaves of different ages. All the analyses were performed as described by Zar (1999) and using GraphPad Instat (1990–1993) software. A value of $p < 0.05$ was considered significant.

RESULTS

Seagrass composition and biomass

The 4 seagrass species *Thalassodendron ciliatum*, *Thalassia hemprichii*, *Cymodocea rotundata*, and *Halodule uninervis* were commonly observed in the intertidal zones at the 2 selected study sites. The seagrass *Halophila ovalis* occurred occasionally at both sites, while *Syringodium isoetifolium* and *Enhalus acoroides* were only found at Sites MJ and OR, respectively. The biomass of each seagrass varied and was generally higher at the less nutrient-rich Site MJ (Fig. 2), with an average of 208 ± 20 g dry wt m^{-2} (Fig. 2a), than at the more nutrient-rich Site OR, with
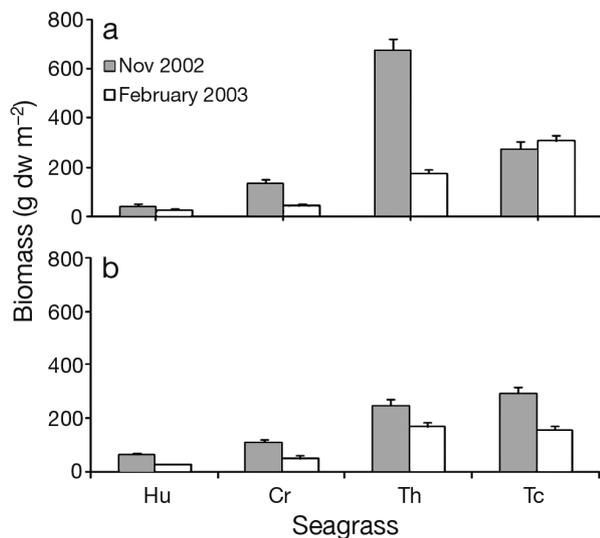
Fig. 2. Biomass of 4 seagrasses commonly found at both sites, (a) Mjimwema and (b) Ocean Road, during the dry season (November) and the rainy season (February) (n = 2). Error bars represent SD. Hu = *Halodule uninervis*, Cr = *Cymodocea rotundata*, Th = *Thalassia hemprichii*, Tc = *Thalassodendron ciliatum*
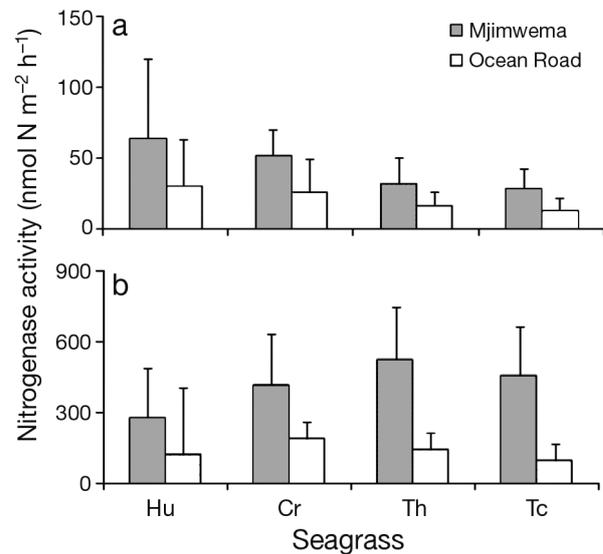

Fig. 3. Nitrogenase activity by 4 seagrass species at the 2 sites examined, Mjimwema and Ocean Road, expressed on the basis of (a) dry weight seagrass biomass, and (b) area (dry weight biomass of seagrass per area) (n = 36). Error bars represent SD. Hu = *Halodule uninervis*, Cr = *Cymodocea rotundata*, Th = *Thalassia hemprichii*, Tc = *Thalassodendron ciliatum*

an average cover of 138 ± 16 g dry wt m^{-2} (Fig. 2b). In general, *H. uninervis* exhibited the lowest biomass and *T. hemprichii* and *T. ciliatum* the highest.

Physico-chemical parameters of the study sites

Using paired *t*-tests, we found nitrate and phosphate levels to be significantly higher (t = 2.75, p = 0.001 and t = 2.34, p = 0.025 respectively) at Site OR than at Site MJ, as expected (see Hamisi et al. 2004). Nitrate and phosphate levels typically ranged from 0.45 to 1.03 μM and 0.03 to 0.09 μM, respectively, for Site OR, while levels were lower at Site MJ, being 0.14 to 0.93 μM and 0.01 to 0.07 μM. There were no significant differences in salinity and temperature between the 2 sites or seasons (Hamisi et al. 2004).

Nitrogenase activity associated with seagrasses

Nitrogenase activity was observed in relation to all 4 seagrasses studied, and the activity was consistently higher at Site MJ than at Site OR. Mean nitrogenase activity (nitrogen fixation) expressed per dry weight of seagrass is given in Fig. 3a. The average nitrogenase activity recorded was 63.5 ± 56.6 nmol N g^{-1} h^{-1} for *Halodule uninervis*, which was the highest activity recorded, followed by *Cymodocea rotundata* at 51.1 ±

18.6 nmol N g^{-1} h^{-1}, *Thalassia hemprichii* at 31.1 ± 19.4 nmol N g^{-1} h^{-1}, and *Thalassodendron ciliatum* at 28.1 ± 12.4 nmol N g^{-1} h^{-1} at Site MJ. At Site OR the trend was the same but the activity values were about half of those recorded at Site MJ (Fig. 3a). There were no significant differences in nitrogenase activity among the seagrass species (ANOVA; F = 1.076, p = 0.45). Expressed on an average seagrass cover area basis (Fig. 3b), nitrogenase activity was highest for *T. hemprichii*, followed by *T. ciliatum*, *C. rotundata*, and *H. uninervis*, but these were not significantly different (F = 0.1472, p = 0.93).

Next, the seagrasses were divided into above- and below-ground parts. The nitrogenase activity of the below-ground parts (rhizosphere) was as high as or nonsignificantly higher than the above-ground parts (phyllosphere). The rhizosphere activity averaged 0.16 ± 0.03 μmol N g^{-1} h^{-1} at Site MJ and 0.05 ± 0.01 μmol N g^{-1} h^{-1} at Site OR. The activity in the phyllosphere averaged 0.12 ± 0.02 and 0.04 ± 0.01 μmol N g^{-1} h^{-1} at the 2 sites, respectively (n = 9).

Leaf age-dependent nitrogenase activity during seasons

Fig. 4 illustrates the nitrogenase activity in leaves of different ages for *Thalassia hemprichii*, *Thalassodendron ciliatum*, and *Cymodocea rotundata* during
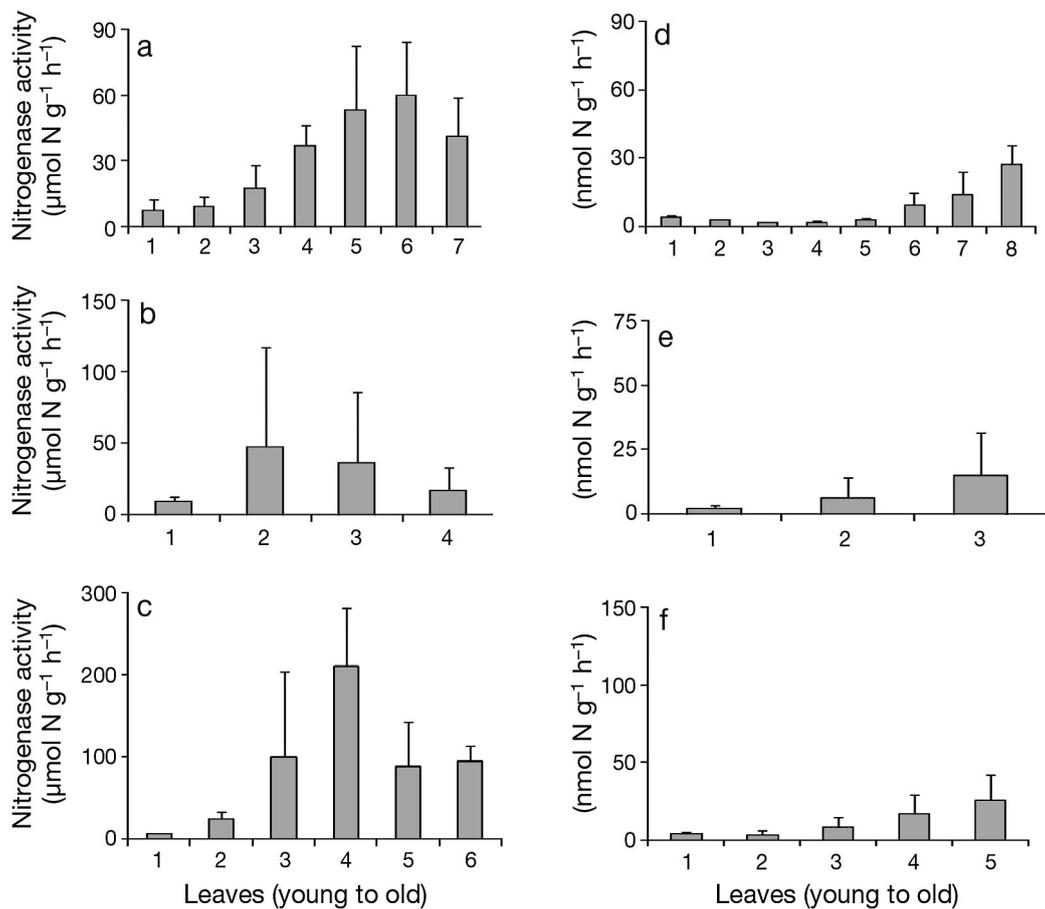
Fig. 4. Nitrogenase activity in seagrass leaves along an age gradient during (a–c) the northeastern monsoon (n = 9) and (d–f) the southeastern monsoon (n = 6). (a,d) *Thalassodendron ciliatum*; (b,e) *Cymodocea rotundata*; (c,f) *Thalassia hemprichii*. Error bars represent SD. Note the different scale bars on the *y*-axes

the northeastern monsoon (NEM) (December to April; Fig. 4a–c) and the southeastern monsoon (SEM) (June to October; Fig. 4d–f). Nitrogenase activity varied significantly between the differently aged leaves during the NEM (Kruskal-Wallis test: p = 0.003, H = 19.92; p = 0.015, H = 20.56; and p = 0.027, H = 21.05 for *T. hemprichii*, *T. ciliatum*, and *C. rotundata,* respectively; n = 9), and during the SEM (Kruskal-Wallis test: p = 0.047, H = 7.94; p = 0.0001, H = 32.12; and p = 0.020, H = 7.02 for *T. hemprichii*, *T. ciliatum*, and *C. rotundata,* respectively; n = 6). The post hoc test (Dunn's multiple comparisons test) showed nonsignificantly higher rates (p > 0.05) in mid-aged leaves compared to young and older leaves during the NEM (Fig. 4a–c). During the SEM, significantly higher rates (p < 0.05) were found in older leaves compared to young and mid-aged leaves (Fig. 4d–f). In general, nitrogenase activity values were considerably higher during the NEM than during the SEM.

Day and night nitrogenase activity

Nitrogenase activities were also recorded during the day (10:00 to 13:00 h) and night (20:00 to 24:00 h) at 3 time points in October, in a mixed community of *Halodule uninervis* and *Cymodocea rotundata* and in the surrounding sediments (Fig. 5). Similar patterns in nitrogenase activity were observed in the 3 sampling time points in both seagrasses and sediments (Fig. 5). Significantly higher nitrogenase activity was apparent during the day compared to the night in the seagrasses (Wilcoxon matched-pairs test, p = 0.0008). On a dry weight basis, nitrogenase activity in the seagrasses ranged from 2.2 to 131.6 nmol N g^{-1} h^{-1} in the daytime and from 0.9 to 69.7 nmol N g^{-1} h^{-1} at night-time (Fig. 5a). In contrast, significantly higher nitrogenase activity (p = 0.0003) was recorded at night compared with daytime in sediments, ranging from 0.14 to 3.59 nmol N m^{-2} h^{-1} during daytime and 1.17 to 7.01 nmol N m^{-2} h^{-1} at night (Fig. 5b).
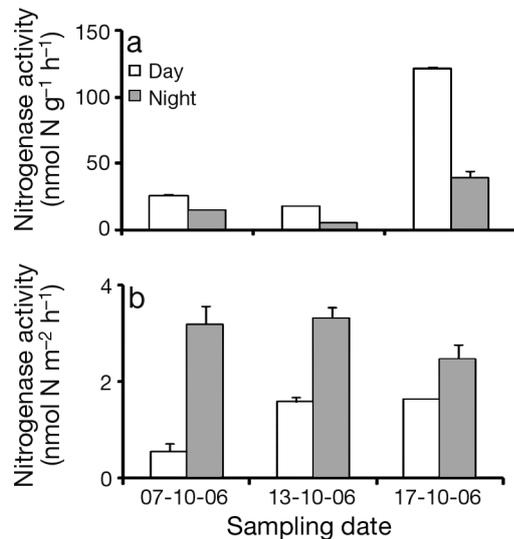
Fig. 5. Day- and night-time nitrogenase activity by (a) epiphytes associated to seagrasses (mixture of *Cymodocea rotundata* and *Halodule uninervis*) and (b) benthic diazotrophs on the seagrass vegetated sediments (n = 9). Error bars represent SD. Dates are dd-mm-yy

Nitrogenase activity in vegetated and unvegetated sediments

To estimate the background nitrogenase activity and the contribution of seagrass cover, activity was measured in the sediments within the seagrass beds (vegetated) and in adjacent bare sediments (unvegetated). Activities were significantly higher (t = 4.021, p = 0.0005) in the vegetated sediments than in the bare sediments (unvegetated) at both sites. The rates of the vegetated sediments ranged from 0.4 to 31.4 µmol N m^{-2} h^{-1}, with an average of 6.7 µmol N m^{-2} h^{-1}, while in the unvegetated sediments rates were undetectable (average of 0.04 µmol N m^{-2} h^{-1}).

Spatial and temporal nitrogenase activity in seagrass meadows (seagrasses and sediments)

The mean total monthly nitrogenase activity recorded for the 4 studied seagrass species (whole seagrass plants) and surface sediments are shown in Fig. 6. The amounts fixed by the seagrasses, expressed per area (using the biomass obtained per unit area), averaged 1.7 and 10.7 µmol N m^{-2} h^{-1} in seagrasses and sediments, respectively, at Site MJ. At the more nutrient-rich Site OR, the nitrogenase levels were lower, averaging 0.6 µmol N m^{-2} h^{-1} in seagrasses and 2.7 µmol N m^{-2} h^{-1} in the sediments. The rates were significantly higher in the sediments than in the seagrasses at both sites (t = 3.654, p = 0.001 and t = 3.21,
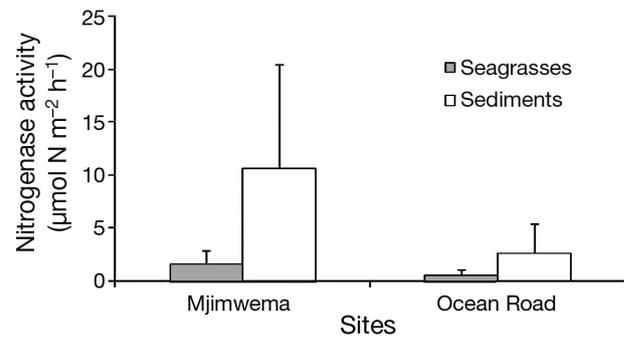

Fig. 6. Nitrogenase activity on seagrasses and on their surrounding sediments at both sites (n = 36). Error bars represent SD

p = 0.004 at Sites MJ and OR, respectively; n = 36). In addition, significantly higher nitrogenase activity was recorded at Site MJ than at Site OR (Mann-Whitney *U*-statistic = 17; U = 127; p = 0.0004).

Furthermore, a distinct annual pattern in nitrogenase activity was apparent when the activities fixed monthly by sediments and the seagrasses at both sites were combined (Fig. 7). Higher nitrogen-fixation rates occurred during October to December at both sites (18.9 and 7.3 µmol N m^{-2} h^{-1} at Sites MJ and OR, respectively) and low rates during June to August. A paired *t*-test showed that there was a significant monthly variation in nitrogenase activity at both sites (p = 0.006 for Site MJ and p = 0.002 for Site OR).

It was also apparent (Fig. 7) that nitrogenase activity during the SEM (June to October) was low, ranging from 3.5 to 11.5 µmol N m^{-2} h^{-1} for Site MJ and 0.5 to 2.7 µmol N m^{-2} h^{-1} for Site OR, compared to during the NEM (December to April) when it ranged from 4.4 to 14.1 µmol N m^{-2} h^{-1} at Site MJ and 0.8 to 5.4 µmol N m^{-2} h^{-1} at Site OR. Statistically the nitrogen-fixation rates were significantly higher (t = 2.56, p = 0.03) during the NEM than during the SEM (see also Fig. 4).
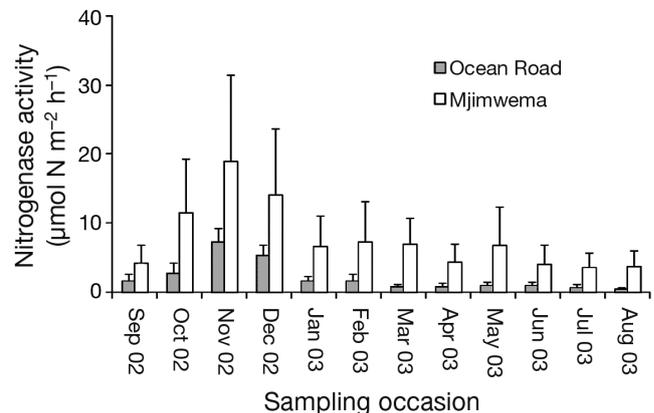

Fig. 7. Monthly nitrogenase activity (2002 and 2003) in seagrass beds at the 2 studied sites (n = 36). Error bars represent SD

DISCUSSION

The present study provides the first detailed information on nitrogen-fixation activity associated with seagrass beds along the Tanzanian coast in the tropical Western Indian Ocean. Our data considerably widens and confirms earlier studies on the importance of seagrass-associated nitrogen fixation in the coastal areas of the Western Indian Ocean. As seen in Table 1, the nitrogen-fixation activity contributed by the seagrass-associated diazotrophs reported here exceeded activities reported from mangrove ecosystems in the region (Lugomela & Bergman 2002, Kyaruzi et al. 2003) and from open intertidal (unvegetated) sediments (Lyimo & Lugomela 2006). Furthermore, the nitrogen-fixation rates of unvegetated sediments corroborate data previously reported within the region (Lyimo & Lugomela 2006).

It is also apparent that the nitrogen-fixation rates were appreciable in all seagrasses, although they varied among the seagrass species examined, often being higher in *Halodule uninervis* than in the others. These results confirm and extend previous observations for other seagrasses (*Ruppia maritime*, *Thalassia testu-*

Table 1. Nitrogenase activity in various habitats in the Western Indian Ocean (WIO) and in some seagrass ecosystems in other geographical regions

Habitat	Locality (season)	N-fixation rate (mg N fixed m^{-2} d^{-1})[a]	Source
Mangrove pneumatophore with epiphytes	Zanzibar Island, WIO	0.23–0.99[b]	Lugomela & Bergman (2002)
Mangrove pneumatophore without epiphytes	Zanzibar Island, WIO	0.05–0.22[b]	Lugomela & Bergman (2002)
Mangrove sediments with microbial mats	Zanzibar Island, WIO	0.05–0.14[b]	Lugomela & Bergman (2002)
Mangrove sediments without microbial mats	Zanzibar Island, WIO	0.01–0.03[b]	Lugomela & Bergman (2002)
Mangrove sediments (sandy)	Zanzibar Island, WIO (yearly)	Mean 0.0053[b]	Kyaruzi et al. (2003)
Mangrove sediments (muddy)	Zanzibar Island, WIO (yearly)	Mean 0.0045[b]	Kyaruzi et al. (2003)
Intertidal sediments	Dar es Salaam, Mafia & Tanga, WIO (dry season)	0.0004–0.053[c]	Lyimo & Lugomela (2006)
Seagrass phyllosphere	Dar es Salaam, Mafia & Tanga, WIO (rain season)	0.006–0.11[c]	Lyimo & Lugomela (2006)
Halodule uninervis	Dar es Salaam, WIO (yearly)	0.03–0.30	Present study
Cymodocea rotundata	Dar es Salaam, WIO (yearly)	0.03–0.38	Present study
Thalassia hemprichii	Dar es Salaam, WIO (yearly)	0.07–0.45	Present study
Thalassodendron ciliatum	Dar es Salaam, WIO (yearly)	0.07–0.33	Present study
Seagrass rhizosphere			
Thalassia hemprichii, *H. uninervis*, and *C. rotundata*	Dar es Salaam, WIO (yearly)	0.90–10.6[c]	Present study
Thalassia hemprichii and *C. serrulata*	Gulf of Carpentaria, Australia (summer)	13–19[d]	Moriarty & O'Donohue (1993)
Zostera capricornia	Moreton Bay, Australia (summer)	25–40[e]	O'Donohue et al. (1991)
Z. marina	Limfjord, Denmark (summer)	4.2–6.0[d]	McGlathery et al. (1998)
Unvegetated sediment	Dar es Salaam, WIO (yearly)	0.005–0.03[c]	Present study

[a]Calculated using a 4:1 conversion ratio in all data from the WIO region
[b]Acetylene added to the headspace of intact corers fixed to a depth of 5 cm
[c]Acetylene added to the headspace of incubation bottles; a corer was used to sample the sediment (only sediments) to a depth of approximately 5 to 10 mm
[d]Measured as acetylene reduction rates using intact core placed in rhizosphere sediments; conversion factor of 3:1 was used
[e]Acetylene injected to the intact core in seagrass bed (rhizosphere and plant); conversion factor used was 3:0.95, calibrated using 15N_2

dinum, *Cymodocea manatorum*, and *Diplanthera wrightii*; Goering & Parker 1972), suggesting that seagrass epiphytes may be common and contribute to 'new' nitrogen inputs in such ecosystems.

The reasons for the activity differences noted among the seagrass species examined here may be related to different colonization patterns or the capacity by the various seagrasses to host diazotrophs. The epiphytic cyanobacterial diversity was for instance previously shown to be higher in *Halodule* spp. than in the other seagrass species examined (Hamisi et al. 2004). Seagrass morphology and metabolic activities may be factors influencing the abundance, composition, and colonization patterns of the epiphytic communities. Plant metabolites such as dissolved inorganic carbon, gaseous exchange, and chemical composition are known to be important factors controlling attached communities in aquatic ecosystems (Wetzel 1993). On a unit area basis, however, the activities associated with *Thalassia hemprichii* were the highest, probably due to its higher biomass and dominance at the study sites. The high variation observed in nitrogen-fixation rates may also be attributed to patchiness in abundance and composition of the diazotrophic organisms.

Nitrogen-fixation rates by below-ground parts (rhizosphere) did not differ significantly from the above-ground parts (phyllosphere) of the seagrasses. Slightly higher activities in the rhizosphere could be due to a high occurrence of heterotrophic nitrogen-fixing bacteria in addition to photoautotrophic cyanobacteria in the phyllosphere. Organic and inorganic compounds from aerial parts of the seagrasses may support a heterotrophic diazotrophic population in the phyllosphere (Capone & Taylor 1980).

Variations in nitrogen-fixation rates among seagrass leaves of different ages suggest differences in colonization patterns by diazotrophs. For instance, the high nitrogen-fixation rates in mid-aged leaves compared to old and young leaves during the NEM correlated positively to the higher cyanobacterial abundance found (microscopic analyses; data not shown). This was the case even though our experiments were conducted during the less-favorable period (NEM) for cyanobacterial colonization according to our previous findings (Hamisi et al. 2004). Potentially, this is due to an ongoing succession in colonization, with cyanobacteria being the first to colonize, subsequently out-competed by micro-algae in older parts (Hamisi et al. 2004). During the SEM, fixation rates were highest in old leaves, possibly due to conditions favoring cyanobacterial colonization and or a colonization of more actively nitrogen-fixing cyanobacteria. This, however, needs to be experimentally verified.

The higher nitrogen-fixation rates in daytime than night-time in the phyllosphere of the seagrass plants,

and vice versa in the rhizosphere, suggest that the filamentous heterocystous cyanobacteria may be the main diazotrophic morphotype on the seagrasses, as these primarily would fix nitrogen aerobically in light (Bergman et al. 1997, Lugomela et al. 2001, Uku et al. 2007, Bauer et al. 2008). Our previous examination showed that the cyanobacterial populations occupying the seagrass plants were dominated by non-heterocystous types (Hamisi et al. 2004) known to restrict their nitrogen fixation to night-time or to micro-aerobic conditions, in order to protect their nitrogenase. However, more recently, Uku et al. (2007), using molecular techniques, identified the heterocystous diazotrophic genus *Calothrix* spp. to be attached to the seagrass leaves. Detailed diurnal and identification studies are therefore warranted for the 2 study sites. In contrast, non-heterocystous filamentous cyanobacteria such as *Oscillatoria* spp. and *Lyngbya* spp., in combination with unicellular and photosynthetic bacteria, were potentially responsible for the high night-time sediment activity (Pereg et al. 1994, Bergman et al. 1997, Pereg-Gerk et al. 2002).

Bare sediments showed an approximately 2 orders of magnitude lower nitrogenase activity compared to sediments within the seagrass meadows. This finding corroborates earlier findings (Capone & Taylor 1980, O'Donohue et al. 1991, McGlathery et al. 1998; see also Table 1), and is supported by previous data showing a higher number of diazotrophic bacteria in the seagrass rhizosphere than in bare sediments (Patriquin & Knowles 1972, McComb et al. 1989, O'Donohue et al. 1991, Hamisi et al. 2004). Together, such observations verify that seagrass meadows selectively attract and function as efficient substrates for nitrogen-fixers compared to bare sediments exposed to the same micro-flora. Seagrass-covered sediments may be less exposed, leading to an increased stability, which in turn stimulates microbial colonization and may release dissolved organic carbon and other growth-promoting molecules that may support growth of diazotrophs.

The monthly variations in nitrogen fixation observed at both sites correlated with the cyanobacterial species diversity and percentage cover found earlier (Hamisi et al. 2004). The higher nitrogen-fixation rates during the NEM compared to the SEM contrast with the higher cyanobacterial diversity and percentage cover during the SEM (Hamisi et al. 2004). However, higher rates during the NEM may be associated with higher temperatures and the occurrence of Fe-containing aeolian dust (Duce et al. 1991, Lugomela 2002), known to promote growth and to stimulate carbon and nitrogen fixation in cyanobacteria (Rueter et al. 1990, Paerl et al. 1994, Lugomela 2002).

The higher nitrogen-fixation rates at Site MJ than at Site OR is likely to be a consequence of the higher lev-

els of terrestrial inputs at Site OR (Lugendo 2000, Hamisi et al. 2004). Increased inorganic nutrient levels, particularly combined nitrogen, may lower or inhibit nitrogenase activity and the competitiveness of dia-zotrophs. Thus, the higher nutrient levels at Site OR stress the importance of lessening eutrophication in favor of promoting atmospheric nitrogen inputs and thereby seagrass productivity in the region.

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