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Full Length Research Paper

The dynamics of bacterial population during growth and decomposition of phytoplankton in a tropical productive pond water ecosystem

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In this study, a field experiment was set up to examine the seasonal dynamics of bacterial population and to investigate the relationship between bacterial abundance and dissolved organic carbon (DOC) produced by phytoplankton in a tropical eutrophic fishpond located in Calabar, South-East Region of Nigeria. Sampling lasted for six months: January to March in the dry season and April to June in the wet season. The highest concentration of DOC released during the dry season was 4.440 mg/L in January, while during the wet season; the highest concentration of 4.992 mg/L was released in April. The patterns of nutrients (nitrate and phosphate) released during both seasons were similar. In the dry season, the range of bacterial count varied between 7.307 x 10^4 and 0.025 x 10^4 CFU/ml while during the wet season it was from 7.909 x 10^4 to 0.019 x 10^4 CFU/ml. The bacteria; *Vibrio, Moraxella* and *Bacillus* species were dominantly present throughout the dry season but during the wet season, the bacteria; *Pseudomonas*, *Flavobacterium, Streptococcus, Bacillus, Corynebacterium* and *Aeromonas* species dominated throughout. Bacterial species succession also took place within seasons. Our results suggest that, during growth and decomposition of phytoplankton, the bacterial community involved shows a successive change not only in generic composition but also in terms of the heterotrophic activity of the bacteria in the community. The correlation analysis between dissolved organic carbon and bacterial count showed a positive correlation (r = 0.95 for the dry season and r = 0.82 for the wet season). There was a significant difference ($p<0.05$) between season in the case of DOC and bacterial count.

Key words: Dissolved organic carbon (DOC), bacteria, nutrient, species, and succession.

INTRODUCTION

Dissolved organic carbon (DOC) is one of the largest reservoirs of reactive carbon in pond water, which play crucial roles in the global geochemical cycle of carbon. The major source for DOC in pond water is biological and related to primary productivity (Tang et al., 2009). Nutrient inputs to lakes, ponds, etc are often dominated by nutrient release from sediments. Rates of nutrient release from the decomposition of sedimentary organic matter such as dead phytoplankton are determined by bacterial demands for food and energy. During phytoplankton growth, dead and decomposition in the pond, primary productivity and its processing by the food web create a heterogeneous environment of particulate, colloidal, and dissolved organic matter in a continuum of size classes and concentrations (Azam, 1998). Major changes in organic matter concentration and composition are expected to occur at different stages of phytoplankton growth and decomposition. The variations in the organic matter regime are typically accompanied by pronounced changes in bacterial abundance, productivity, ectohydrolase activities, and colonization of particles (Smith et al., 1995). For instance, dominance of bacteria

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specialized in colonizing and hydrolyzing phytoplankton detritus would greatly influence the biogeochemical transformation of the detritus. At the same time, free-living species adapted for efficiently utilizing dissolved organic matter could prevent large-scale dissolved organic matter accumulation despite its high production rates typical in phytoplankton growth and bloom (Koike et al., 1990; Smith et al., 1995). Van Hannen et al., (1999) showed that the origin of detritus can indeed affect the structure of the bacterial community.

However, a number of studies have also demonstrated that flagellate grazing has the potential to affect the species composition of a mixed bacterial community. The extent of flagellate grazing of bacteria is affected by bacterial size and motility (Gonzalez et al., 1993; Pinhassi et al., 2004; Pisman et al., 2005; Simek et al., 1992), and protozoa consume and digest various bacterial species with variable efficiencies (Gonzalez et al., 1990). Furthermore, Caron (1987) showed a strong specialization among flagellate species in the ability to graze free-living and attached bacteria and Simek et al. (1997) observed a shift in the numerical dominance of different subdivisions of the class *Proteobacteria* as a consequence of heavy flagellate grazing. However, studies that simultaneously measure changes in the bulk biochemistry and phylogenetic composition of the bacterial community are just beginning to evolve (Rooney-Varga *et al.,* 2005).

The present study was designed to investigate the seasonal fluctuation of the number and type of bacteria, dissolved organic carbon and inorganic nutrients (nitrate and phosphate) during growth and decomposition of phytoplankton in a productive pond water ecosystem. It also attempted to investigate the relationship between bacterial abundance and DOC that may enhance these observed dynamics in bacterial population.

MATERIALS AND METHODS

Study area

The University of Calabar (Unical) fish farm is located in the vicinity of the University of Calabar staff quarters at approximately 04.56°, 020'N and 08.20°, 456'E in Cross River State, Nigeria. The climate of the area is governed by its latitude and to a large extent by the two dominant winds, the Southeast monsoon and the northeast trade winds common in most of West Africa. The area is also characterised by distinct wet and dry seasons. Mean annual rainfall is about 2000 mm and temperatures generally range from 22°C in the wet to 35°C in the dry season (Akpan and Offem, 1993).

Experimental design

The field experiment was mounted at the University of Calabar fish farm. Three tanks, each of about 3000 L volume capacity (two experimental tanks and one control tank) were filled with pond and bore-hole water respectively. The experimental tanks were each pre-treated with 100 g of the fish pond fertilizer 20-20-0 as additional source of nutrient to facilitate phytoplankton growth and bloom. This was necessitated by the low levels of nutrients initially measured (0.01 mg/L for phosphate and 0.4 mg/L for nitrate). The tanks were left to stand for two days exposed to natural environmental conditions before sampling. Just prior to sampling, the tanks were gently stirred with a polyvinyl chloride pipe to resuspend any particulates accumulating around the bottom edges of the tanks. Sampling was accomplished by siphoning 1 L of water from the center of each tank with silicone tubing into polycarbonate carboys. All variables were daily monitored. Aliquots for bacterial count were fixed immediately after sampling, and identification was initiated thereafter. Samples for nutrients and DOC were processed and then immediately frozen for later analysis.

Phytoplankton enumeration and identification

Bulk samples of 50 mL were fixed with Lugol's solution in sedimentation chambers (2.5-10 mL) and cells were enumerated with an inverted microscope according to Utermohl technique as reported by Edler and Elbrachter (2010). Phytoplankton species were identified with the help of taxonomic catalogue proposed by Botes (2003).

Determination of dissolved organic carbon (DOC)

The high temperature combustion method outlined by Bruckner (2011) was used for DOC measurement. According to this method, sample preparation and processing protocol is outlined as follows:

1) The sample was collected in a glass container that was baked in the laboratory at 550°C for 2-4 h (the baking process removes any residual carbon in or on the collection vessel that may cause contamination); 2) A known volume of sample (1000 mL) was filtered through a 0.22 µm pore size nitrocellulose filter with the aid of the vacuum pump. Filters were cleaned by passing deionised water through them before collection to prevent DOC leaching: 3) The filtered samples were combusted at high temperature which involves conversion of inorganic carbon to dissolved CO_2, and purging this from the sample and 4) the remaining (organic) carbon was then oxidized at a high temperature to CO_2 which was detected by the instrument's non dispersive infrared (NDIR) sensor and directly correlated to total organic carbon (TOC) content.

Nutrient analysis

Water samples were filtered through 0.45 µm pore size membrane filters and preserved by freezing at – 10° C.

Determination of phosphate concentration

The Molybdenum blue method reported by Kolo et al. (2010) was used. 5 ml of mixed reagents (mixture of ammonium molybdate solution, sulphuric acid solution, ascorbic acid solution and potassium antimonyl tartrate) in the ratio 1:2.5:1:0.5 was added to 50 ml of the sample and allowed to stand for 30 minto 1 h for colour development. It was read at 885 nm with spectrophotometer.

Determination of nitrate concentration

The Cadmium reduction method of Parsons et al. (1984) was used. The cadmium was shaken several times in copper sulphate solution. Slurry was used to pack the column. The column was washed several times with ammonium chloride buffer. The column was drained and 100 ml of sample was allowed to pass through it. The initial 25-30 ml was discarded. 50 ml was collected from the

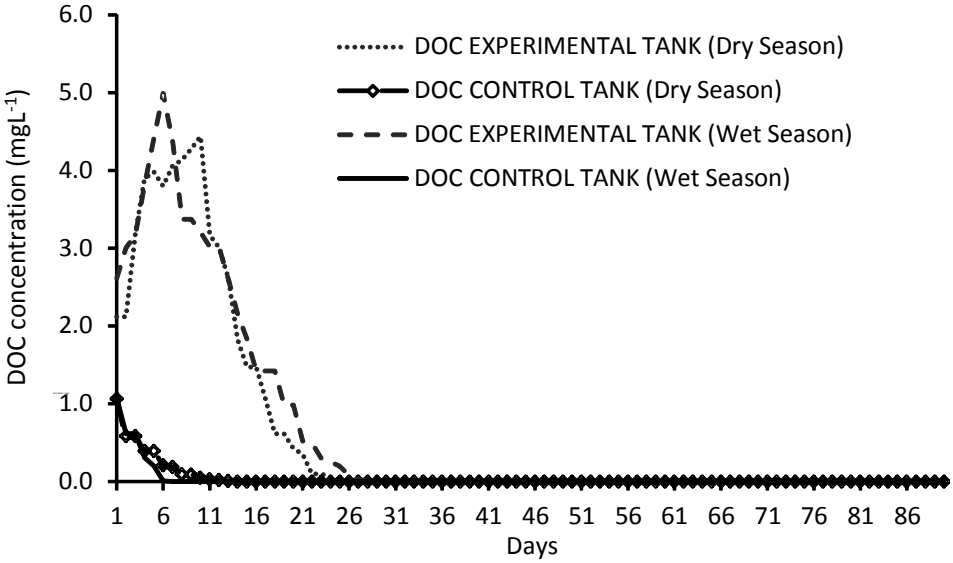

Figure 1. DOC concentrations (mgL^{-1}) during dry and wet seasons.

column as reduced sample used for the analysis. 2 ml of sulphonilic acid was added to the 50 ml reduced sample. It was thoroughly mixed and after 2 to 8 min, 1 ml of naphthylethylene diamine (NED) was added. After 20 min, the concentration was measured at 540 nm against blank.

Enumeration and identification of bacteria

Bacterial cell numbers were counted by staining with 4', 6 – Diamidino - 2 - phenylindole followed by Epifluorescence Microscopy according to the procedure of Porter and Feig (1980). Bacterial cells were also grown on nutrient agar medium by the pour plate method. Isolated colonies were subjected to morphological characterization, differential staining and biochemical testing and, identified to genus level with the aid of Bergey's manual.

Statistical method

Correlation and regression analysis was carried out between dissolved organic carbon and bacterial count.

RESULTS

Phytoplankton abundance

Phytoplankton species richness and successions were observed during both seasons with the class Chlorophyceae (>99%) predominantly present throughout both seasons (data not shown). The phytoplankton community was dominated by *Crucigenia lauterbornei, Selenastrum gracile, Chlorella vulgaris, Gloeobotryls limnetica,* and *Dinema griseolum* during the dry season and by *Scenedesmus dentriculatus, Lobocystis dichotoma, Scenedesmus quadricauta, Goeobotryls limnetica* and *Euglenopsis vorax* during the wet season (data not shown).

DOC concentrations

The DOC concentrations increased up to day 10 and day 6 of the dry and wet seasons respectively to maximum and then started to decline in the experimental tanks. The DOC concentrations in control tanks decreased continuously from onset. As can be seen in Figure 1, the highest concentration of DOC released by phytoplankton during the dry season was 4.440 mg/L, while a higher concentration of 4.992 mg/L was released during the wet season.

Nutrients patterns

The patterns of both nutrients (nitrate and phosphate) analysed during the dry and wet periods were similar. In all cases, a slight increase in nutrient concentration was observed on day 2 of sampling. It then decreased, increased later and then decreased to the end. Also, higher values in nutrient concentrations were observed during the wet season than the dry season (Figures 2a and b).

Bacteria abundance

The fluctuation patterns for bacterial abundances were very similar during both seasons. The highest count of 7.307×10^4 CFU/ml and lowest count of 0.025×10^4 CFU/ml were observed on days 4 and 17 respectively during the dry season. Much higher count of 7.909×10^4 CFU/ml and lowest count of 0.019×10^4 CFU/ml were observed on days 3 and 85 respectively during the wet season. Generally, much higher values of bacterial count were observed during the wet than the dry season (Figure 3).

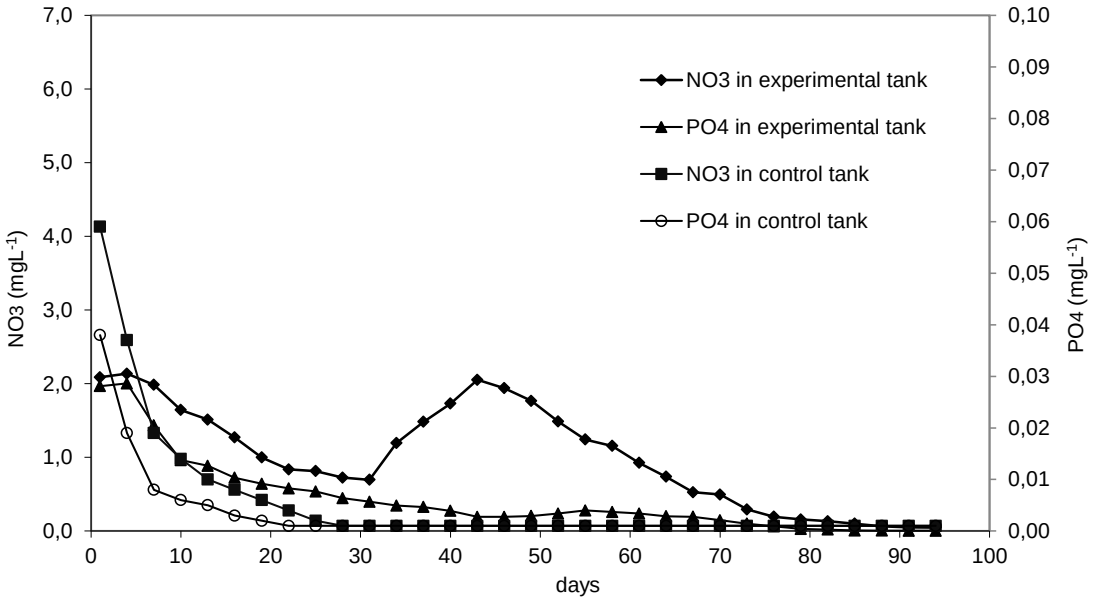

Figure 2a. Nutrient concentrations during the dry season (January to March).

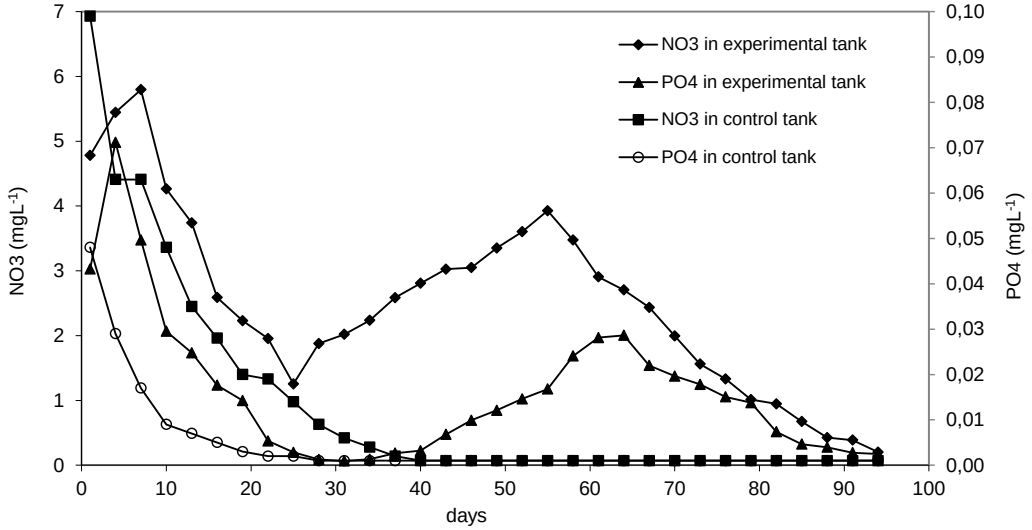

Figure 2b. Nutrient concentrations during the wet season (April to June).

Bacteria species

From biochemical characterization and identification, the bacteria; *Vibrio spp., Moraxella spp* .and *Bacillus spp.* were predominantly present throughout the dry season. The early part of this season was dominated by *Pseudomonas spp.*, *Flavobacterium spp.* and *Micrococcus spp.*, while the later part was dominated by *Corynebacterium spp* and *Streptococcus spp.* (Figure 4a). But during the wet season, the bacteria; *Pseudomonas spp., Flavobacterium spp., Streptococcus sp.p, Bacillus spp., Corynebacterium spp.* and *Aeromonas spp.* were predominantly present throughout. The early part of this period was dominated by *Vibrio spp., Micrococcus spp.* and *Moraxella spp.* (Figure 4b).

Correlation and regression analysis

The correlation analysis between dissolved organic carbon and bacterial count shows a positive correlation between these two variables with linear correlation coefficients (r) of 0.95 and 0.82 for the dry and wet seasons respectively and that, there is a high significant difference (p = 0.05) between DOC and bacterial count during both seasons.

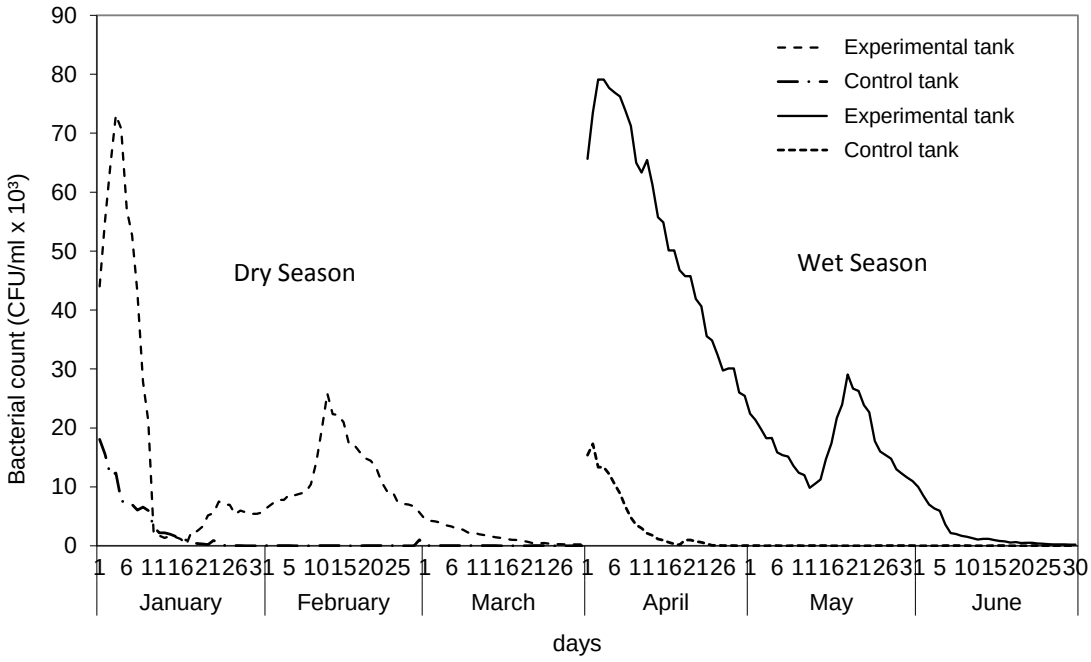

Figure 3. Heterotrophic bacteria distribution during dry and wet seasons.

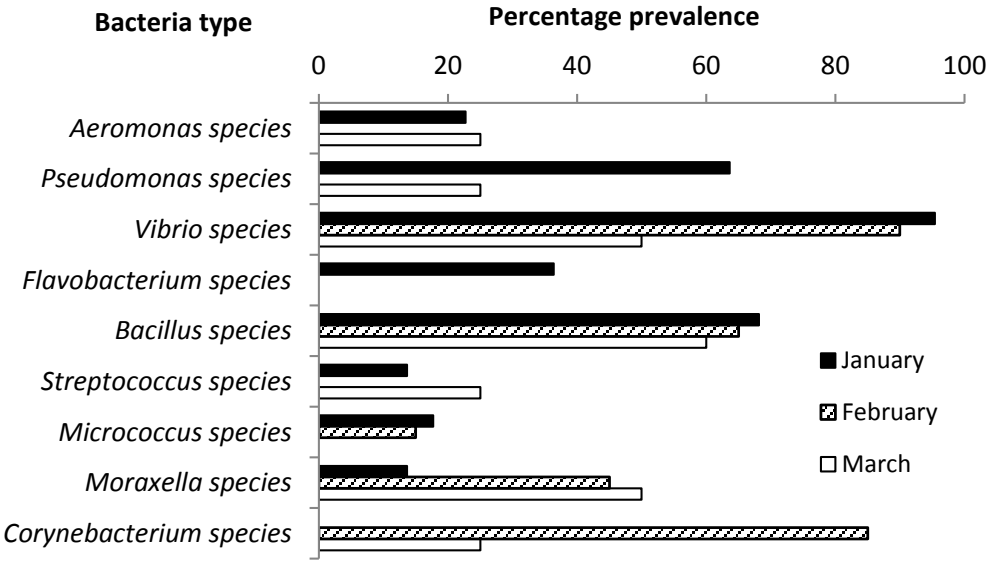

Figure 4a. Percentage prevalence of bacterial species isolated during the dry season.

DISCUSSION

DOC was released mainly through excretion and lysis of phytoplankton and it had a dramatic increase from on set and then decreased later throughout both seasons. The highest concentrations observed (4.440 mg/L for dry season and 4.992 mg/L for wet season) correspond to phytoplankton growth peak where maximum bloom occurs (Figure 1). Bloom may continue until nutrients are exhausted or grazing balances production (Maddocks, 1998). Brussaard (2004) reported that through cellular lysis, viruses indirectly affect the fluxes of energy, nutrients, and organic matter, especially during phytoplankton bloom events when biomass is high. The decreasing phase in DOC corresponds to the dead and decomposition of phytoplankton due to nutrient exhaustion and grazing by zooplankton, and also due to certain chemical substances produced by certain bacteria which show antimicroalgal

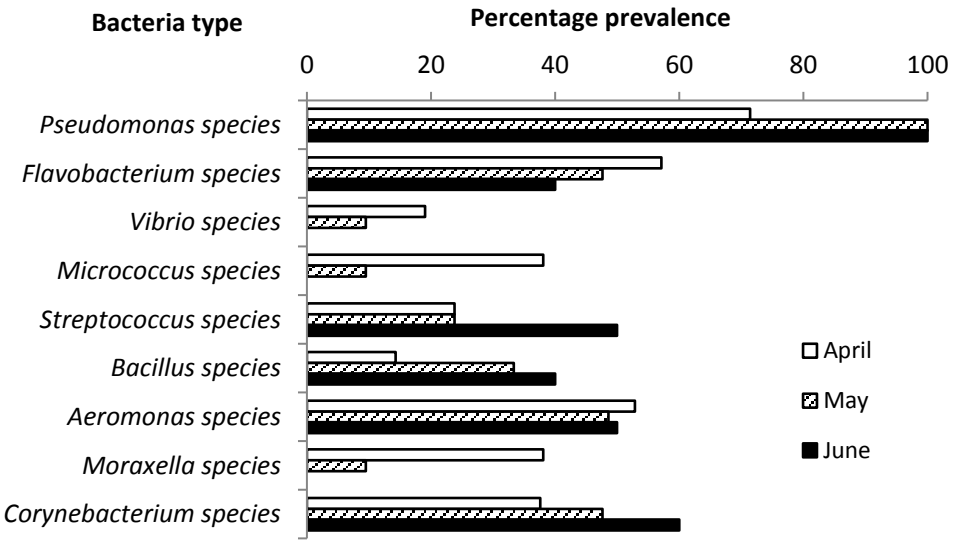

Figure 4b. Percentage prevalence of bacterial species isolated during the wet season.

activity (kawano et al., 1997; Ichiro-lmai, 1997). Tang et al. (2009) reported that this decrease resulted from the absorption of DOC by bacteria.

The periods of increase in nutrients (Figures 2a and b) could be explained by the continuous action of bacteria on dead phytoplankton cells, thereby unlocking or regenerating nutrients for re-use by a new generation leading to succession. The decrease observed in nutrient concentration during both seasons was due to increasing competition by the relatively increasing phytoplankton and bacterial population (Zevenboom and Westeyn, 1990), and also since the phytoplankton dead cells produced at the surface do not decompose in the euphotic zone but reaches greater depths before decomposition, thus transporting nutrients from the surface to deeper water masses. The rate at which this enriched water returns to the surface for re-utilization is very slow (Maddocks, 1998). Nutrient inputs to ponds are often dominated by nutrient release from sediments. Schultz and Urban (2007) explained that rates of nutrient release from the decomposition of sedimentary organic matter are determined by bacterial demands for food and energy.

During growth and decomposition of phytoplankton, fluctuations in the number of heterotrophic bacteria showed trends similar to those reported previously (Fukami et al., 1985). The heterotrophic bacterial count increases sharply from onset to maximum values of 7.307×10^4 and 7.909×10^4 CFU/ml during the dry and wet seasons respectively (Figure 3).This first phase of increase to maximum value was due to the presence of DOC released by the growing phytoplankton, in addition to nitrate and phosphate in the water, which were all utilized by the bacterial community for growth. The second period of slight increase in bacteria count observed was due to nutrient regenerated from dead phytoplankton cells by the decomposition activity of bacteria and according to Riemann et al. (2000), during this phase, attached bacteria could be one or two order of magnitude more than free - living bacteria. There are specific interactions between phytoplankton and the bacteria attached to them, and these interactions influence the composition of both communities (Rooney-Varga *et al*., 2005). Such interactions increase aggregate formation and particle sinking and thus may enhance the efficiency of the biological pump (Gardes et al., 2011). The decreasing phases observed during both seasons were due to the decreasing nutrient concentration and grazing by zooplankton. Tijdens et al. (2008) explained that such temporal changes in bacterial abundances could be significantly related to viral community assemblage, and vice versa, suggesting an interaction between viral and bacterial communities.

Bacterial species succession was observed from month to month within both seasons and from season to season as shown in Figures 4a and b. This is in conformity with some recent studies which have demonstrated bacterial succession both seasonally in the field (Riemann et al., 2008; Tian et al., 2009). Riemann et al. (2000) reported that, the observed changes could have resulted from community succession with bacteria with inherently different metabolic capabilities predominating at different stages of phytoplankton growth and decomposition. Species successions could then affect as well as reflect bacterial-organic matter coupling and biogeochemical fate of phytoplankton growth and decomposition.

From correlation and regression analysis, there exist a positive correlation ($r = 0.95$ for the dry season and $r = 0.82$ for the wet season) and a high significant difference ($p = 0.05$) between DOC and bacterial count during both seasons indicating that, as DOC increases so do bacterial count. This suggests that the DOC produced by phytoplankton through excretion and lysis was the main

source of carbon for the bacterial community. Although potentially toxic phytoplankton would have been involved, they have been shown to produce DOC which was accessible to the bacterial community for growth (Armando, 2001).

Conclusions

This study shows that, growth and decomposition of phytoplankton in a tropical productive pond water ecosystem is accompanied by major changes in bacterial community composition. It further shows the highly dynamic nature of bacterial community composition and strongly suggests that nutrient-induced changes in natural phytoplankton communities lead to significant effects on the structure and functioning of bacterial assemblages as well as on the nature and the rates of bacterially mediated organic matter cycling. This study further emphasises the need to incorporate community composition into our conceptual thinking of the biogeochemical activities of aquatic microbial assemblages.

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