International Research Journal of Natural and Applied Sciences
Vol. 3, Issue 1, Jan 2016 IF- 2.818 ISSN: (2349-4077)
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POTENTIAL NITRIFICATION ACTIVITY AND AUTOTROPHIC NITRIFYING BACTERIA IN HYDROCARBON-IMPACTED MANGROVE SEDIMENT OF THE NIGER DELTA, NIGERIA.

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ABSTRACT

The activities of nitrifying bacteria in the sediment of Qua Iboe River, Cross River and Imo River estuary mangrove ecosystems were investigated using bacteriological technique. Samples were collected over eight month period from the micro-habitat (epipellic and benthic sediment). Potential nitrification activity was measured by short-term assay (< 4 hours) by amending the sediment samples with sodium chlorate to inhibit the oxidation of NO_2-N to NO_3-N throughout the eight month study period and the rates ranged from below detection level to 1.5 nmol N/g dry weight sediment (DWS)/h. The abundance of ammonia-oxidising bacteria (AOB) also varied by site and time and ranged from 10^4 to 10^7 AOB per gram dry weight sediment. Ammonia-oxidising bacteria (AOB) were higher than NOB. Significant ($P<0.05$) correlations were observed between nitrifying activity and denitrifying activity ($r=0.622$) and AOB ($r== 0.682$) and between AOB and NOB ($r=0.686$). This was also observed among other microbiological factors (potential denitrifying activity, Pseudomonas aeruginosa and Alcaligenes faecalis). The significant correlation found for numbers and activities which has rarely been reported, suggests that large proportion of the counted bacteria can also be rapidly activated in short-term activity assays. Weak correlations were generally exhibited among nitrification measures (potential activity and AOB/NOB abundance) and soil parameters (temperature, pH, moisture content, % organic matter, % nitrogen, % carbon and KCl-extractable ammonia and nitrate). This indicates the apparent moderate rate in nitrification which may be as a result of immobilization of ammonium by hydrocarbon-stimulated microbial activity. It also indicates that potential nitrifying activity and

the abundance of micro-organisms responsible for the activity were quite moderate compared to unpolluted soil. Hence, the potential nitrification activity and nitrifying bacterial community in mangrove sediment may not be entirely determined by the absence or presence of hydrocarbon contamination in the environment but by the availability of the dominant factors that control nitrification.

Key word: Potential nitrification activity; presence of nitrifying bacteria; hydrocarbon-impacted mangrove sediment.

Introduction

Estuaries, which are dynamic, complex and unique systems, are among the most productive marine ecosystems in the world (Chapman and Wang, 2001). The pollution problems are characterized by interconnected, complicated interactions, often making the interpretation of the disturbance effects in such ecosystems complex and confusing. Nitrification, a microbial aerobic autotrophic oxidation of ammonia is one of the ammonia removal processes in environment and one of the prominent biochemical processes in the global nitrogen (N) cycle and individual ecosystems. Galloway *et al*. (2004) reported that 30% of fixed-N loss globally occurs in the sediment of estuarine and continental-shelf. Coupled nitrification and denitrification in the estuaries also play a role in critical removal processes of 10 – 80% of anthropogenic N pollution (Seitzinger, 1988). Furthermore, approximately 6 -70% of the N_2 generated from denitrification process was produced from nitrogenous oxides (nitrate and nitrite) derived from nitrification (Nishio *et al*., 1983).

Nitrification is generally favoured by increasing the availability of NH_4^+, the initial substrate for nitrification. It is favoured at moderate pH and in well-aerated soils, but declines as soils become very dry. The temperature response of nitrification is approximately bell-shaped with an optimum between 20^0C and 35^0C. The decline at higher temperatures may be partially due to the increased biological O_2 consumption (Prosser, 1989; Grundmann *et al*, 1995; Parton *et al*, 2001; Avrahami *et al*, 2003). Denitrification is generally favoured by high availability of Labile C as a source of energy and of NO_3^- as an electron acceptor. It is favoured in poorly aerated soils, with a pH close to neutrality. The response of denitrification to temperature is similar to that of nitrification, but can have a higher temperature maximum (Merrill and Zak 1992; Weier *et al*., 1993; Strong and Fillery, 2002; Simek and Cooper, 2002). This biological process has received

significant attention because the conversion of ammonia to nitrate has a great impact on the environment, such as pollution of domestic water with nitrate, eutrophication of surface and groundwater (Kurtz, 1980; Bock *et al*, 1989) and production of greenhouse gases (Tortoso and Hutchinson, 1990). It was reported that nitrifying bacteria, principally ammonia oxidizers, are capable of oxidizing various hydrocarbons and halogenated derivatives (Hymans *et al*. 1998; Keen and Prosser, 1988; Vanelli *et al*, 1990). However, oxidation of hydrocarbons is apparently not done heterotrophically, because the products are neither assimilated nor metabolized by ammonia-oxidising bacteria (AOB) but accumulate out of bacterial cells.

It has been established that all known AOB are obligate chemolithoautotrophs (Watson *et al.*, 1989). Yet recent data have shown that some ammonia oxidizers take up pyruvate (Koops *et al.*, 2003), which challenge the concept of obligate chemolithoautotrophy for AOB. Despite the importance of nitrogen for high productivity of the estuaries of the Niger Delta, nitrification as one of the biochemical processes in the nitrogen cycle has not been studied hitherto.

However, if bacteria are to be used effectively in bioremediation schemes, it is important to obtain information concerning the nitrification process that occurs in the presence of hydrocarbon in a natural soil medium; studies performed with pure cultures ignore interactions of bacteria and environmental components and bacterial diversity (Stephen *et al*, 1998; 1996). The objective of the present study was to determine the potential nitrification activity and the presence of nitrifying bacteria in hydrocarbon-impacted mangrove sediment of the Niger Delta region of Nigeria.

Materials and methods

Study area

The study area of this investigation is the coastal zone of Nigeria in West Africa, which lies between the Niger Delta (7^0 30^0E) and Rio del Ray (8^0 30^0) in the Cameroon Republic (Tahal Consultants, 1979, Tuegels *et al.*, 1992). Within this coastal stretch, mangrove occurs in the estuaries of Imo River, Qua Iboe River and Cross River (Fig 1). The three estuaries connected to each other by means of interriverine creeks constitute a homogenous ecological unit. In this study, only the mangrove swamp ecosystems of the freshwater dominated Cross River, brackish Qua Iboe and Imo River estuaries were investigated.

Sampling procedure

Sampling was conducted between June, 2009 and February, 2010 covering the peak of the wet and dry season in Nigeria. Coastal sediment samples were collected in two ways. Intertidal

sediment samples were obtained with a gravity corer (6.5cm diameter and length of 100cm) to a depth of 10cm. Subtidal sediment samples were collected using Shipek grab sampler (Stainless Steel, 472 x 638 x 442mm size with approximate weight of 60kg. All containers were rinsed at least three times with the water being sampled before collection.

Triplicate samples were usually obtained at each station (Popek, 2003; Radojevic and Bashkin, 1999). Sediment from the various sampling locations were separately mixed and composite sub-samples from the three stations of each ecosystem were placed in glass bottles, kept at lower temperatures in ice cooler to reduce microbial activity. They were transported to the laboratory for analysis. Prior to analysis, the sediment samples were air dried and 2mm sieved.

Sediment physicochemical parameters

Composite sediment sub-samples were removed to determine moisture content (mass basis) and pH (3:1 distilled water: soil). Moisture content on a mass basis (Θm) was measured by weight differences upon drying at 105^{o}C. Total carbon (TC) and total nitrogen (TN) were measured on the dried composites samples using a Leco CN-2000 carbon/nitrogen analyzer (Leco Corp., St. Joseph, Mich). Exchangeable ammonium, nitrate, and nitrite were determined after extraction of the soil samples (three replicates) with 1M KCl (1:5 w/v) for 2h using a Tecator Aquatec® 5400 autoanalyzer. The detection limit was of 0.1mg/kg N for all three compounds. Mineral nitrogen was quantified colorimetrically by the indophenols (ammonium) and cadmium reduction (nitrate) methods (Schmidt and Belser, 1982). Particle size distribution (grain size analysis) was determined by the hydrometer method (AOAC, 1975; Juo, 1979). Additional sample were removed to determine the most probable number for nitrifying bacteria, denitrifying activity and potential nitrification activity. Biological analysis was conducted within 24 hours of sampling.

Enumeration of nitrifying bacteria

Ammonia and nitrite-oxidizing bacteria were enumerated by a Most-Probable-Number (MPN) procedure (Schmidt and Belser, 1994). Suspensions of 5.0g of moist soil and 45ml of sterile phosphate buffer (diluted 1:10), containing 139mg of K_2HPO_4 and 27mg of KH_2PO_4 per litre (pH7.0), were shaken at 100 rpm for 2 hours. (Deni and Pennick, 1999).

Subsamples of the suspensions were diluted in sterile microtitre plates containing the appropriate medium for the ammonium and nitrite oxidizing bacteria. (John *et al*, 2012). Three replicates were made per diluton. Samples were incubated for 14 days at 28^{0}C in the dark. The present of NO_2 was detected with the gricss reagent (Schmidt and Belser, 1982). MPN data with

95% confidence levels were obtained using Cochran's tables (Cochran, 1950) to determine the number of nitrifying bacteria.

Denitrification activity

Denitrification activity assays were performed by the method of Tiedje, 1994. Flasks (500ml) containing 20g of fresh sediment and 50ml of the following medium (gram per liter): KNO_3, 1.01; K_2HPO_4, 0.14; KH_2PO_4, 0.027; glucose.H_2O, 1.98 and chloramphenicol, 0.1. The flasks were flushed with N_2 (purity > 99.9%). After the addition of 10 kPa of acetylene to inhibit nitrous oxide reduction, the flasks were incubated in a horizontal position on a rotary shaker (180 rpm, 20^oC). Nitrous oxide production was measured by injection of head space samples into a gas chromatograph equipped with an electron-capture detector and Hayesep Q column (80^oC) for gas separation. Denitrification activity was calculated from the slope of the linear progression curve of nitrous oxide concentrations during 8h of incubation. Accumulation of nitrous oxide in that period was always linear ($R^2 > 0.90$). The applied concentration of chloramphenicol effectively inhibited the de novo enzyme synthesis (data not shown).

Potential nitrification activity

Short term potential nitrification activity was determined using the procedure described by Schmidt and Belser, (1994). Sediments were slurried by adding 2.5g of moist sediment to a flask containing 50ml of phosphate buffer (1m*M*) and $(NH_4)_2SO_4$ solution (0.250m*M*). Flasks were placed on a reciprocating shaker where 1.0ml of chlorate solution (1*M*) per flask was added. At periodic intervals ranging from 15min to 1hr, a 1.0ml aliquot was removed and frozen in liquid nitrogen to stop the biological reaction. Rates were calculated by a linear regression of nitrite accumulation per gram of oven dried soil during a 2 to 4hours incubation.

Statistical analyses

Statistical analyses were performed using the GLM procedure of the SAS package (SAS Institute, Inc, 1998). Multiple comparisons were conducted using the Student-Newman-Keuls (SNK) procedure. The linear correlation and regression were conducted using the REG procedure.

Results and discussions

Sediment Quality Characteristics

Table 1- 4 shows mean values and standard deviation of pH, total organic carbon (%), total nitrogen content (%), nutritive salts (mg/kg), salinity (%) and percentage sand, silt and clay in sediment studied. The sediments of the three estuary mangrove ecosystems have shown varying

pH from slightly acidic for epipellic sediment (mean values of 5.85) in Qua Iboe estuary during dry seasons and neutral in benthic sediment (mean values of 7.78) of Cross River during wet season. The acidic nature is mainly pronounced in epipellic sediment during the dry season, and this can be attributed to the decomposition of mangrove litter and hydrolysis of tannin in mangrove plants releasing various kinds of organic acids (Liao, 1990). The sediment samples show a variable admixture of sand, silt and clay. Results show that sand (>63μm) was the main component of all sediment samples, with a range from 60.97% to 67.58%. Mean clay contents were in the range of 20.78% to 28.33%. The dominance of sand fraction might be as a result of high energy level in the estuaries, giving the depositional area a sandy beach environment. A variable amount of erosion and decompositions in these estuaries in both seasons is reflected from variable admixture of sand and clay fractions in individual sediment. The high levels of nutritive salts (CO_3^{2-}, SO_4^{2-}, Cl^-, NH_4^+ and NO_3^-) in sediments analyzed for both seasons were indicative of the influences of human mediated activities. Crude oil pollution has also been associated with increase in nutritive salt and salinity levels of aquatic ecosystems (Rhykered *et al*., 1995) and may have contributed to the high concentrations of SO_4^{2-}, Cl^-, and NH_4^+ salts in the epipellic sediments of the three estuary mangrove ecosystems during both seasons.

The mean TOC levels in all sediment analyzed ranged from 4.2% to 13.5%. The TOC levels was higher in epipellic sediments (11.97% and 13.78%) in both wet and dry seasons than that of benthic sediments (5.69% and 6.97%) recorded in the three ecosystems (Qua Iboe, Cross River, Imo River estuaries) Precious studies have found that higher TOC (>3.0%) levels are typically associated with fine sediments and lower TOC levels with coarse sediments (Cho *et al*., 1999). The relatively high concentrations of TOC in epipellic sediments studied reflect "high" organic matter flux to epipellic sediments of Cross River Estuary mangrove ecosystem which can be related to the decomposition of mangrove litter and hydrolysis of tannin in mangrove plants releasing various kinds of organic matter and acids. (Liao, 1990). The total organic nitrogen (TON) ranged from 0.23% to 0.59%. The benthic sediment collected during the dry season has the least nitrogen content of 0.23%, while others fell within the same ranged of 0.4 – 0.59%. However, water sample had the lowest of these variables.

Table 1: Physicochemical characteristics of sediment of Qua Iboe estuary mangrove swamp.

Parameters	**Wet season: June 2009 – Sept 2009**		**Dry season: Nov 2009 – Feb 2010**	
	Epipellic Sediment	Benthic sediment	Epipellic sediment	Benthic sediment
Temp.^{0}C	28.1-29.05 (28.24±0.24)	27.2-27.8 (27.36±0.29)	28.14-29.94 (29.24± 0.4)	27.3-27.4 (27.22± 0.4)
pH	6.24-6.5 (6.34± 0.08)	7.3-7.8 (7.6± 0.26)	5.8-6.5 (6.41± 0.07)	7.1-7.4 (7.21± 0.12)
Moisture content (%)	11.8-13.2 (12.5±1.4)	11.20-14.7 (12.95±0.08)	8.3-10.6 (10.95±1.8)	10.5-12.1 (11.3±0.05)
Organic matter (%)	60.7-67.5 (64.1±1.4)	65.8-70.2 (68±0.04)	60.4-62.6 (61.5±1.2)	54.8-60.9 (57.85±1.12)
TOC(%)	12.2-15.1 (13.56± 1.32)	4.3-5.5 (4.9± 0.46)	9.68-10.64 (10.2± 0.46)	5.3-6.3 (5.69± 0.45)
TON(%)	0.4-0.62 (0.52± 0.12)	0.2-0.26 (0.23± 0.02)	0.21-0.28 (0.25± 0.03)	0.22-0.32 (0.26± 0.04)
C/N	26.1	21.3	40.8	21.9
Nutritive salts				
CO_3^{2-}	90.15-99.6 (95.01± 3.14)	55.6-72.1 (60.48± 9.15)	76.5-99.94 (86.15± 10.18)	49.2-56.1 (53.92± 3.22)
Cl^-	113.13-150.8 (136.84± 17.60)	3.6-7.9 (4.85± 2.16)	86.32-160.25 (121.26 ±32.2)	5.42-5.56 (5.44± 0.04)
SO_4^{2-}	30.14-46.64 (36.86± 6.66)	14.7-22.1 (17.8± 2.32)	33.45-58.8 (47.75± 10.11)	17.1-19.75 (18.68± 1.11)
NO_3^{2-}	30.18-33.1 (31.55± 0.8)	28.9-35.5 (33.45± 3.08)	14.9-18.3 (16.28± 1.11)	7.68-11.8 (8.9± 1.2)
NH_4^+	148.1-205.1 (177.61± 27.7)	10.4-44.7 (20.77± 15.1)	64.6-90.45 (75.65± 11.05)	9.2-9.96 (10.2± 0.78)
THC	21.32-86.19 (50.63± 26.9)	14.30-17.30 (15.57± 1.16)	48.24-132.12 (116.67± 54.62)	37.1-210.42 (131.46± 85.1)
PSD %				
Sand (<50µm)	58.45-64.18 (61.31± 3.91)	60.15-68.4 (64.27± 9.6)	59.9-68.14 (64.22± 0.31)	64.84-68.91 (66.88± 0.85)
Silt (>2-63µm)	10.2-12.62 (11.41± 0.95)	8.5-10.34 (9.42± 2.34)	9.68-10.66 (10.17± 0.4)	9.1-9.78 (9.44± 0.06)
Clay (<2µm)	25.3-29.98 (27.29± 30.1)	14.1-39.56 (26.33± 8.1)	21.54-30.88 (26.21± 0.95)	21.90-25.46 (23.68± 0.70)

All measurements are in mg/kg, except otherwise indicated. Ranges (means, ± SD, n =12)

PSD = Particle Size Distribution
TOC = Total Organic Carbon
TON = Total Organic Nitrogen
THC = Total Hydrocarbon Content

Table 2: Physicochemical characteristics of sediment of Cross River estuary mangrove swamp.

Parameters	**Wet season: June 2009 – Sept 2009**		**Dry season: Nov 2009 – Feb 2010**	
	Epipellic sediment	Benthic Sediment	Epipellic sediment	Benthic Sediment
Temp.^{0}c	26.4-27.5 (27.2 ± 0.3)	27.1 - 27.6 (27.3 ± 0.3)	27.2-28.6 (27.85 ± 0.68)	27.7-27.96 (27.23 ± 0.53)
pH	6.2-6.7 (6.5 ± 0.2)	7.08 - 7.6 (7.23 ± 0.26)	6.85-6.18 (5.98 ± 0.3)	7.3-7.68 (7.5 ± 0.15)
Moisture content (%)	10.10-12.11 11.1±0.5	11.15-12.10 11.63±0.8	9.15-10.20 9.68±11	9.60-11.66 10.63±0.8
Organic matter (%)	62.11-65.62 63.87±3.8	68.5-72.4 70.45±0.9	57.21-61.52 59.37±1.18	58.04-62.54 60.29±2.13
TOC(%)	9.5-11.2 (10.3 ± 0.6)	4.48 - 9.54 (6.97 ± 2.78)	10.66-12.82 (11.47 ± 0.76)	3.8-4.6 (4.2 ± 0.38)
TON(%)	0.26-0.66 (0.4 ± 0.1)	0.32 - 0.55 (0.46 ± 0.07)	0.46-0.69 (0.59 ± 0.09)	0.26-0.48 (0.38 ± 0.11)
C/N	25.8	15.2	19.4	11.1
Nutritive salts				
CO_3^{2-}	62.98-69.6 (65.8 ± 2.8)	48.88 - 52.4 (50.03 ± 2.29)	98.22-108.80 (103.5 ± 5.58)	43.68-53.22 (49.86 ± 3.55)
Cl^-	20.4-24.36 (22.93 ± 1.36)	2.8 - 17.4 (6.93 ± 7.18)	148.25-174.4 (166.9 ± 14.51)	3.5-4.35 (3.91 ± 0.34)
SO_4^{2-}	17.2-20.84 (18.78 ± 1.77)	17.7 - 20.1 (18.95 ± 1.12)	28.4-38.18 (33.97 ± 3.68)	10.8-13.6 (11.68 ± 1.70)
NO_3^{2-}	16.3-24.92 (19.02 ± 4.96)	8.9-35.4 (18.88 ± 12.84)	12.48-13.88 (43.97 ± 6.40)	9.3-10.2 (9.8 ± 0.6)
NH_4^+	46.24-56.82 (50.09 ± 4.56)	10.1-11.9 (13.8 ± 5.6)	36.66-54.76 (43.97 ± 6.40)	9.3-10.88 (9.8 ± 0.6)
THC	1.24-1.65 (1.44± 0.05)	1.11-1.35 (1.23 ± 0.16)	1.55-1.86 (1.7±0.05)	1.4-1.77 (1.58 ±0.25)
PSD %				
Sand (<50µm)	59.4-68.4 (63.90 ± 5.07)	58.2-66.75 (62.48 ± 5.72)	66.74-68.42 (67.58 ± 1.32)	64.02-70.24 (67.13 ± 1)
Silt (>2-63µm)	12.3-18.42 (15.36 ± 3.04)	14.4-16.1 (14.4 ± 1.76)	8.88-12.96 (10.9 ± 0.47)	10.4-12.24 (11.32 ± 1.66)
Clay (<2µm)	11.88-29.62 (20.74 ± 1.08)	19.05-27.2 (23.12 ± 1.88)	21.96-22.7 (22.33 ± 1.04)	18.64-24.46 (21.55 ± 2.06)

All measurements are in mg/kg, except otherwise indicated. Ranges (means, ± SD, n =12)

PSD = Particle Size Distribution
TOC = Total Organic Carbon
TON = Total Organic Nitrogen
THC = Total Hydrocarbon Content

Table 3: Physicochemical characteristics of sediment of Imo River estuary mangrove swamp.

Parameters	**Wet season: June 2009 – Sept. 2009**		**Dry season: Nov 2009 – Feb 2010**	
	Epipellic Sediment	Benthic Sediment	Epipellic sediment	Benthic Sediment
Temp.^{0}C	28.5-29.1 (28.8 ± 0.09)	27.2-27.88 (27.51 ± 0.30)	28.22-29.32 (28.78 ± 0.31)	27.32-28.2 (27.95 ± 0.29)
pH	6.34-6.9 (6.54 ± 0.09)	7.6-7.78 (7.67 ± 0.28)	5.94-6.45 (6.36 ± 0.14)	7.1-7.28 (7.19 ± 0.31)
Moisture content (%)	8.9-15.5 (12.95±0.2)	11.5-15.8 (13.65±0.15)	8.6-10.5 (9.7±0.08)	9.7-12.4 (11.05±1.02)
Organic matter (%)	50.4-58.2 (54.3±0.22)	63.5-68.4 (65.95±0.25)	50.9-59.8 (55.35±0.11)	52.6-60.5 (56.55±0.26)
TOC (%)	12.9-14.9 (13.78 ± 1.34)	4.66-5.84 (5.14 ± 0.52)	9.62-10.78 (10.15 ± 0.35)	5.2-6.2 (5.51 ± 0.53)
TON (%)	0.42-0.68 (0.53 ± 0.14)	0.21-0.28 (0.25 ± 0.04)	0.2-0.28 (0.25 ± 0.08)	0.2-0.3 (0.25 ± 0.04)
C/N	26	18.64	38.48	22
Nutritive salts				
CO_3^{2-}	91.8-98.7 (95.88 ± 3.12)	54.4-70.58 (59.95 ± 9.05)	74.3-96.82 (83.99 ± 10.62)	49.8-55.82 (52.16 ± 8.5)
Cl^-	118.8-140.9 (129.93 ± 17.4)	3.58-7.85 (4.98 ± 2.18)	80.9-155.1 (116.85 ± 20.2)	5.35-5.68 (52.16 ± 8.5)
SO_4^{2-}	35.2-48.5 (42.35 ± 6.82)	12.63-21.56 (17.43 ± 2.34)	33.86-56.7 (47.52 ± 22.4)	17.85-19.85 (18.77 ± 2.12)
NO_3^{2-}	30.2-39.2 (34.7 ± 0.83)	29.82-36.80 (33.99 ± 3.02)	12.88-18.44 (16.19 ± 1.10)	7.4-11.8 (8.84 ± 10.5)
NH_4^+	164.8-202 (172.8 ± 26.9)	13.89-42.82 (21.5 ± 15.3)	54.78-92.2 (74.17 ± 20.5)	9.4-10.65 (10.06 ± 0.45)
THC	1.5-1.68 (1.59±0.02)	1.3-1.60 (1.45±0.12)	1.8-1.96 (1.88± 0.28)	1.6-1.72 (1.66± 0.05)
PSD %				
Sand (<50µm)	59.8-68.6 (64.2 ± 36.6)	60.4-66.4 (63.4 ± 9.52)	52.95-58.4 (61.17 ± 8.23)	52.4-69.54 (60.97 ± 9.20)
Silt (>2-63µm)	10.8-14.9 (12.85 ± 0.98)	11.3-17.1 (14.2 ± 2.32)	9.6-11.4 (10.5 ± 2.56)	10.26-16.98 (13.61 ± 2.21)
Clay (<2µm)	19.52-25.4 (22.95 ± 30.13)	17.1-27.7 (22.40 ± 8.05)	20.2-36.46 (28.33 ± 10.14)	21.36-29.49 (25.42 ± 7.14)

All measurements are in mg/kg, except otherwise indicated. Ranges (means, ± SD, n =12)

PSD = Particle Size Distribution
TOC = Total Organic Carbon
TON = Total Organic Nitrogen
THC = Total Hydrocarbon Content

Table 4: Relationship nitrifying and denitrifying activity with the environmental factors

	Nitrifying activity	AOB	NOB	Denitrifying activity	Sediment Temp.	Moisture Content	pH	NO_3^-	NH_4^+	%Org. Matter	%N
Nitrifying Activity		0.682*	0.596*	0.622*	-0.046	-0.065	0.281	-0.234	-0.266	0.082	0.243
AOB	0.682*		0.686*	0.314*	0.326	0.282	0.330	-0.398*	-0.388*	-0.063	0.098
NOB	0.596*	0.686*		0.398*	0.364**	0.208	0.373**	-0.216	-0.314*	-0.211	0.024

Values with * are significant at the 0.05 level. Values with ** are at the 0.1 level.

Nitrifying bacteria in polluted sediment

Nitrifying bacteria were detected in the hydrocarbon-impacted sediment despite the absence of energy supply (Figure 1- 6). Higher number of nitrifying bacteria determined by classical MPN procedure was detected in the polluted sediment (John, 2012).

Generally, the density of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) ranged from $6.18 \times 10^4 – 1.57 \times 10^7$ and $4.86 \times 10^2 – 3.19 \times 10^4$ cells/g of dry weight sediment (DWS) respectively (Fig. 1 & 2). High prevalence rate of nitrifying bacteria was recorded in the epipellic sediment than the benthic sediment (Fig.4 & 5). Sediments collected during the wet season (June - October) showed high AOB and NOB density in the ranged of $4.16 \times 10^4 – 1.54 \times 10^7$ and $4.46 \times 10^2 – 3.11 \times 10^5$ cells/g DWS respectively. In contrast, the densities of AOB and NOB were much lower in the sediment collected during dry season (November - February) ranging from $2.15 \times 10^2 – 3.18 \times 10^3$ and $2.19 \times 10^2 – 2.96 \times 10^3$ cells/g DWS respectively. However, we recorded higher total hydrocarbon content (THC) in Qua Iboe and Imo River mangrove ecosystems than in Cross River estuary which also accounted for the proliferation of nitrifying bacteria in Cross River estuary than Qua Iboe and Imo River estuaries mangrove ecosystems (Fig 4 & 5). Nevertheless, the survival mechanism of nitrifiers in oxygen-limited environments where they probably have to compete for the available oxygen with heterotrophic bacteria as well as biochemical processes is not known. The competitive ability for a limiting substrate is defined as the ratio of the maximum consumption capacity (ie V_{max}) and the affinity constant (ie K_m) (Hearly, 1980). Nitrifying bacteria are poor competitors for oxygen due to their low V_{max} and High K_m values, respectively; compared with heterotrophs (Laaubrock *et al*., 1994;

Laaubrock and Gerards, 1993) thus nitrifying bacteria in sediment is only possible when there is oxygen, assuming that ammonia is not limiting and that there is no spatial separation between nitrifier and heterotrophs.

Potential Nitrification Activity

In this study, sodium chlorate was used to inhibit the oxidation of NO_2-N to NO_3-N.This allows nitrification to be determined by measuring the accumulation of NO_2-N production. Respective concentrations of ammonium, nitrite and nitrate ranged between 1.2nmol to 1.5nmol N/g DWS/h between the month of June and October, 2009 in all the sampling stations and were below the detection limit of 0.5nmol N/g DWS/h between the month of November, 2009 and February, 2010.These low concentration may be due to the absence of accumulation of any form of inorganic nitrogen and low total nitrogen content that resulted in the absence of sufficient organic nitrogen substrate for nitrifying bacteria. The lack of accumulation of nitrite in the presence of hydrocarbons indicates that hydrocarbons did not affect the oxidation of nitrite to nitrate by nitrite-oxidizers. NH_4-N was the predominant inorganic N in the sediment collected from all sampling stations. Among the sampling points, concentration of ammonia was higher in the epipellic sediment than in the benthic sediment. NO_2-N concentration exhibit relatively similar concentration in the sediment collected from all sampling station. Concentration of NO_3-N in the sediment collected from Qua Iboe was below detectable limit. Imo River mangrove ecosystems had lower concentration than that of Cross River mangrove ecosystem. The absence of inhibition of nitrifying bacteria by hydrocarbons was also confirmed by measurement of the titre in bacteria. However, contaminated soil is a nitrogen-limited environment and the adaptations which we observed may be very important to nitrifier survival in ammonium-limited soil. Whether these adaptations are due to physiological plasticity or to the presence strains specialized for living in an ammonium limited and hydrocarbon- polluted soil which can be examined by using the new molecular techniques for studying the diversity of these organisms (Kowalchuk *et al*., 1997).

Potential nitrifying activity was higher during the wet season (June- October) than the dry season (November - February) (Fig. 3). It was higher in the epipellic sediment of Cross River and Imo River mangrove ecosystems respectively but very low in Qua Iboe mangrove. The later may be attributed to high concentration of crude in the sediment. Nitrification activity was higher than the potential nitrifying activity. Nitrification activity was very higher in the epipellic sediment of Cross River estuary compare with that of Qua Iboe and Imo River mangrove ecosystem (Fig. 6)

Denitrification activity

The results of the denitrifying activity measurements that were determined in the presence of chloramphenicol to inhibit de novo enzyme synthesis shows that denitrification activity was higher in the polluted sediment which may account for the higher rate of denitrifying bacteria (*Pseudomonas aeruginosa, Alcaligenes faecalis, Micrococcus* sp, *Bacillus subtilis, Arthrobacter* sp) in the ecosystem.

Correlation with environmental factors

Table 5 shows the correlation coefficient of the measured variables in relationship with nitrifying and denitrifying activity. Nitrifying activities significantly correlated with denitrification activities ($P<0.05$, $r= 0.622$) and between AOB and NOB ($P<0.05$, $r= 0.686$) in the epipellic sediment. The correlation between the numbers of ammonia- and nitrite-oxidizing bacteria and denitrification activities was clearly evident ($P<0.05$, $r= 0.314$ and $r= 0.398$ respectively). Nitrification activities and numbers were only correlated in the epipellic sediment, whereas this relationship was much weaker in the benthic sediment. Caffrey *et al*. (2007) have found that potential nitrifications in estuarine sediments with different histories of hypoxia do not correlate to abundance of AOB, but correlate positively to abundance of ammonia oxidizing archaea (AOA). Similar results have been reported by Lam *et al*. (2008) in the Endeavour hydrothermal plume. These reports suggest that AOA may play a significant role in nitrogen cycling especially in ammonia oxidation. The ammonium content of the sediment was insignificantly correlated ($P<0.05$, $r= -0.388$ and $r= -0.314$) with the number of ammonia- and nitrite-oxidizing bacteria respectively but the proliferation of nitrifying bacteria was not correlated with the amount of nitrate production.

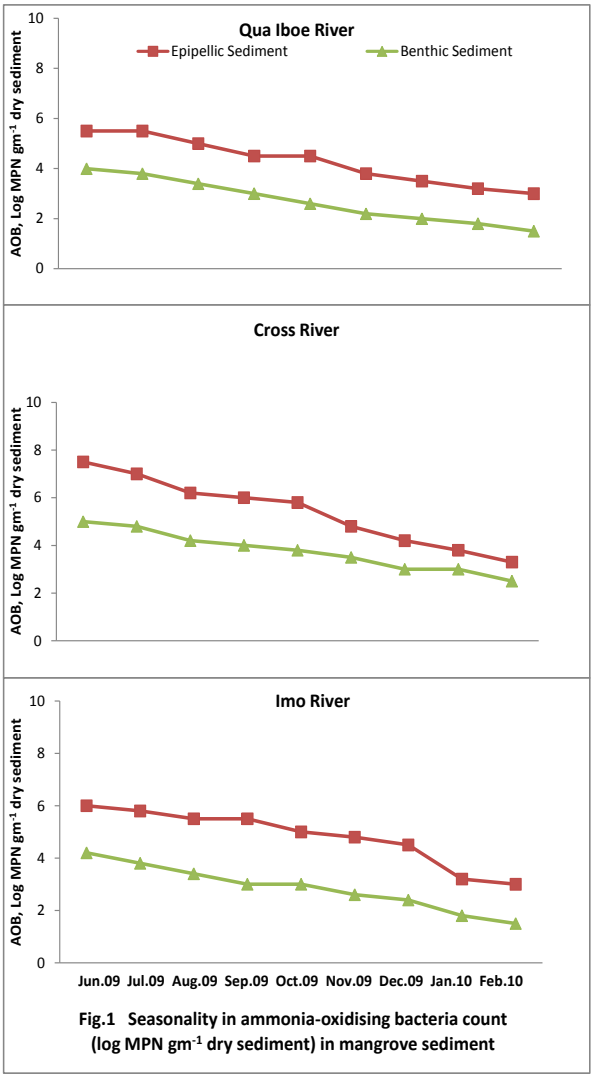

Fig.1 Seasonality in ammonia-oxidising bacteria count (log MPN gm^{-1} dry sediment) in mangrove sediment

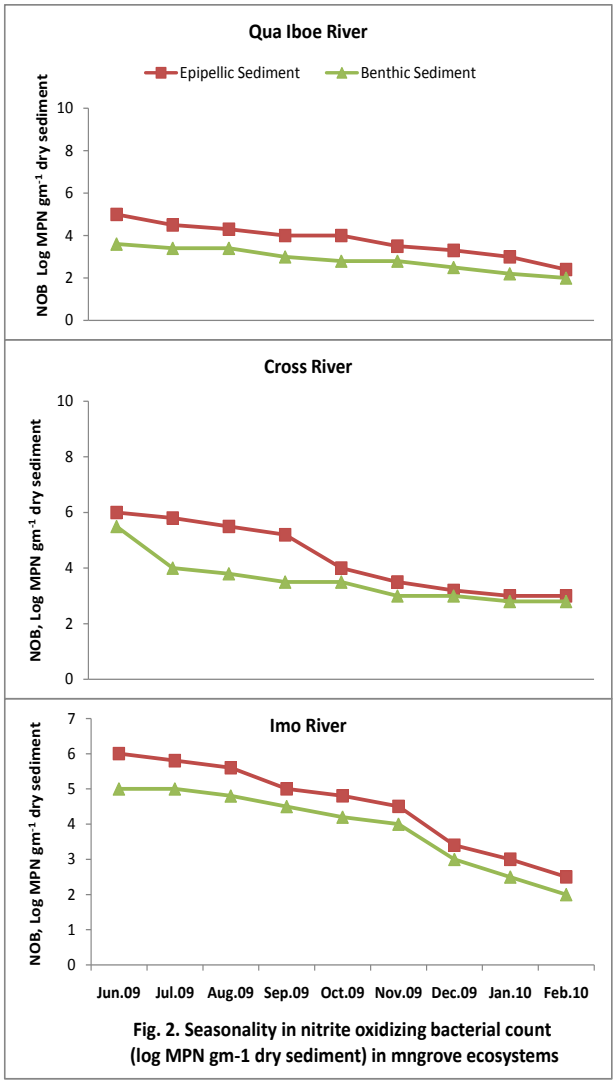

Fig. 2. Seasonality in nitrite oxidizing bacterial count (log MPN gm-1 dry sediment) in mngrove ecosystems

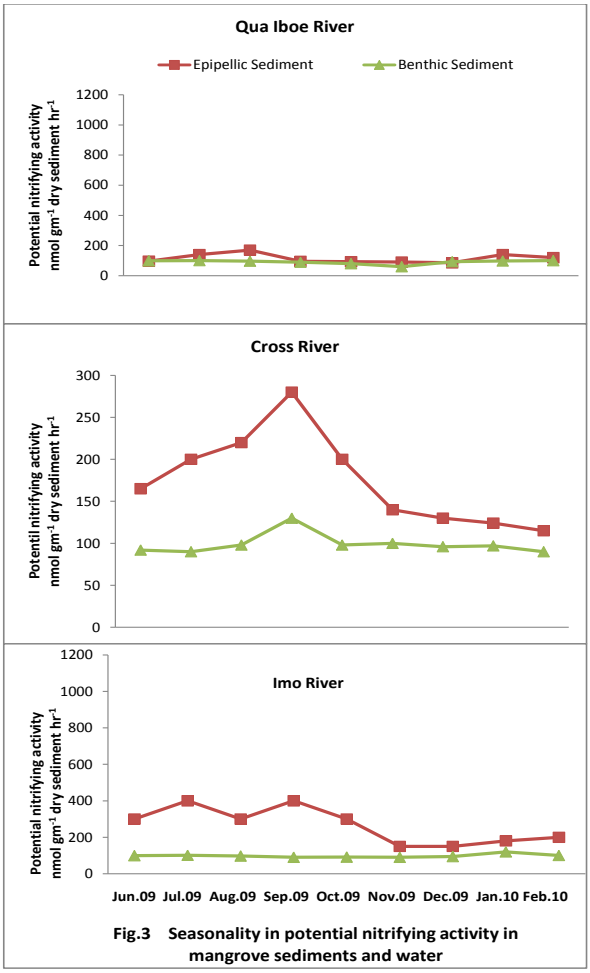

Fig.3 Seasonality in potential nitrifying activity in mangrove sediments and water

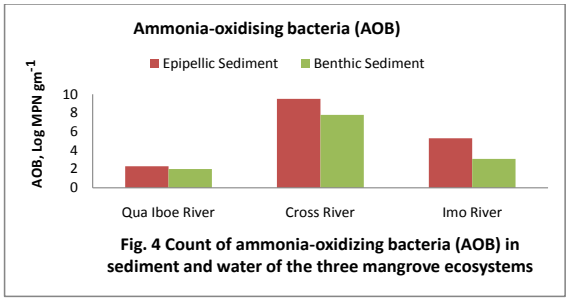

Fig. 4 Count of ammonia-oxidizing bacteria (AOB) in sediment and water of the three mangrove ecosystems

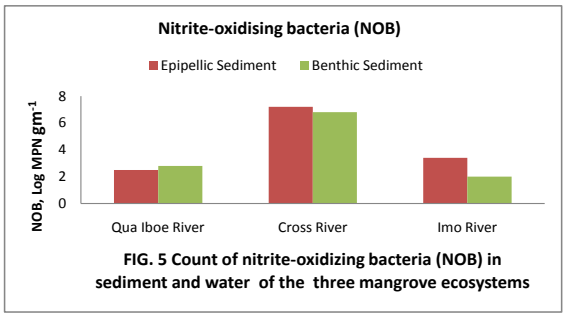

FIG. 5 Count of nitrite-oxidizing bacteria (NOB) in sediment and water of the three mangrove ecosystems

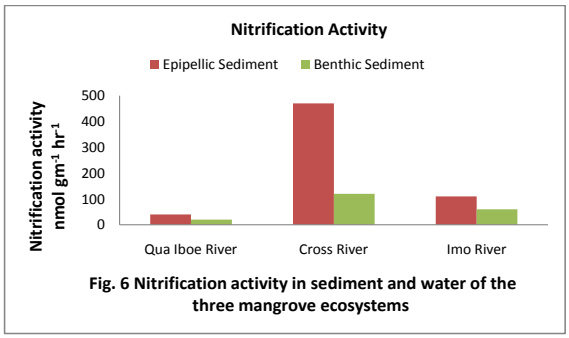

Fig. 6 Nitrification activity in sediment and water of the three mangrove ecosystems

Conclusion

As ammonium and nitrate are the substrate and final production of nitrification, respectively, nitrification is likely to be one of the important processes for supplying nitrate to microorganisms and generally in the nitrogen cycle in the Niger Delta mangrove ecosystems. The rate of ammonium oxidation is significantly affected by the nature of nitrifying bacteria and variety of environmental factors. The dominant factors that control the rates of nitrification in many soils are (i) the supply of NH_4^+ substrate (ii) the acidity (iii) the water content and (iv) the temperature (Bremner and McCarty, 1993; John, 2012). These factors were moderate for nitrification in the different sampling stations use in this investigation. Spatial differences were observed in the activity and distribution of nitrifying microorganisms even when the seasonal effects were minimal. However, correlation between counts of nitrifier, potential activities and environmental factors indicate that sediment temperature, pH, and ammonium may influence nitrifier in mangrove sediment.

References

AOAC(1975). Methods for soil analysis (12th ed.) Washington, DC: Association of Official Analytical Chemist

Avrahami S, Liesack W, Conrad R (2003) Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers. *Environ. Microbiol*., 5, 691– 705

Bremner JM, McCarty GW (1993) Inhibition of nitrification in soil by allelochemicals derived from plants and plants residues, p. 181–218. *In* J. M. Bollag and G. Stotzky (ed.), Soil biochemistry. Maecel Dekker, Inc., New York, N.Y.

Bock E, Koop HP, Harms H (1989) Nitrifying bacteria in: Schlegel, H. G., Bowien, B., (eds), Autotrophic bacteria. Science Tech. Publishers, Madison, WI, pp. 80-96

Caffrey JM, Bano N, K. Kalanetra, Hollibaugh JT (2007). Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. *ISME J*., 1: 660-662.

Chapman PM, Wang F (2001) Assessing sediment contamination in Estuaries. *Environ. Toxicol. Chem*. 20:3-22

Cho YG, Lee CB, Choi MS (1999) Geochemistry of surface sediments off the southern and western coast of Korea. *Marine Geology*, 159: 111-129

Cochran WG (1950) Estimation of bacterial densities by means of the "most probable number." *Biometrics* 6:105–106

Deni J, Penninckx MJ (1999) Nitrification and Autotrophic nitrifying bacteria in a hydrocarbon-polluted soil. *Appl.Environ.Microbiol*. 9:4008-4013

Galloway, J. N., F. J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, D.M. Karl, A.F. Michaels, J.H. Porter,

A.R. Townsend and C.J. Vörösmarty, (2004). Nitrogen cycles: past, present, and future. *Biogeochemistry*, 70: 153-226.

Grundmann, G. L., P. Renault, L. Rosso and R. Bardin (1995). Differential effects of soil water content and temperature on nitrification and aeration. *Soil. Sci. Soc. Am* . J. 59:1342-1349

Healy, F. P. (1980). Slope of the monod equation as an indicator of advantage in nutrient competition. *Microb. Ecol*. 5:281–286.

Hyman, M. R., I. B. Murton, and J. D. Arp. (1998). Interaction of ammonia monooxygenase from *Nitrosomonas europaea* with alkanes, alkenes, and alkynes. *Appl. Environ. Microbiol*. 64:3187–3190.

John, R. C. (2012). Ecology and hydrocarbon degrading capabilities of Nitrifying and denitrifying bacteria from sediment of the Niger Delta mangrove ecosystems. Ph. D. Thesis, University of PortHarcourt, Nigeria, p 425

John, R. C. and Okpokwasili, G. C. (2012). Crude oil-degradation and plasmid profile of nitrifying bacteria isolated from oil-impacted mangrove sediment in the Niger Delta of Nigeria. *Bull. Environ. Contam. Toxicol*. 88: 1020-1026

Juo, A. S. R. (1979). Selected methods for soil and plant analysis: Manual series. p.70 Ibadan: International Instittute of Tropical Agriculture (IITA).

Keen, G. A., Prosser, J. I. (1988). The surface growth and activity of Nitrobacter. *Microb. Ecol*. 15: 21-39

Koops, H. P., Purkhold, U., Pommerening-Roser, A., Timmermann, G., Wagner, M.(2003). The lithoautotrophic ammonia oxidizers. In:Dworkin, M., *et al*., eds), The prokaryotes: An Evolving Electronic Resource for the microbiological community. Springer, New York.

Kowalchuk, G. A., J. R. Stephen, W. De Boer, J. I. Prosser, T. M. Embley, and J. W. Woldendorp. (1997). Analysis of ammonia-oxidizing bacteria of the b-subdivision of the class *Proteobacteria* in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. *Appl. Environ. Microbiol*. 63:1489–1497.

Kurtz, L. T., (1980). Potential of nitrogen loss. In: nitrification inhibitors-potentials and limitations, ASA Special Publication No. 38 American Society of Agronomy, Soil Science Society of America, Madison WI, pp 1-18

Laanbroek, H. J., and S. Gerards. 1993. Competition for limiting amounts of oxygen between *Nitrosomonas europaea* and *Nitrobacter winogradskyi* grown in mixed continuous cultures. *Arch. Microbiol*. 159: 453–459.

Laanbroek, H. J., and J. W. Woldendorp (1995). Activity of chemolithotrophic nitrifying bacteria under stress in natural soils, p. 275–304. *In* J. Gwynfryn Jones (ed.), Advances in microbial ecology,vol. 14. Plenum Press, NewYork.

Lam, P., J.P. Cowen, B.N. Popp and R.D. Jones (2008). Microbial ammonia oxidation and enhanced nitrogen cycling in the Endeavour hydrothermal plume. *Geochimica et Cosmochimica Acta*, 72: 2268-2286.

Liao, J. F. (1990). The chemical properties of the mangrove solonchak in the Northeast part of Hainan Island. Acta Scientiarum Naturalium Universitis Sunyatseni, 9 (4), 67-72 supp.

Merril, A. G. and D. K. Zak (1992). Factors controlling denitrification rates in upland and swamp forests. *Can. J. For. Res*. 22: 1597-1604

Nishio, T., I. Koike and A. Hattori (1983). Estimates of denitrification and nitrification in coastal and estuarine sediments. *App. Environ. Microbiol*., 45: 444-450.

Parton, W, J.,E.A.Holland, S. J. Del Grosso, M. D. Hartman, R. E. Martin, A. R. Mosier, D. S. Ojima and D. S. Schimel (2001). Generalized model for NO_X and N_2O emissions from soils. J. Geophy. Res. 106:17,403-17419

Popek, E. P. (2003). Sampling and analysis of environmental pollutants: a complete guide p. 356. USA:Academic.

Prosser, J. I. (1989). Autotrophic nitrification in bacteria. *Adv. Microb.Physiol*. 30:125–181.

Radojevic M. and Bashkin, V. N. (1999). Practical environmental analysis. Royal society of Chemists.465p

Rhykered, R. I.,Weaver, R. W. and Mclnnes, K. J. (1995). Influence of salinity on bioremediation of oil in soil. *Environ. Pollut*.: 90:127-130

SAS Institute. 1998. *SAS/STAT User's Guide*. Version 7. Vol. 2. 4th ed. Cary, N. C.: SAS Institute.

Schmidt, E. L., and L. W. Belser. (1982). Nitrifying bacteria, p. 1027–1042. *In* A. Page (ed.), Methods of soil analysis, part 2. Chemical and microbiological properties. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wis.

Schmidt, E. L., and L. W. Belser. (1994). Autotrophic nitrifying bacteria, p.159–177. *In* R. W. Weaver, J. S. Angle, and P. S. Bottemley (ed.), Methods of soil analyses. Part 2:

Microbiological and biochemical properties. Soil Science Society of America, Inc., Madison, Wis.

Seitzinger, S. (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnol. Oceanograph*. 33: 702-724.

Simek, M. and J. E. Cooper (2002). The influence of soil ph on denitrification: progress towards the understanding of this interaction over the last 50 years. *Eur. J. Soil Sci*. 53: 345-354

Stephen, J. R., A. E. McCaig, Z. Smith, J. I. Prosser, and T. M. Embley. (1996). Molecular diversity of soil and marine 16S rDNA sequence related to b-subgroup ammonia-oxidizing bacteria. *Appl. Environ. Microbiol*. 62:4147–4154.

Stephen, J. R., G. A. Kowalchuk, M. V. Bruns, A. E. McCaig, C. G. Phillips, T. M. Embley, and J. I.Prosser. (1998). Analysis of b-subgroup proteobacterial ammonia oxidizer population in soil by denaturing gradient gel electrophoresis analytical and hierarchical phylogenetic probing. *Appl. Environ. Microbiol*. 64:2958–2965.

Strong, D. T. and I. R. P. Fillery (2002). Denitrification response to nitrate concentrations in sandy soils. *Soil Biol. Biochem*.34: 945-954

Tahal Consultants (1979). (Nigeria) Ltd. Qua Iboe River Basin. Pre-feasibility study. Cross River Basin Development Authority, Nigeria. Vol 2, Annex II: A1-14

Teugels, G.C.,Reid, F.M. and King,R.P. (1992).Fishes of the Cross River Basin (Cameroun-Nigeria): taxanomy, zoogeography, ecology and conservation. *Annals Science Zoologiques*. 1:216-248

Tiedje, J. M. (1994). Denitrifiers, p. 245–267. *In* R. W. Weaver, J. S. Angle, and P. S. Bottemley (ed.), Methods of soil analysis. Part 2. Microbiological and biochemical properties. Soil Science ociety of America, Inc.,Madison, Wis.

Tortoso, A. C., Hutchinson, G. L. (1990). Contribution of autotrophic and heterotrophic nitrifiers to soil NO and N_2O emissions. *Appl. Environ. Microbiol*. 56:1797-1805

Vanelli, T., Logan, M., Arciero, D. M., Hooper, A. B.(1990). Degradation of halogenated aliphatic compounds by ammonia-oxidizing bacterium: Nitrosomonas europaea. *Appl. Environ. Microbiol*. 56:1169-1171

Watson, S.W., Bock, E., Harms, H., Koops, H.-P., and Hooper, A.B. 1989. Nitrifying bacteria. *In* Bergey's manual of systematic bacteriology. Vol. 3. *Edited by* J.T. Stanley. Williams and Wilkins, Baltimore, Md. pp. 1808–1834.

Weier, K. L., J. W. Doran, J. F. Power, and D. T. Walters (1993), Denitrification and the dinitrogen / nitrous oxide ratio as affected by soil water, available carbon, and nitrate *Soil. Sci. Soc. Am. J.,* 57, 66– 72.