Ammonia Oxidation Potential and Microbial Diversity in Sediments from Experimental Bench-Scale Oxygen-Activated Nitrification Wetlands

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation/thesis of JENNIFER ALLEN find it satisfactory and recommend that it be accepted.

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(Wiebner *et al*., 2002; Jespersen *et al*., 1998; Inubushi *et al*., 2002; Yoshida, 1981, Wu *et al.*, 2001). Rhizosphere oxidation could also explain the pattern of ammonia oxidation potential in my studies control mesocosms. Sediments from the central sampling sites had the highest ammonia oxidation potential and *Typha spp.* densities in the mesocosm.

Because nitrification can occur when in oxygen concentrations are as low as 0.05 mg/L, ammonia availability often limits nitrification rates (Bothe *et al*., 2000; Albeliovich, 1987). The comparatively higher ammonia oxidation potentials documented in this study are partially attributed to enhanced ammonia availability. Ammonium oxidation is the rate-limiting step in nitrification and slow growing nitrifying bacteria are sensitive to ammonia concentrations (Chu *et al*., 2008, Limpiyakorn *et al*., 2007); high substrate availability enhances substrate utilization and subsequently increases ammonia oxidation potentials (Tchobanoglou*s et al.*, 2003). Similar conclusions were established when Gorra *et al*. (2007) documented specific effects of ammonium concentration on ammonia oxidation potentials in sediments from a CTW. Sediments from an established CTW were treated with 2.5 mM and 25 mM ammonium. Ammonia oxidization potential associated with the 25 mM treatment was consistently and significantly higher. The results indicated that ammonia oxidation potentials were limited by nitrifying species' sensitivity to low ammonium concentrations. Arable soils treated with ammonia reflected similar results. Comparing ammonia oxidation potentials of arable soils pre-incubated in ambient air, ammonia, carbon monoxide and methane, ammonia oxidation potentials were highest in the ammonia incubation (Bender and Conrad, 1994). Increasing ammonia concentrations also increases ammonia oxidizing bacterial cell counts. Okano *et al*. (2004) compared ammonia oxidizing bacterial growth yields of soils treated with 1.5 mM and 7.5 mM ammonia. At the completion of the seven-day study, these soils had growth yields of 5.6

x 10^6 cells/µmol and 1.8 x 10^7 cells/µmol. Prior to the treatment, AOB were approximately 0.4 % of the total bacterial populations. Post-treatment AOB populations increased to 3.1 and 5.7% of total bacterial populations in the 1.5 and 7.5 mM treatments.

The estimated AOB cell counts in our study (6 x 10^7 cells/g-dw in the entrance and exit of control mesocosms to a peak of 5 x 10^8 cells/g-dw in the entrance of the oxygen-activated mesocosms) were higher than cell counts in wastewater treatment plant sludge and arable soils, as determined by Okano *et al.* (2004) (0.5-1.5 x 10^7 cells/g-dw) and Mendum, *et al.* (1999)(1.4 x 10^4-6.5 x 10^6 cells/g-dw), but comparable to those documented by Urakawa *et al*. (2006)(5.7-8. x 10^8 cells/g-dw) in a canal receiving wastewater. Other AOB counts in fertilized arable soils (~6.2x 10^7 cells/g -dw) were very similar to the estimated values of this study (Hermansson and Lindgren, 2001). Unfertilized soils evaluated in this latter study had AOB populations approximately one-third of the fertilized cell counts, corroborating the researchers' hypothesis that N fertilization enhances AOB biomass yield and nitrification. Total bacterial cell counts could not be estimated in our samples via the nitrification assay or conventional PCR, so it is impossible to determine the fraction of AOB to total bacteria in sediments from the wetland sediments. But the ongoing application of real time PCR will allow for such an evaluation (see Future Research subsection).

Microbial Diversity

A key finding in this study was the presence of *Nitrosomonas oligotropha,* a common AOB, in sediments from the oxygen-activated wetland mesocosms; no AOB were isolated in sediments from control mesocosm. *Nitrospira spp*., a nitrite oxidizer, was found in sediments

from both mesocosms. Three key observations in the sediments from the oxygen-activated mesocosms, the presence of AOB, and high rates of ammonia oxidation potential, supports the contention by Palmer and Beutel (2009) that oxygenation will 'activate' wetland sediments and led to higher rates of biological ammonia oxidation in the experimental wetlands. Results from the present study regarding the dominant species of nitrifying bacteria in oxygen-activated sediments are similar to a number of studies of AOB species diversity in wastewater treatment reactors. Ammonia utilization varies among AOB (Metcalf and Eddy, 2003). As a result, species diversity will also vary with ammonia concentration, which has been the focus of a number of AOB studies of nitrifying activated sludge. Limpiyakporn *et al*. (2007) treated reactors with four different ammonium concentrations to determine effects on ammonia oxidizing community. Results indicated *Nitrosomonas oligotropha* dominance at 2 mM, 5 mM and 10 mM ammonium treatments. *Nitrosomonas europaea* and *Nitrosococus mobilis* were dominant in the 30 mM ammonium treatment. Although present at the beginning of the 30 mM treatment, *N. oligotropha* was undetected in the reactor by the second week, indicating that *N. europaea* and *Nitrosococus mobilis* out-competed *N. oligotropha* at high ammonium concentrations. Suwa *et al.* (1994; 1997) and Bollmann and Laanbroek (2006) documented similar *N. oligotropha* dominance at low ammonium concentrations in both activated sludge and estuarine sediments.

DO utilization varies among species, indicating that oxygen concentration will also affect community diversity and activity. Guo *et al.* (2009) documented nitrification and total AOB population changes during high and low oxygen treatments. Nitrification rates were greater in the high DO reactor. The accompanying flouresence in-situ hibridization (FISH) analysis confirmed that AOB populations in the high and low DO reactors ranged from 9-12% and 6-8%,

respectively, and signified that high DO enhances both nitrification and AOB populations. In a study documenting the effects of DO on specific ammonia-oxidizing bacterial communities, Park and Noguera (2004) documented a clear species differential between the high and low DO chemostat reactors (~8.5 and < 0.24 mg-DO/L) within the first 56 days after start up. *N. oligotropha* dominated in the high DO chemostat reactor during the first four months of study, at which time dominance shifted to *N. europaea*. *N. europaea* remained dominant in the low DO reactor (<0.24 mg/L) throughout the duration of the study. Similar to findings by Beutel and Palmer (2008), the high DO reactor nitrified over 90% of the ammonium within days of initial start up. Complete nitrification was eventually reached in the low DO reactor but it took approximately one month. Community changes to a full scale WWTP were also examined in the Park and Noguera (2004) study. At the completion of the three month study, *N. oligotropha* and *N. europaea* were dominant in the high and low DO reactors (<7.4 and < 0.8 mg/L).

Some studies have examined AOB in environmental systems, but only a fraction of these have looked at nitrifier diversity in sediments from aquatic settings such as lakes, rivers and wetlands. A study examining ammonia-oxidizing communities in wetlands found that the dominant species were member of the "phylogenically young" *Nitrosospira* lineage (Gorra *et al.*, 2007). *Nitrosospira spp.* are beta-proteobacterial AOB but they do not belong to the same genera as *Nitrosomonas* (Dworkin *et al.*, 2006). *Nitrosospira* is often the dominant AOB genera in submerged soil systems like wetlands (Haleem *et al.*, 2003; Ikenaga *et al.*, 2003, Hails *et al.* 2004, *Ibekwe, et al.*, 2003). This is a noted difference from wastewater treatment plants, which are generally dominated by *Nitrosomonas spp.* (Suwa *et al.*, 1994; Suwa *et al.*, 1997; Bollmann and Laanbroek, 2006; Park and Noguera, 2004; Limpiyakorn *et al.*, 2005; Limpiyakorn *et al.*, 2007). The fact that *Nitrosomonas oligotropha*, rather than *Nitrosospira spp.*, was the dominant

AOB in oxygenated sediments from the experimental mesocosms suggests that the oxygen-activated treatment wetlands were more of a 'treatment systems' than a 'natural' wetland. Bernhard *et al.* (2005) determined that salinity affected AOB community diversity. The results from low, mid, and high salinity locations showed that seasonal and community diversity decreased as salinity increased. The dominant species were *N. oligotropha* and *N. ureae*, which correlated to findings in other estuaries (Bollman and Laanbroek, 2002).

Species richness was comparable in the separate treatments (Fig. 4); approximately forty different species were identified in each treatment (Table 2). Although some species were present in both treatments, the majority of the identifiable species were unique to each treatment. The control and oxygen-activated lineage diversities were comparable to those found in a shallow eutrophic lake (Tamaki *et al.*, 2005). Other studies outlining wetland species diversity showed that plants had negligible effects on diversity (Baptista *et al*., 2008; Gorra *et al*., 2007). Because the wastewater fed to each of the treatments was identical, the oxygenation likely had the largest effect on species diversity.

The low DO in the control wetland mesocosms did not reduce microbial species diversity. Instead, bacteria with low substrate utilization rates proliferated by out-competing other organisms. Filamentous cyanobacteria diversity burgeoned in the low DO control mesocosms (Table 1 and 2), which coincided with findings by Metcalf and Eddy (2003) in low oxygen wastewater reactors. Other species lack the low oxygen substrate utilization rates characteristic of filamentous bacteria, which allows these bacteria to out compete other species. Large portions of the identified species in the control mesocosms were these filamentous aerobic phototrophs (Fig. 5). Nitrite oxidizing species were among the bacteria found in the control mesocosm. Ammonia oxidization is the rate-limiting step during nitrification (Chu *et al*., 2008,

Limpiyakorn *et al*., 2007) but oxygen concentrations in the control mesocosms were high enough for some nitrification to proceed (Bothe *et al*., 2000; Albeliovich, 1987). Thus, it is unlikely that the low oxygen concentration in the control is solely responsible for the absence of AOB and the low oxidation rates. Instead, fast growing heterotrophic bacteria likely out competed the slow growing lithotrophic AOB for the limited oxygen supply (Metcalf and Eddy, 2001; Madigan and Martinko, 2004). In contrast, the high oxygen concentrations in oxygen-activated mesocosms may have been toxic to some heterotrophic bacteria, which provided comparatively slower growing lithotrophic AOB with opportunities to prosper. Mikell *et al.* (1986) documented the biomass of four heterotrophic bacteria in benthic sediments underlying perpetually high DO waters of an Antarctic lake. Maximum cell density fell as a result of the high DO concentrations, suggesting that elevated DO concentrations inhibited the heterotrophic species diversity. Thus, oxygen-activation could have inhibited heterotrophic diversity while simultaneously enhancing lithotrophic diversity.

Sulfur- and iron-reducing bacteria were identified in both treatments, but more sulfur-reducing species were identified in the control mesocosms. In addition, nitrate-reducing (denitrifying) bacteria were identified only in the control sediments. Thus, while high oxygen concentrations in the oxygen-activated mesocosms enhanced ammonia oxidation, it appears to have inhibited anaerobic metabolic processes such as sulfate and nitrate reduction. Similarly, fermenting bacteria were identified in both treatments but none of the species were held in common. Again, this difference is attributed to different oxygen tolerance levels of fermenting bacteria, as well as the difference in overall environmental conditions in the oxygen-activated and control mesocosms.

CONCLUSIONS

The primary goal of this research was to determine the effects of oxygenation on microbial activity and diversity in CTW sediments. Using the chlorate inhibition technique, the ammonia oxidation potential was calculated as nitrite mass from the soil accumulated over time. Traditional PCR techniques were also used to identify the microbial communities in each treatment. During the nitrification assay, all sediment samples from oxygen-activated mesocosms accumulated high levels of nitrite while the control mesocosms showed a slow nitrite accumulation over the course of the assay. Pooling and comparing the samples by treatment, ammonia oxidation potentials were significantly higher in the oxygen activated mesocosms (2.6 ± 0.80 mg-N/g-dw/d) then the control mesocosms (0.48 ± 0.20 mg-N/g-dw/d) and values documented in other studies. The increased rates under oxygenated conditions are attributed to enhanced DO availability at the sediment-water interface in the wetlands, and resulting increased rates of ammonia oxidation by AOB. This proposition is supported by the observation that nitrification potential in sediments dropped from the inlet to the exit as oxygen and ammonia were consumed along the length of the activated mesocosms, a pattern absent from the control mesocosm. *Nitrosomonas oligotropha* and *Nitrospira* sp. were identified in the oxygen activated sediments; no AOB were isolated in sediments from control mesocosm. The presence of AOB combined with the high rates of ammonia oxidation potential in oxygen-activated sediments supports the contention by Palmer and Beutel (2009) that oxygenation 'activates' wetland sediments and leads to higher rates of biological ammonia oxidation in the experimental wetlands. Species richness was comparable in each treatment. The oxygenation did not enhance

diversity but appears to have selected for a different cohort of species able to thrive in a more oxygenated sediment-water interface.

FUTURE RESEARCH

Real Time PCR for AOB and AOA

Traditional PCR efforts indicated that a disparity exists between the nitrifying communities in the sediments of the oxygen-activated and control communities. Real-time PCR is a method of quantifying the number total bacteria and target bacteria. A number of studies have used real-time PCR to quantify AOB populations in sludges and sediments (Ward *et al.*, 2000; Limpiyakorn *et al.*, 2005). Using real-time PCR, quantification of total and ammonia oxidizing bacteria from sediments in this study is currently under way. This will help to determine if oxygenation enhanced only the nitrification process or if it successfully augmented the fraction of AOB to total bacterial cells, which then subsequently increased nitrification rates. Expanding research on the importance of ammonia oxidizing archaea (AOA) in aquatic and soil systems suggests that AOA may be partially responsible for the high ammonia oxidation potentials (Chen *et al*., 2008; Leininger, *et al*., 2006). Quantifying the AOA populations, in addition to AOB, through real-time PCR will help to identify the species responsible for the majority of ammonia oxidation in the mesocosms. The primer sets quantifying total bacteria and archaea and ammonia oxidizing bacteria and archaea were selected from published studies (Quan *et al.*, 2008; and He *et al*., 2007). Results of the analysis are pending. These efforts are currently underway in collaboration with ongoing PhD student Stephen Dent and Dr. Doug Call's research staff.

Trace Gas Emissions

A growing issue related to CTWs is their tendency to emit significant amounts of two key trace gasses responsible for global warming: methane (CH_4) and nitrous oxide (N_2O) (Søvik and Kløve, 2007). While nitrous oxide emissions from CTWs tend to be an order of magnitude lower than methane emission (Søvik *et al*., 2006), nitrous oxide is over ten times as potent as methane from a warming perspective (IPCC, 2001); thus both trace gasses are of concern. To implement CTWs on a more sustainable basis, we must fully understand what environmental factors control greenhouse gas emissions from CTWs. In collaboration with research staff from the WSU Laboratory for Atmospheric Sciences, I performed preliminary trace gas emission measurements on the experimental wetland mesocosms described in this study. Our main question was whether the oxygen-activated nitrification wetlands exhibited higher rates of nitrous oxide emissions associated with elevated levels of nitrification and denitrification, thereby offsetting the overall environmental benefit of ammonia removal. Nitrous oxide is a common intermediate nitrogen-oxide species emitted during the reduction of nitrate to dinitrogen gas (Firestone, 1982). Nitrous oxide has also been measured during nitrification in soils and marine environments, presumably forming as an intermediate during the oxidation of hydroxylamine to nitrite (Schmidt, 1982). Using standard protocols for the environmental measurement of trace gas fluxes (TGPDC, 2003), we measured nitrous oxide fluxes from duplicate oxygen-activated and control mesocosms (Fig. 6). Preliminary results showed that nitrous oxide fluxes from oxygen-activated wetlands were about twice those in the controls (3-5.5 versus 1.5-3 $\mu l/m^2/d$). Further research is needed to document trace gas emissions from oxygen-activated wetlands and, in a broader context, to determine how and if wetland oxygenation might be used to minimize emissions of trace gas from CTWs.

Treatment of Halogenated Aliphatic Compounds

Nitrifying bacteria employ very unique enzymes including ammonia monooxygenase to convert ammonia to hydroxylamine and hydroxylamine oxdioreductase to convert hydroxylamine to nitrite. A number of studies have documented the degradation of a wide range of halogenated compounds by ammonia oxidizing bacteria including dichloromethane, dibromomethane, chloroform, trichloroethane, trichloroethylene (TCE) and vinyl chloride (Yang *et al*., 1999; Vannelli *et al*., 1990; Arciero *et al*., 1989). These studies are typically done in highly controlled environments using well defined isolates and pure cultures. Further study is needed in the context of natural treatment systems with mixed and highly diverse microbial populations, such as oxygen-activated nitrification wetlands, to evaluate their potential to degrade halogenated aliphatic compounds and resulting changes in microbial communities associated with such treatments. In collaboration with Dr. Richard Watts, an expert in *in situ* chemical oxidation of organic pollutants, I performed an initial experiment to evaluate the capacity of the experimental wetland mesocosms in this study to degrade TCE. Approximately 10 mg/L of TCE and 20 mg-N/L of ammonia were added to each mesocosm, which were operated in batch mode (i.e., influent flow was discontinued). Ammonia and TCE (via gas chromatography) were tracked over time. Unfortunately, at the beginning of the batch experiment an excess of organic matter was also added to the mesocosms, resulting in an extreme drop in dissolved oxygen in both the oxygen-activated and control mesocosms. As a result, nitrifying microorganisms, which are particularly sensitive to environmental conditions and not at all resilient (Schmidt, 1982), were inhibit and/or kill. This was confirmed by a total lack of ammonia removal in oxygenated wetlands during the batch experiment. Experimental

results of the effort were inconclusive. It is quite possible that oxygen-activated nitrification wetlands, with their extreme rates of ammonia removal as documented by Palmer and Beutel (2009), could also yield high rates of degradation of a range of halogenated aliphatic compounds.

Immobilized Cell Biotechnology

A relatively new approach to enhance ammonia removal in a range of wastewaters is immobilized-cell biotechnology (Jung *et al*., 2004; Rostron *et al*., 2001). Cell immobilization involves the purposeful entrapment and encapsulation of select microbial cells in small beads or pellets of support media made up of natural (e.g., calcium alginate) or synthetic polymers (e.g., polyethylene glycol). Because these pellets can easily be retained in treatment reactors using screens, immobilized nitrifying microorganisms do not get washed out of the reactors resulting in more effective treatment (i.e., shorter hydraulic retention times and smaller reactors). In one study that looked at ammonia removal in livestock wastewater, the use of immobilized nitrifiers resulted in a drop in the required hydraulic retention time from 10 days to 12 hours (Vanotti and Hunt, 2000). Further research is needed to evaluate how immobilized-cell biotechnology could interface with CTWs to enhance ammonia removal. Perhaps immobilized nitrifiers in biodegradable media, in concert with oxygenation or without oxygenation, could be intermittently added to a CTW with the result of enhancing rates of nitrification and shrinking the required size and cost of the treatment wetland.

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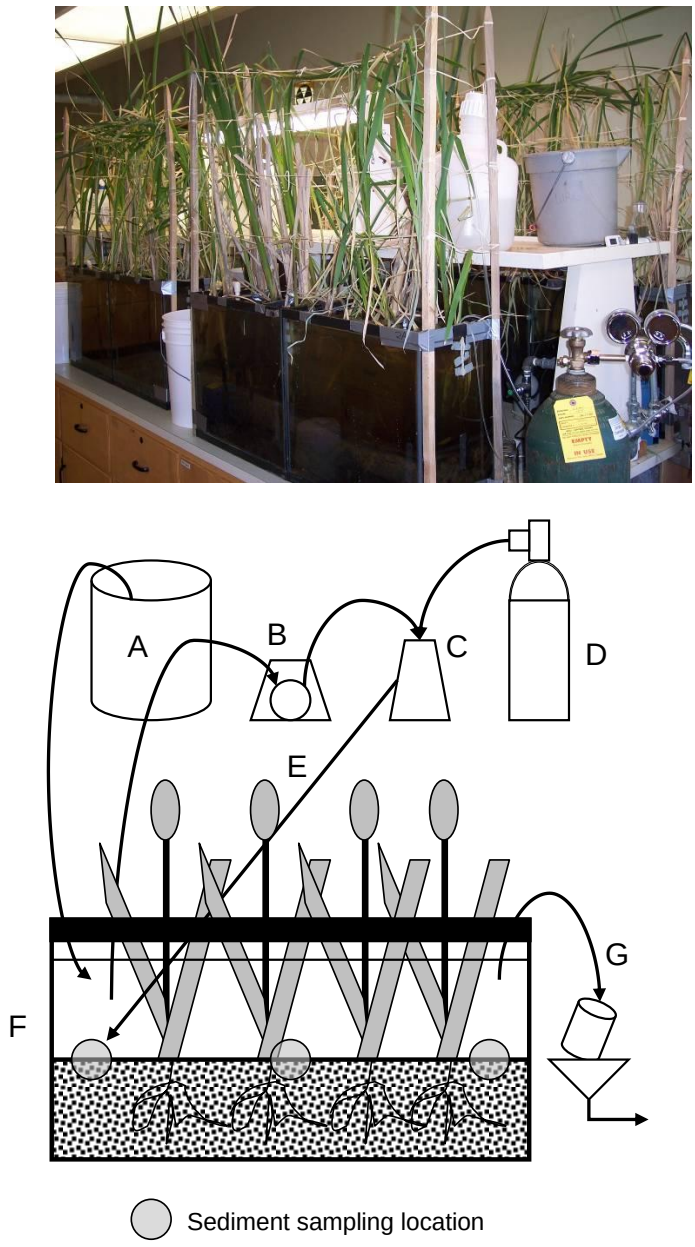

Figure 1. Top: Photo of experimental setup. Bottom: Schematic of oxygen-activated wetland mesocosm and sediment sampling locations. Note that control wetland mesocosms had no side-stream oxygenation components. (A) synthetic wastewater inflow via gravity-feed line; (B) side-stream pump feeding oxygenation chamber; (C) oxygenation chamber; (D) pure oxygen gas cylinder feeding oxygenation chamber; (E) oxygenated side-stream return line; (F) aquarium with water, wetland sediment, and cattail plants; and (G) siphon outlet structure and effluent sampling point. Modified from Palmer and Beutel (2009).

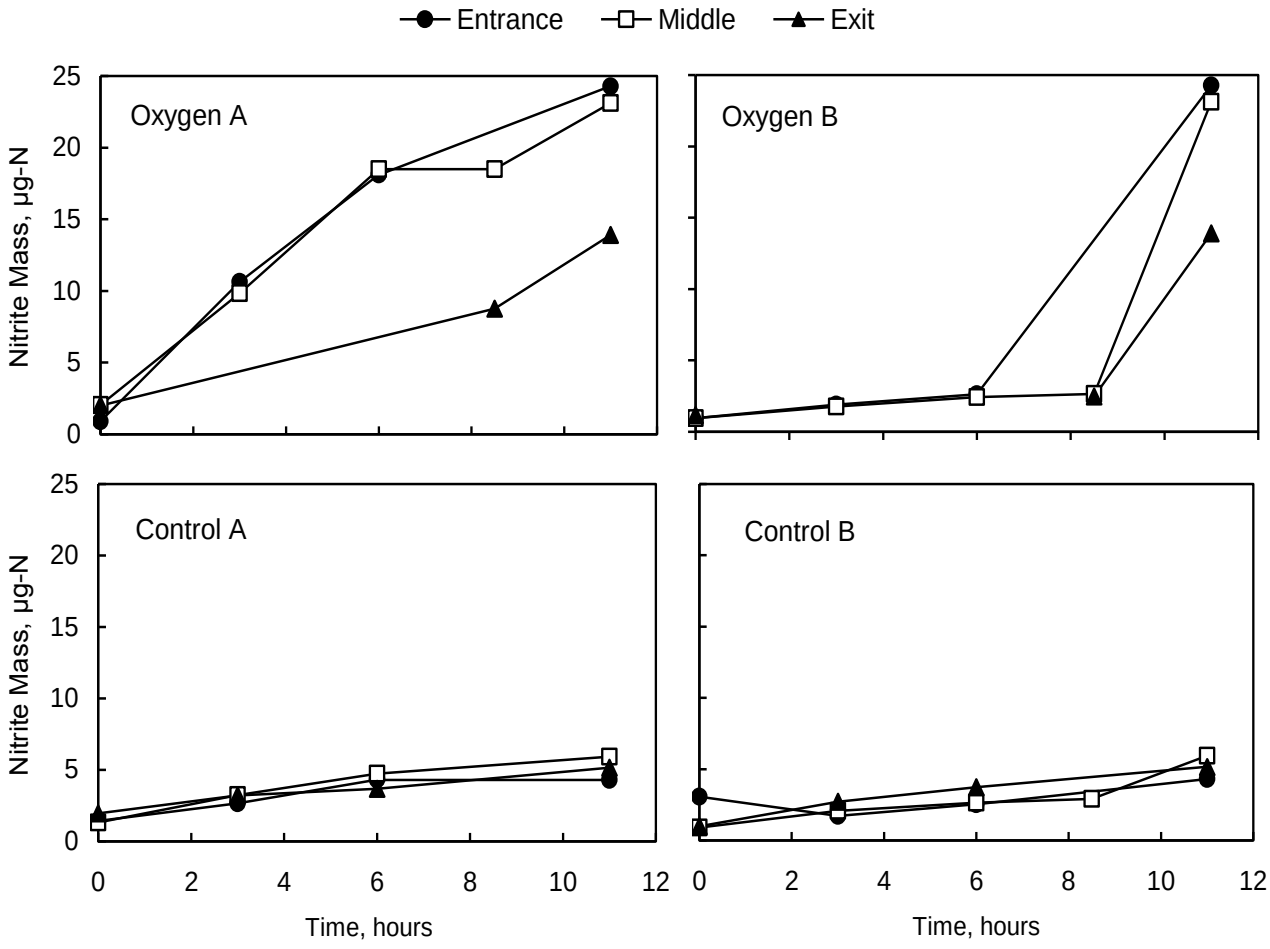

Figure 2. Accumulation of nitrite mass during ammonia oxidation potential assays with surficial sediments sampled at the entrance, middle and exit of duplicate oxygen-activated wetland mesocosms (Oxygen A, Oxygen B) and duplicate control wetland mesocosms (Control A, Control B).

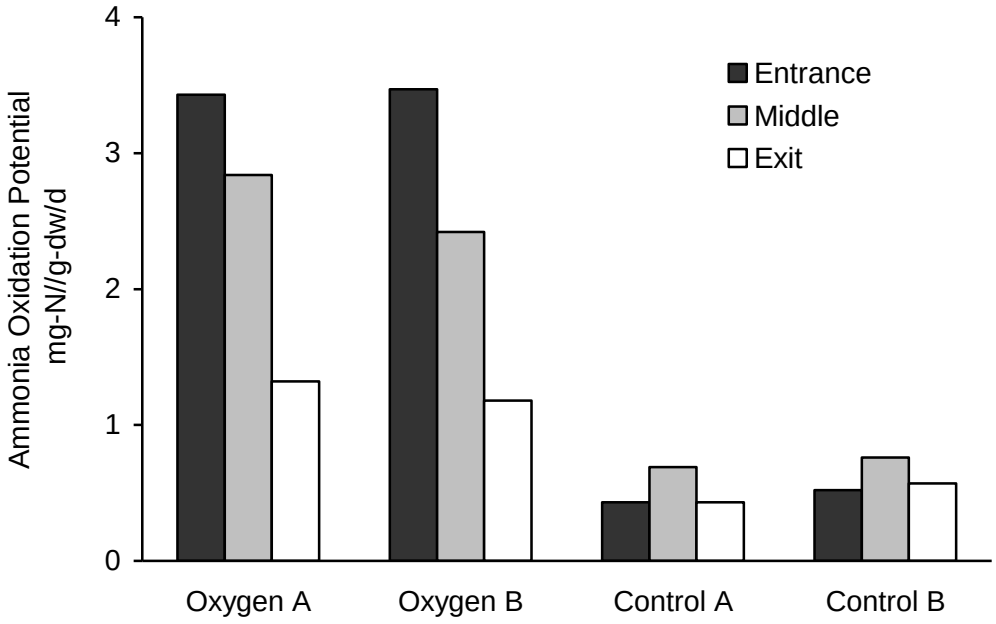

Figure 3. Ammonia oxidation potential measured in surficial sediments sampled at the entrance, middle and exit of duplicate oxygen-activated wetland mesocosms (Oxygen A, Oxygen B) and duplicate control wetland mesocosms (Control A, Control B).

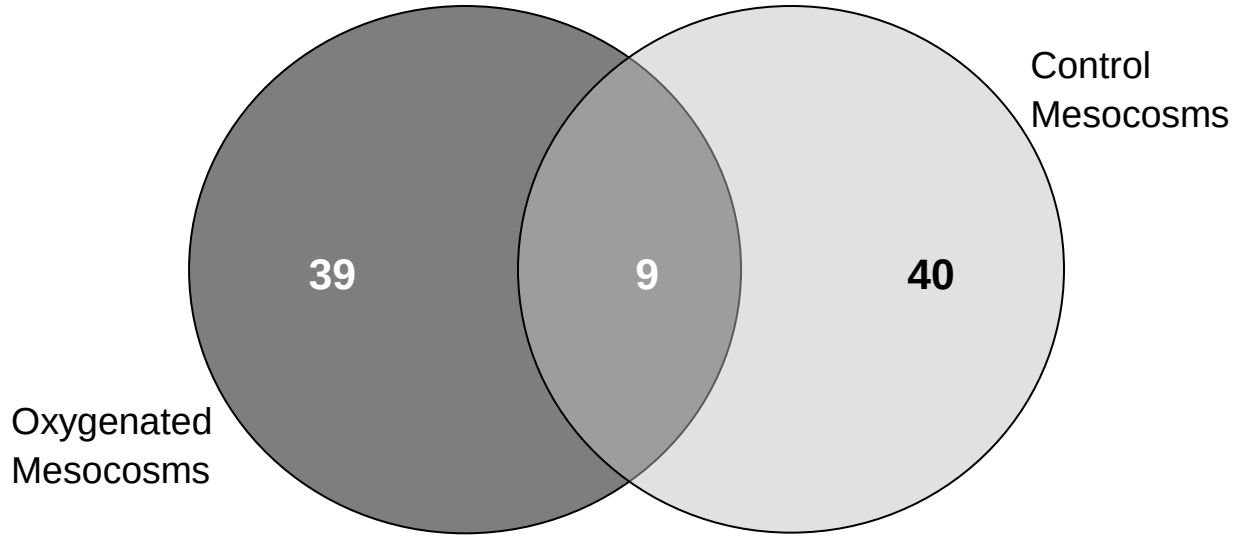

Figure 4. Venn diagram of identifiable microbial species measured in surficial sediments from oxygen-activated and control wetland mesocosms.

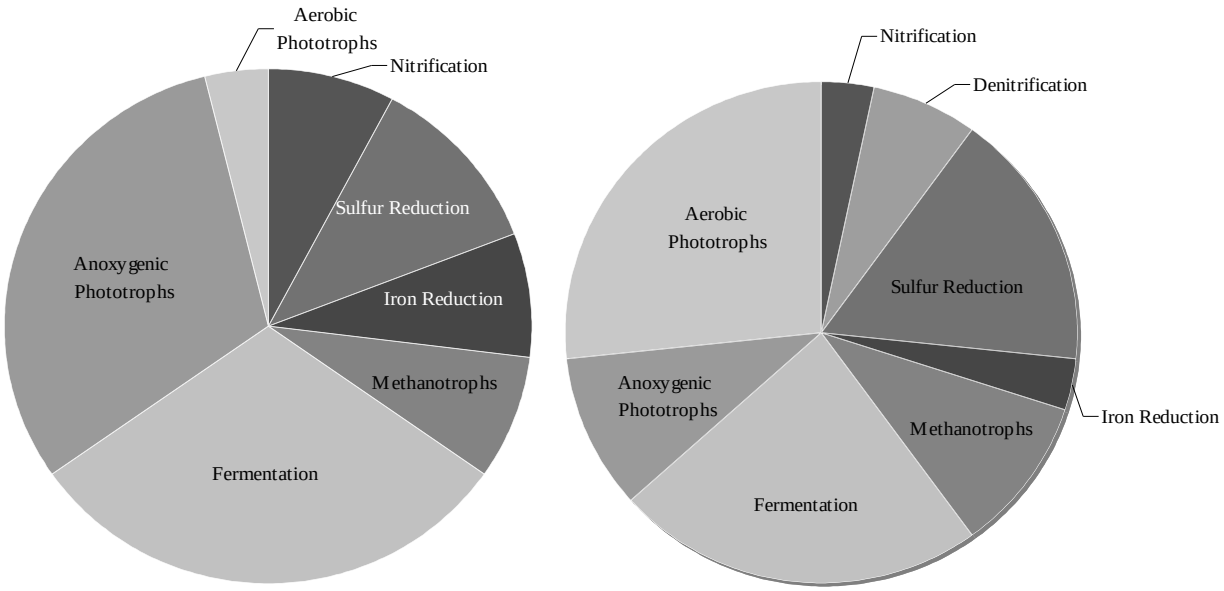

Figure 5. Microbial species richness measured in surficial sediments from oxygen-activated (left) and control (right) wetland mesocosms according to preferred metabolic capability. Note that areas represent number of species and not the microbial numerical populations.

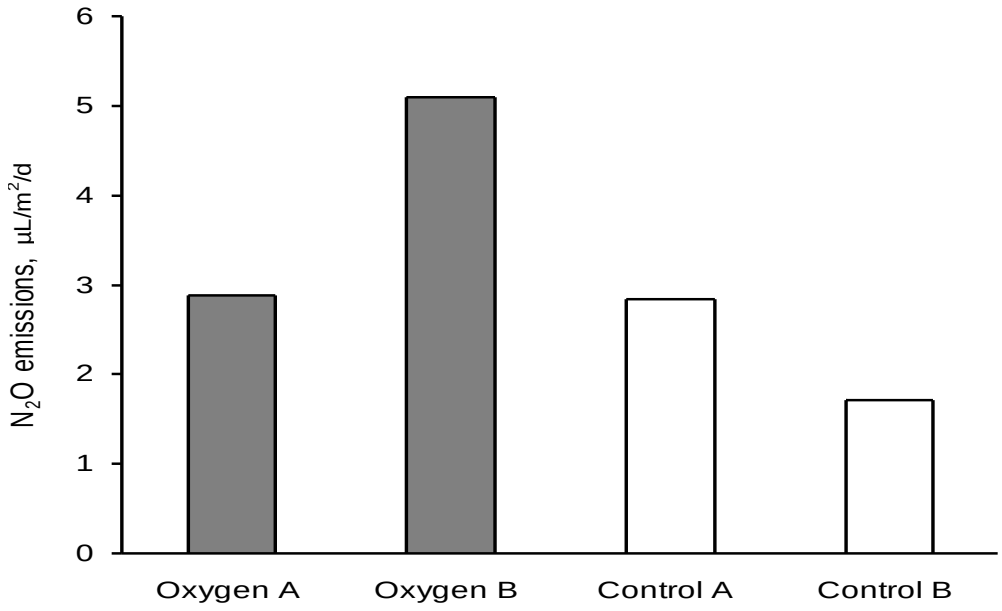

Figure 6. Preliminary results from trace gas measurements made in duplicate oxygen-activated wetland mesocosms (Oxygen A, Oxygen B) and duplicate control wetland mesocosms (Control A, Control B). Measurements were made in collaboration with staff from the WSU Laboratory for Atmospheric Research following standard protocol detailed in TGPDC (2003).

1 Table 1. Ammonia oxidation potentials of terrestrial soils and aquatic sediments under various treatment regimes.

Ammonia Oxidation Potentials

Media	Treatment/Soil Zone	Ammonia Oxidation Potential (mg-N/g dry weight◆d)	Reference
Experimental constructed wetland sediments	Oxygen-activated	1.2-3.5	This study
	Control	0.43-0.76	
Constructed wetland sediments	25 mM ammonium treatment:		Gorra *et al*., 2007
	root zone	0.049-1.2	
	bulk zone	0.031-0.38	
	2.5 mM ammonium treatment:		
	root zone	0.024-0.31	
	bulk zone	0.0010-0.31	
Rice field soils	Root Zone	0.0046-0.015	Bodelier and Frenzel, 1999
	Bulk Zone	0.0033-0.0047	
Rice field soils	Surface soil	0.018	Nicolaisen *et al*., 2004
	Bulk soil	0.0094	
	Rhizosphere	0.013	
Grass land and Forest	Forest	0.6-1.8	Hoffman *et al*., 2007
	Agriculture	1.9	
	Natural Grassland	0.3-0.25	
Meadow, forest, rice paddy	Air	0.015	Bender and Conrad, 1994
	Methane	0.016	
	Ammonia	0.020	
	Carbon Monoxide	0.016	
Agricultural soils		0.0012-0.0067	Kurola, et. al. 2005
Agricultural soils		0.0024-0.0029	Berg and Rosswall, 1987
Agricultural soils (long-term fertilization)	Fallow	0.050	He *et al*., 2007
	No fertilizer	0.036	
	Fertilizer	0.0062-0.024	
	Fertilizer + Organic Matter	0.11	

Table 2. A comparison of bacterial species identified in surficial sediments from oxygen-activated and control wetland mesocosms.

Species Diversity (according to BLAST results)		
Oxygen-Activated Mesocosms	**Both**	**Control Mesocosms**
Acidobacterium sp.	*Chlorobium phaeobacteroides*	*Acidithiobacillus thiooxidans*
Actinopolymorpha rutilus	*Anaerolinea thermolimosa*	*Amphora coffeaeformis*
Agrobacterium tumefaciens	*Caldilinea aerophila*	*Anaerolinea thermolimosa*
Arthrobacter oxydans	*Chloroflexus sp.*	*Arenimonas sp.*
Arthrobacter rhombi	*Dechloromonas sp.*	*Attheya longicornis*
Azospira oryzae	*Desulfobacca acetoxidans*	*Azospira oryzae*
Bacillus benzoevorans	*Desulfobacterium indolicum*	*Bacillus longiquaesitum*
Blastopirellula sp.	*Flavobacterium sp.*	*Bacillus nealsonii*
Chlorobium limicola	*Nitrospira sp.*	*Bacteriovorax sp.*
Clostridium aldrichii		*Brevundimonas vesicularis*
Clostridium beijerinckii		*Clostridium acetobutylicum*
Clostridium carboxidivorans		*Clostridium cellulosi*
Cystobacter violaceus		*Clostridium hveragerdense*
Cytophaga sp.		*Codakia orbicularis*
Dechloromonas hortensis		*Cystobacter ferrugineus*
Desulfobulbus rhabdoformis		*Dechloromonas hortensis*
Geobacter lovleyi		*Desulfocapsa sp*
Geobacter pelophilus		*Desulfofaba fastidiosa*
Haliscomenobacter sp.		*Desulforhopalus sp.*
Magnetic coccus		*Dickieia ulvacea*
Nitrosomonas oligotropha		*Flavobacterium aquatile*
Nocardioides sediminis		*Flavobacterium hercynium*
Phenylobacterium lituiforme		*Flavobacterium succinicans*
Pirellula staleyi		*Geobacter sp.*
Pseudomonas aeruginosa		*Geothermobacter ehrlichii*
Pseudomonas nitroreducens		*Holophaga sp.*
Rhodobacter changlensis		*Methylobacter tundripaludum*
Rhodobacter ovatus		*Methylocystis parvus*
Rhodobacter sphaeroides		*Methyloversatilis sp.*
Rhodoplanes sp.		*Microlunatus phosphovorus*
Rhodopseudomonas palustris		*Nanofrustulum shiloi*
Smithella sp.		*Navicula phyllepta*
Spirobacillus cienkowskii		*Nitzschia closterium*
Sterolibacterium denitrificans		*Phaeodactylum tricornutum*
Syntrophorhabdus aromaticivorans		*Rhodocyclus sp.*
Syntrophus aciditrophicus		*Salinisphaera sp.*
Syntrophus gentianae		*Salinispora arenicola*
Syntrophus sp.		*Sphingobacterium sp.*
Thalassiosira gravida		*Sphingomonas sp.*
		Syntrophus sp.

Table 3. Common metabolic processes and associated species found in surficial sediments of the oxygen-activated and control wetland mesocosms.

	Bacterial Species (according to BLAST analysis)	
Metabolism	Oxygen-Activated Mesocosms	Control Mesocosms
Nitrification	*Nitrosomonas oligotropha* *Nitrospira sp.*	*Nitrospira sp.*
Denitrification		*Pseudomonas aeruginosa* *Pseudomonas nitroreducens*
Sulfur Reduction	*Desulfobacca acetoxidans* *Desulfobacterium indolicum* *Desulfobulbus rhabdoformis*	*Desulfobacca acetoxidans* *Desulfobacterium indolicum* *Desulfocapsa sp. Cad626* *Desulfofaba fastidiosa* *Desulforhopalus sp.*
Iron Reduction	*Geobacter lovleyi* *Geobacter pelophilus*	*Geobacter sp.*
Methanotroph	*Bacillus longiquaesitum* *Bacillus nealsonii*	*Methylobacter tundripaludum* *Methylocystis parvus* *Methyloversatilis sp.*
Fermentation	*Syntrophorhabdus aromaticivorans* *Syntrophus aciditrophicus* *Syntrophus gentianae* *Syntrophus sp.* *Thalassiosira gravida* *Clostridium aldrichii* *Clostridium beijerinckii* *Clostridium carboxidivorans*	*Clostridium acetobutylicum* *Clostridium cellulosi* *Clostridium hveragerdense* *Anaerolinea thermolimosa* *Flavobacterium aquatile* *Flavobacterium hercynium* *Flavobacterium succinicans*
Anoxygenic Phototrophs	*Rhodobacter changlensis* *Rhodobacter ovatus* *Rhodobacter sphaeroides* *Rhodoplanes sp.* *Rhodopseudomonas palustris* *Anaerolinea thermolimosa* *Caldilinea aerophila* *Chloroflexus sp.*	*Anaerolinea thermolimosa* *Caldilinea aerophila* *Chloroflexus sp.*
Aerobic Phototrophs	*Thalassiosira gravida*	*Phaeodactylum tricornutum* *Attheya longicornis* *Navicula phyllepta* *Amphora coffeaeformis* *Dickieia ulvacea* *Nanofrustulum shiloi* *Nitzschia closterium* *Navicula phyllepta*