The distribution of ammonia-oxidizing betaproteobacteria in impounded Black mangroves (*Avicennia germinans*)

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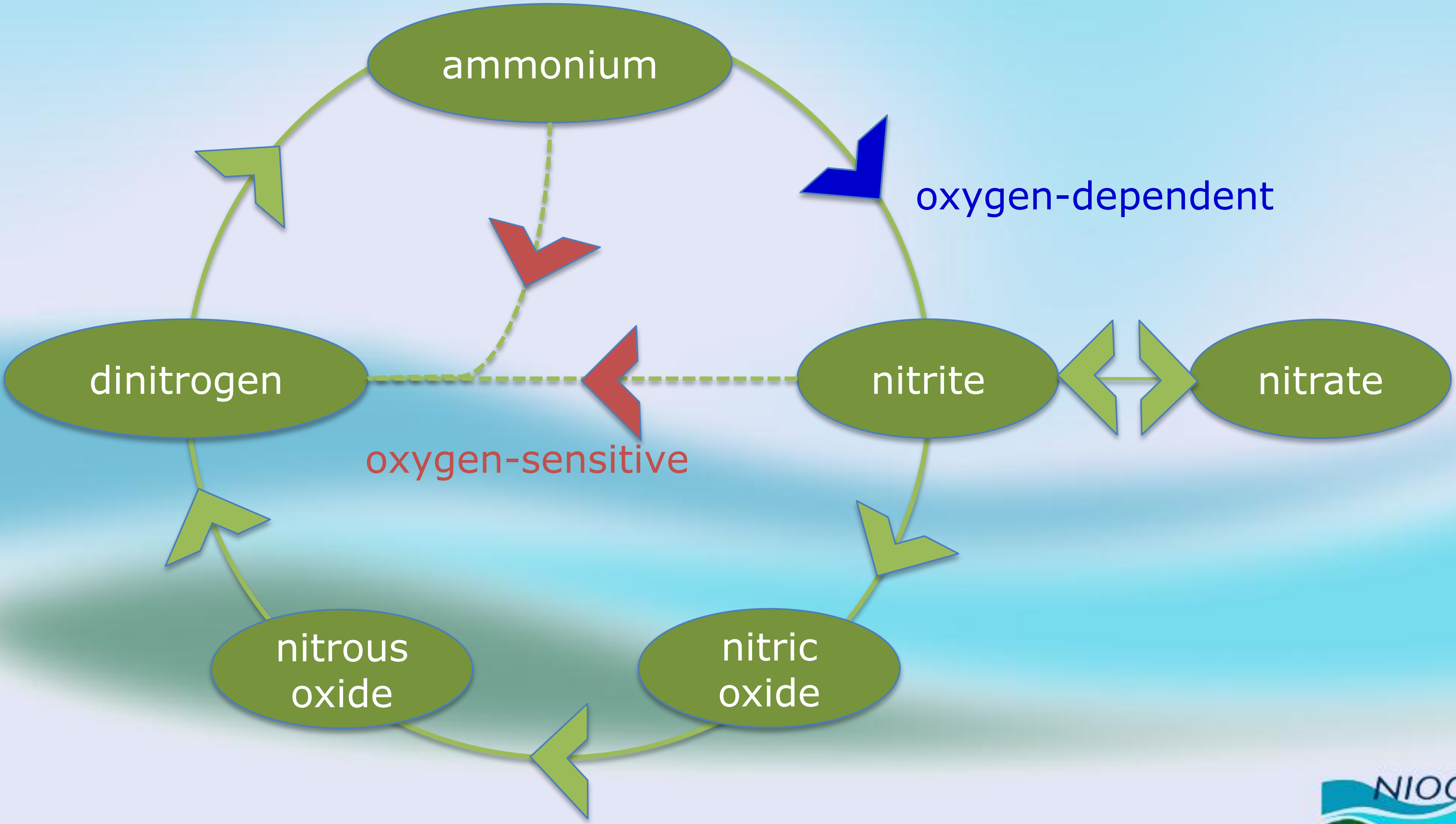
Biogeochemical oxidation of ammonia: Key process in the nitrogen cycle



Geochemical process of ammonia oxidation

Oxic conditions

- Ammonia oxidation by proteobacteria and thaumarchaea both producing nitrite

Anoxic conditions

- Ammonia oxidation with the concomitant reduction of nitrite to dinitrogen gas by planctomycetes (Anammox bacteria)

Geochemical process of ammonia oxidation

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Ammonia-oxidizing betaproteobacteria (β-AOB)

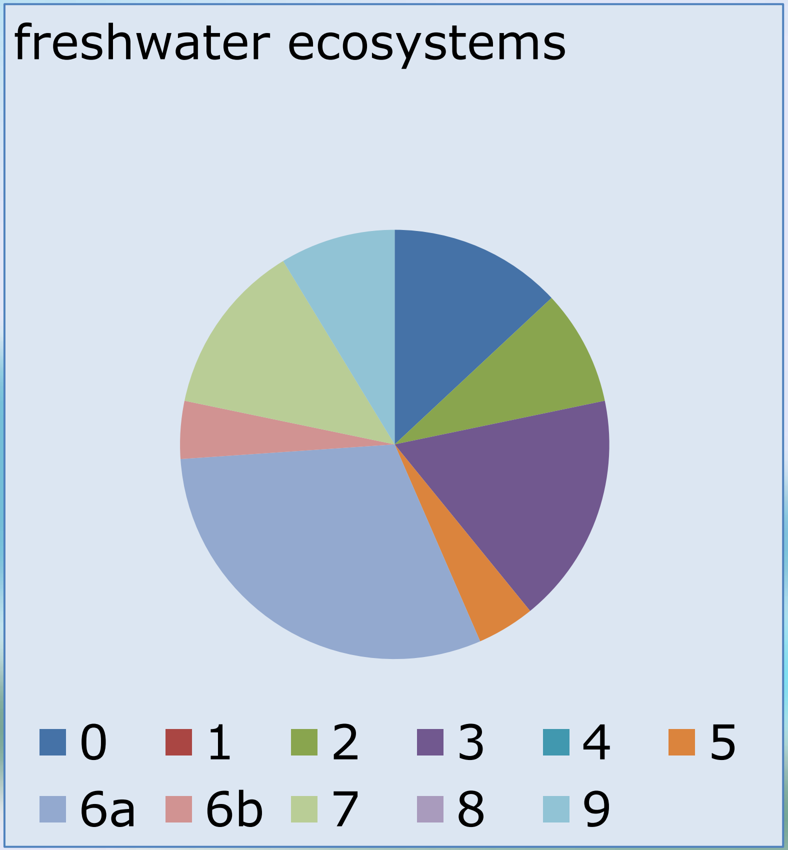
Simple metabolism

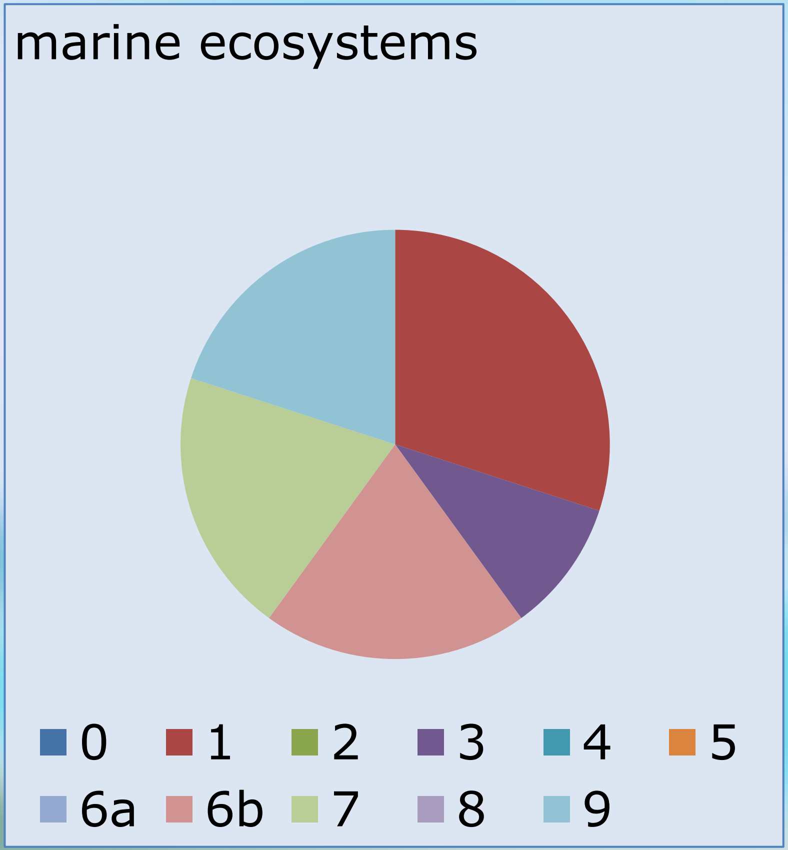
- Oxidation of ammonia in the presence of oxygen
- Carbon dioxide fulfills the carbon requirements

Steering factors

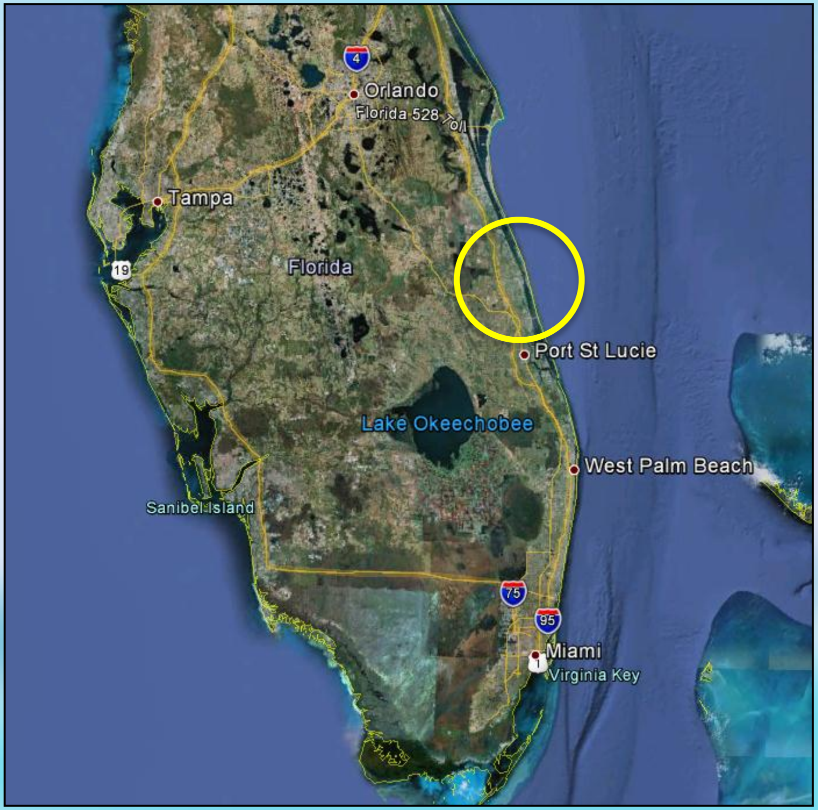
- Ammonia concentration
- Oxygen availability
- Carbon dioxide accessibility
- pH
- Iron and other metals
- Salinity
- Temperature

Occurrence of β-AOB clusters in different ecosystems (based on the 16S rRNA gene)





The distribution of β-AOB in impounded Black mangroves



Location of Impoundments #23 and #24 on North Hutchinson Island, St. Lucie, Fl.

Management history of mangroves on North Hutchinson Island, St Lucie, Fl.

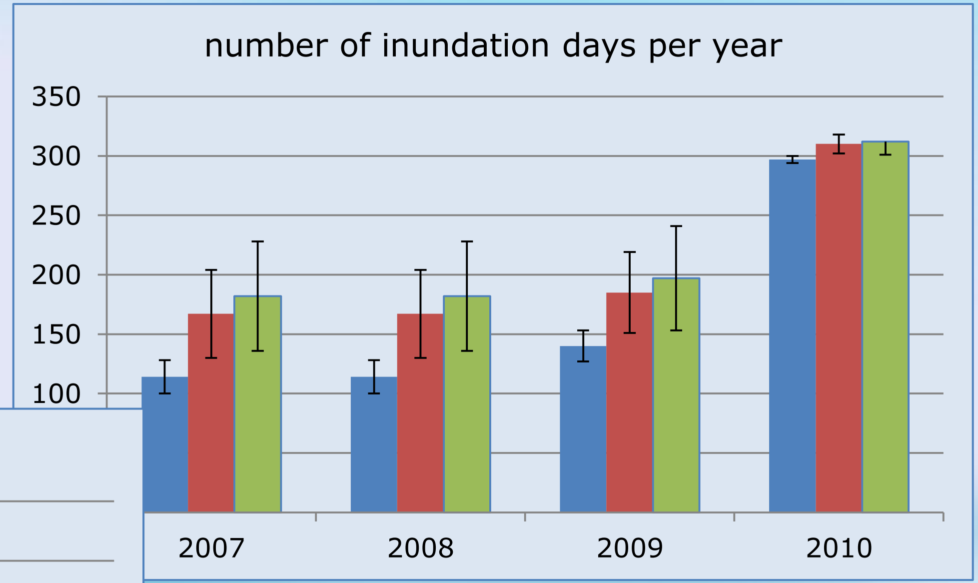
Event	Impoundment 23	Impoundment 24
Closing of the dike	1966	1970
Dike breach	1974	
Placement of one culvert		1985
Placements of four additional culverts		1987
Installing rotational impoundment management		early spring 2009

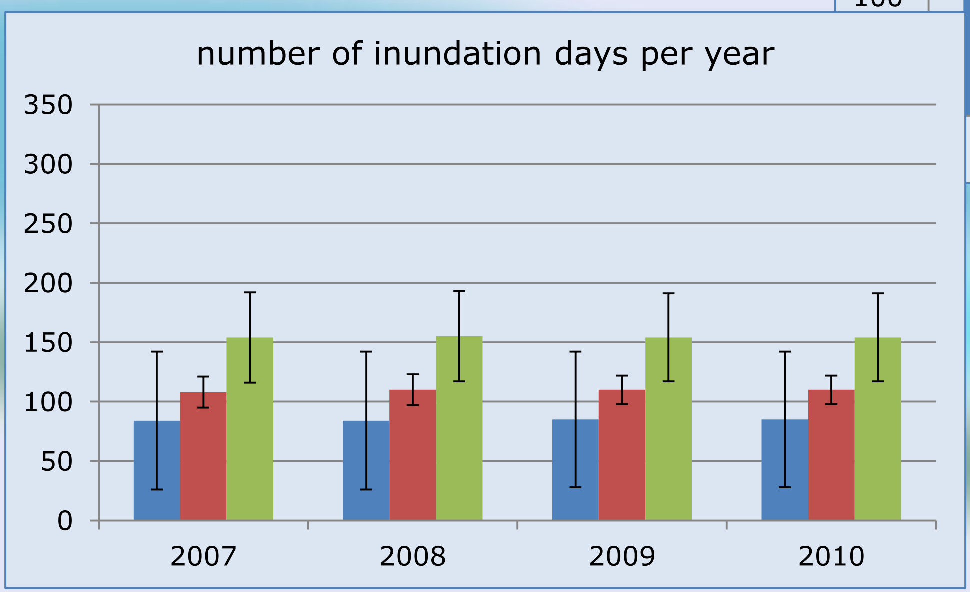
Partial restoration of the tide movements and subsequently mangrove vegetation

Modeled number of inundation days in Impoundments #23 and #24

Vegetations:
- Dwarf
- Sparse
- Dense

24





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Three different Black Mangrove habitats on North Hutchinson Island, St. Lucie, Fl.

dwarf

sparse

dense

Steering factors for mangrove growth

- N and P concentrations
- Frequency of flooding
- pH
- Salinity
- Predation

Steering factors for mangrove growth and for β-AOB

- N and P concentrations
- Frequency of flooding
- pH
- Salinity
- Predation

- Ammonia concentration
- Oxygen availability
- Carbon dioxide accessibility
- pH
- Salinity
- Iron and other metals

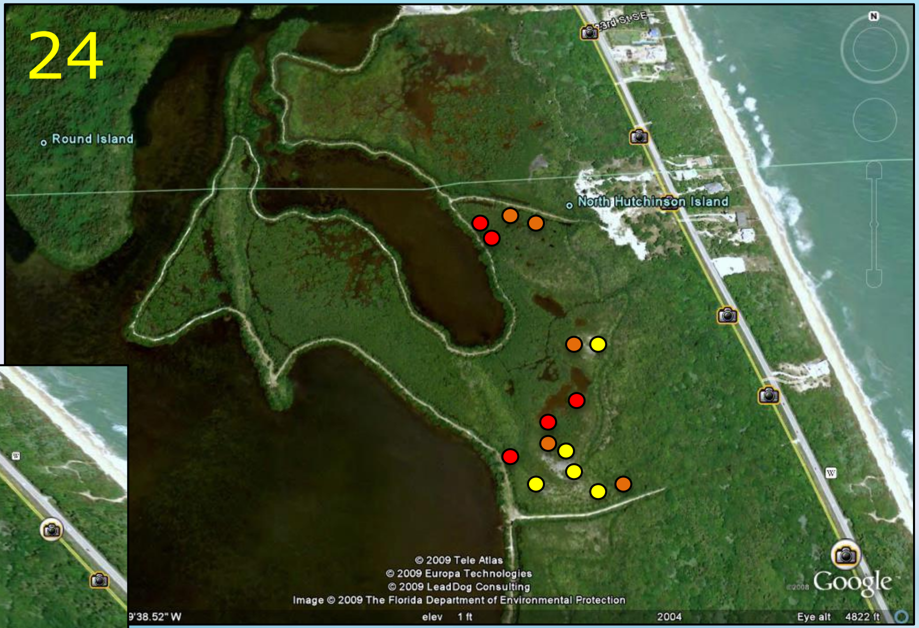
Research questions

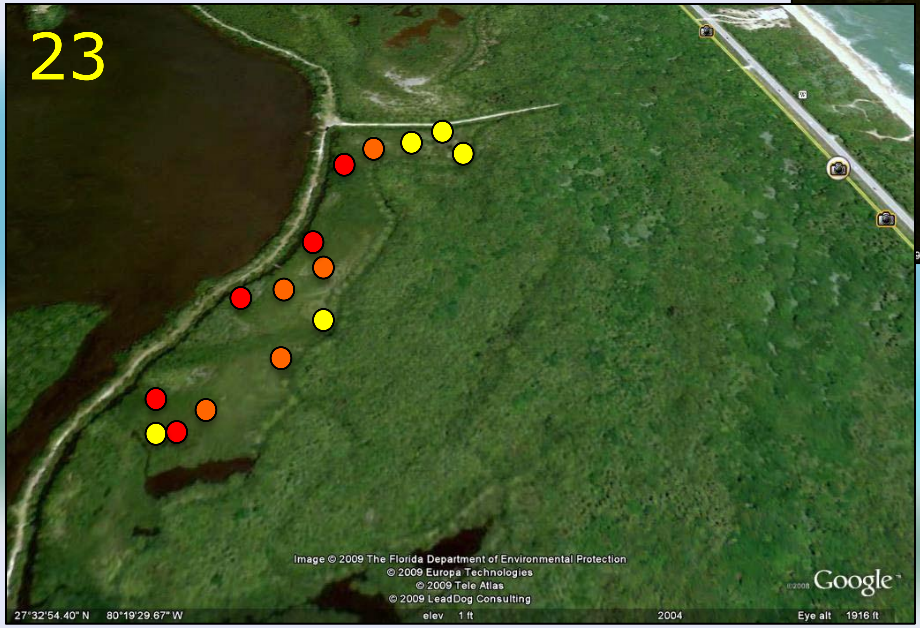
I. Is there a similarity between the distribution of lineages of β-AOB and the distribution of Black mangrove vegetation types imposed by soil conditions?

II. Is the distribution of lineages of β-AOB affected by the new flooding regime starting spring 2009?

Sampling times and locations of Black mangrove habitats

March 2009
March 2010





- dwarf (yellow)
- sparse (orange)
- dense (red)

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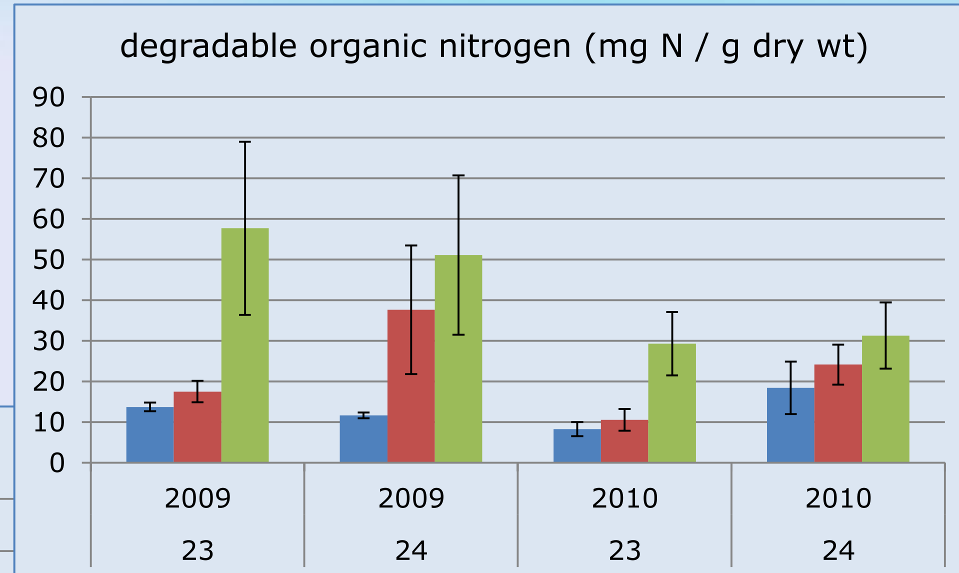
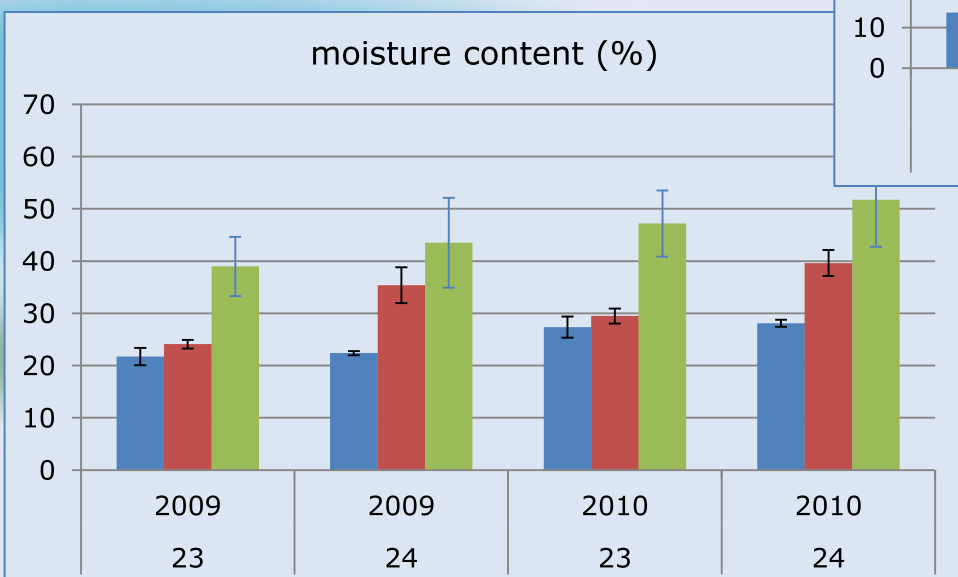
Soil characteristics of sampling locations: Moisture and degradable organic nitrogen

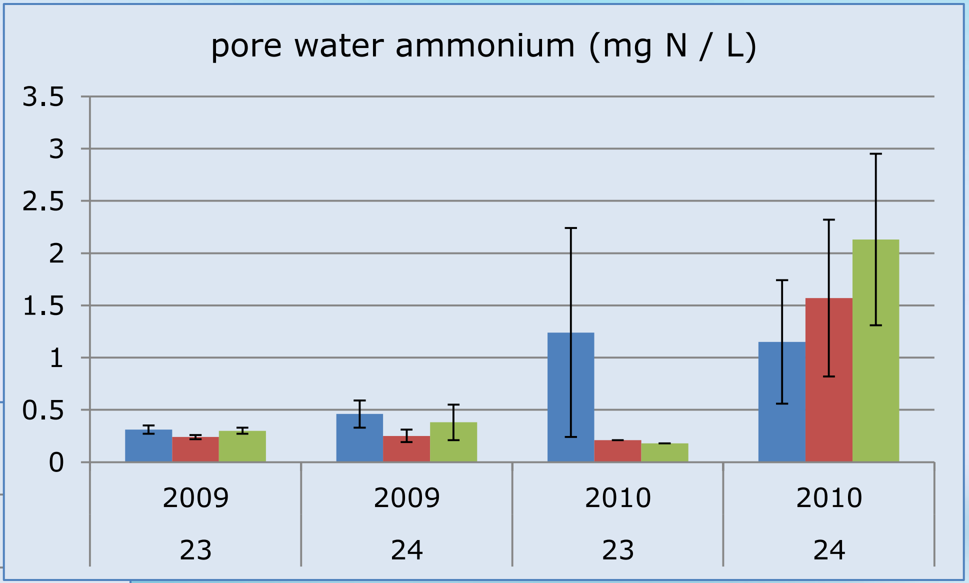
Vegetations:
- Dwarf
- Sparse
- Dense

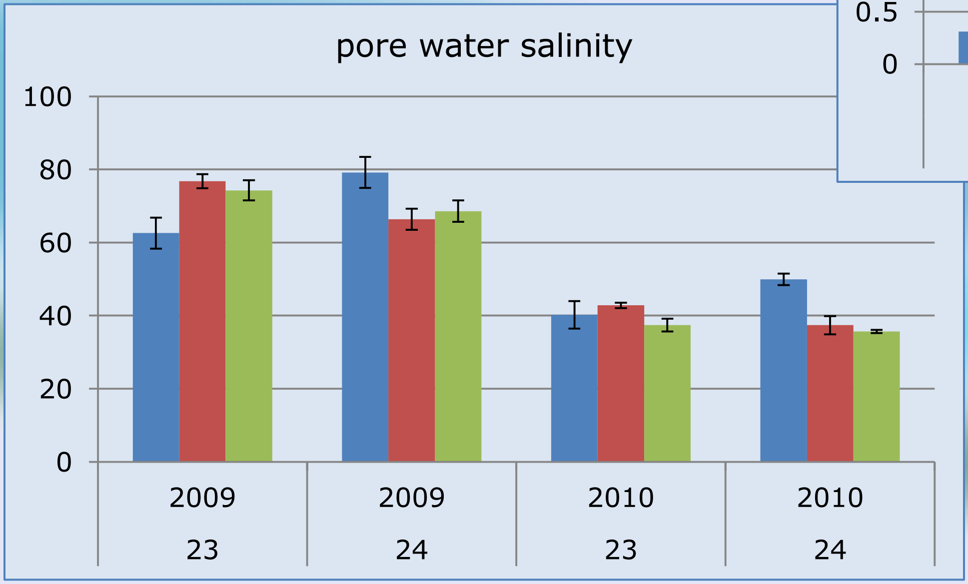




Soil characteristics of sampling locations: Pore water salinity and ammonium

Vegetations:
- Dwarf
- Sparse
- Dense





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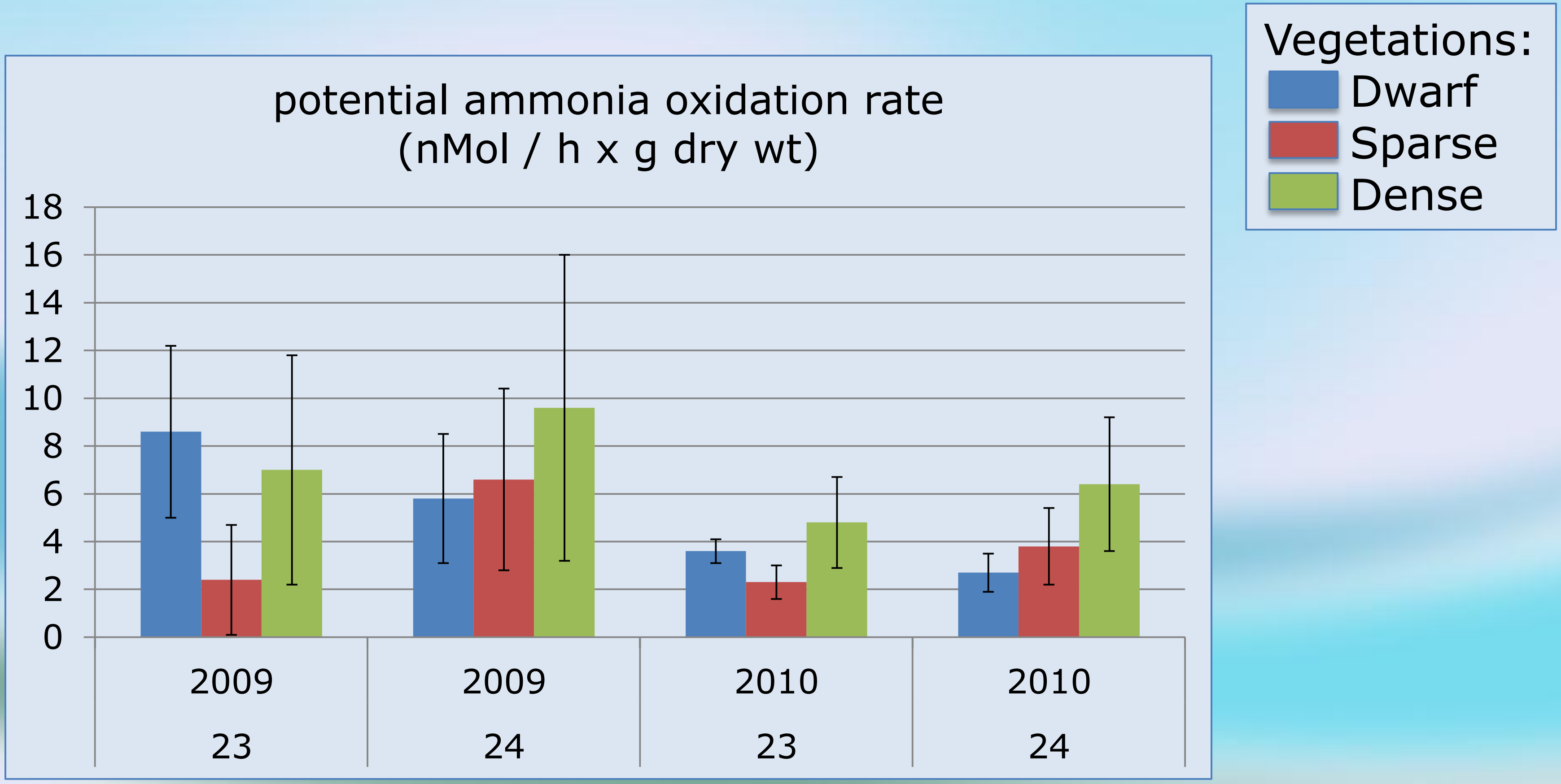
Potential ammonia-oxidizing activities in the different mangrove habitats



OTUs of β-AOB based on 97% 16S rRNA similarity (440-460 bp)

OTU #	**Numbers**	**BLAST analysis of OTU's representatives**		
		Closest type strain β-AOB lineage	% similarity	Origin closest relative
01	206	*Nitrosomonas aestuarii*	97	High altitude saline wetland
02	120	*Nitrosospira tenuis*	96	Deep-water sponge
03	115	*Nitrosomonas* Nm143	97	Estuarine sediment
04	58	*Nitrosomonas aestuarii*	98	Prawn farm sediment
05	14	*Nitrosomonas europaea*	100	Nitrifying bioreactor
06	11	*Nitrosomonas aestuarii*	97	Estuarine sediment
07	10	*Nitrosomonas aestuarii*	98	Estuarine sediment
08	6	*Nitrosomonas aestuarii*	96	Coastal marine sediment

Dissimilarities between communities of β-AOB at 97% mutual difference

Groups	Comparison	R-value	P-value
Impoundments	23 *versus* 24	0.058	0.046 *
Sampling years	2009 *versus* 2010	0.063	0.036 *
Mangrove habitat type	All habitats	0.215	0.001 ***
	Dwarf *versus* sparse	0.326	0.001 ***
	Dwarf *versus* dense	0.248	0.002 **
	Sparse *versus* dense	0.076	0.069

Of the 24 environmental soil parameters measured, the combination of **moisture** content, **C/N** ratio and the amount of **magnesium** in the pore water explained **15%** of the observed variation in community composition.

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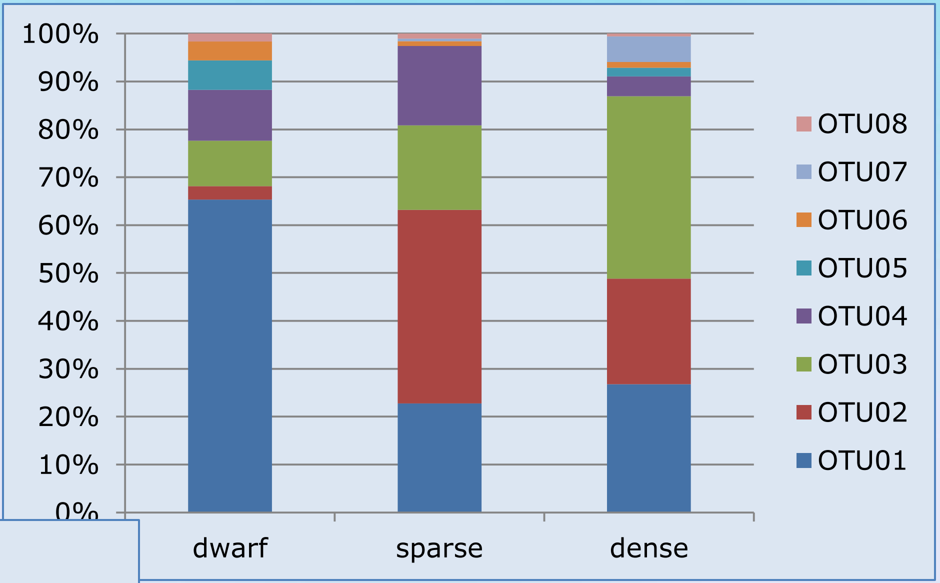
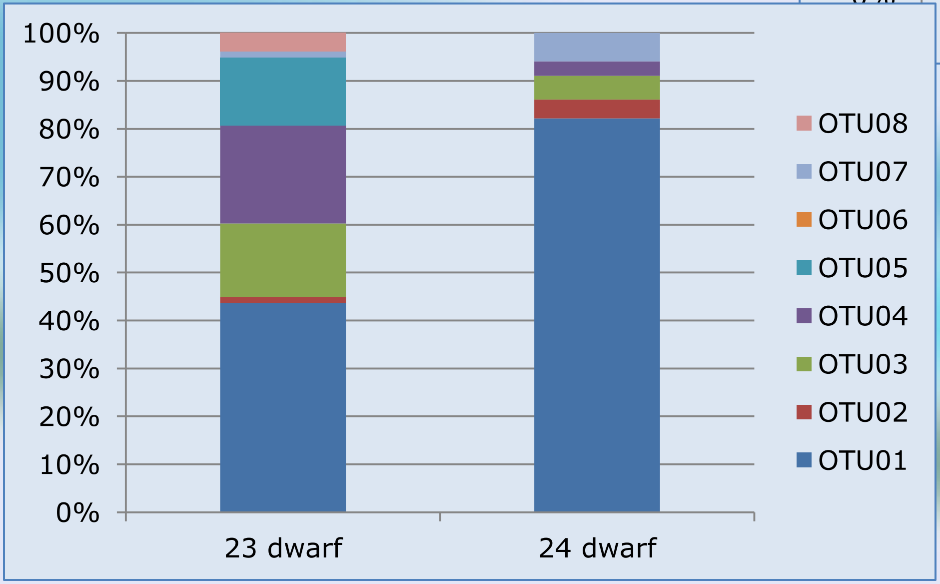
Frequency distribution of OTUs of β-AOB between impoundments at 97% similarity



Overall distribution

Distribution in the dwarf vegetation samples



Niche differentiation among β-AOB?

- OTU01 (*Nitrosomonas aestuarii*) seems to be negatively affected by the more moist conditions in the dense vegetation and also in 2010 after flooding. Oxygen limitation?
- OTU02 (*Nitrosospira* cluster 1) was most prominent in the sparse vegetation. Adaptation to conditions of starvation?
- OTU03 (*Nitrosomonas* sp. Nm143) seems to be positively affected by more moist and rich conditions. Adapted to lower oxygen concentrations?

Research questions

I. Is there an agreement between the distribution of lineages of β-AOB and the distribution of mangrove vegetation types as constraint by soil conditions?

II. Is the distribution of lineages of β-AOB affected by flooding?

III. Is the distribution of lineages of β-AOB affected by the supply of extra ammonium and/or oxygen?

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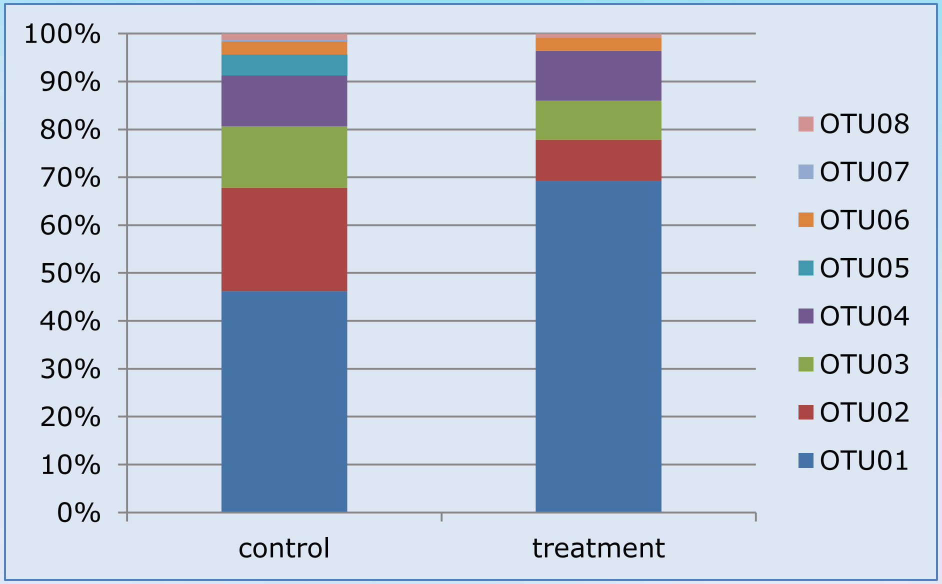
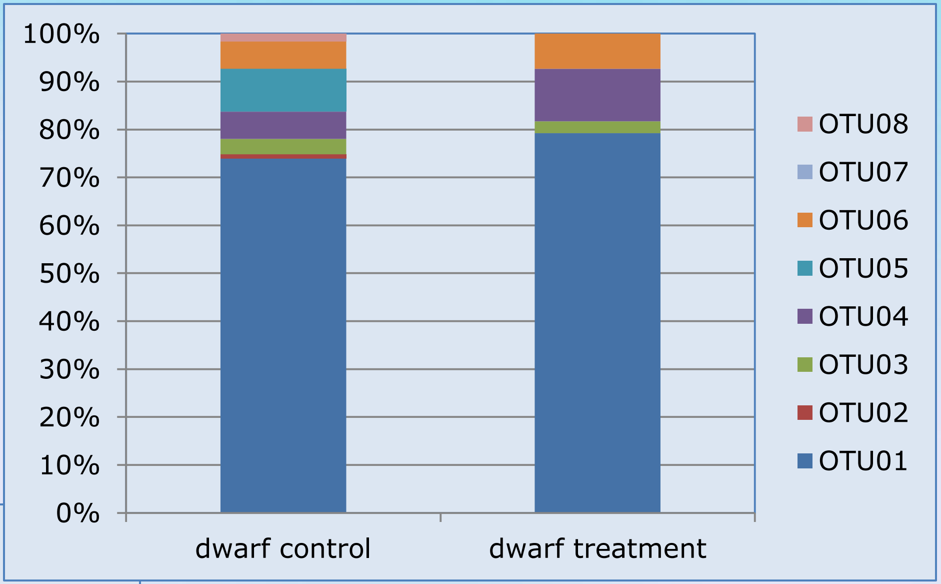
Effect of ammonium addition on the frequency distribution of OTUs of β-AOB at 97% similarity

Comparison	R-value	P-value
Control *vs* treatment	0.084	0.050 *



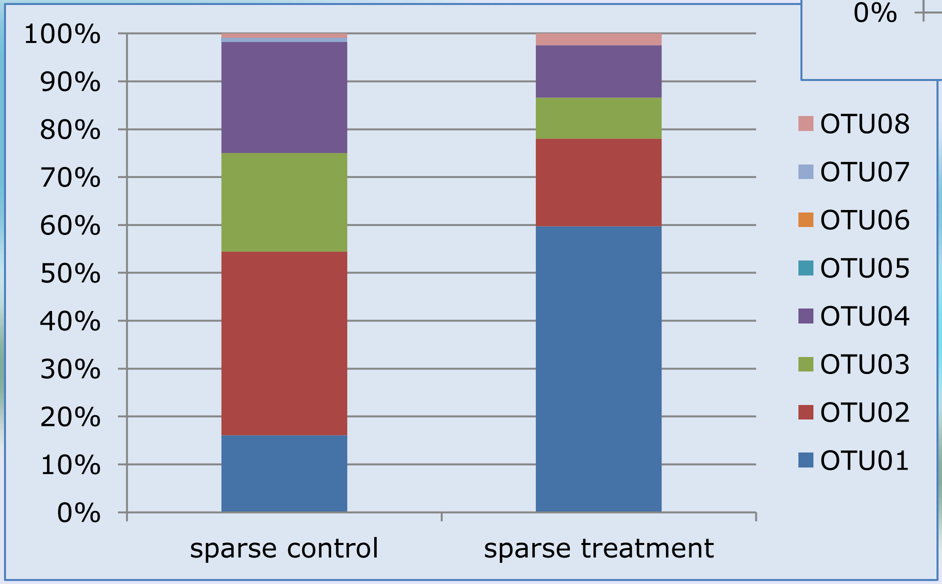
OTU #	Average abundance		Average dissimilarity	Contribution (%)	Cumulative contribution (%)
	control	treatment			
01	0.43	0.70	22.90	39	39
02	0.25	0.09	14.00	24	63
04	0.10	0.11	8.62	14	77
03	0.13	0.08	8.02	14	91

Effect of ammonium addition on the frequency distribution of OTUs of β-AOB at 97% similarity



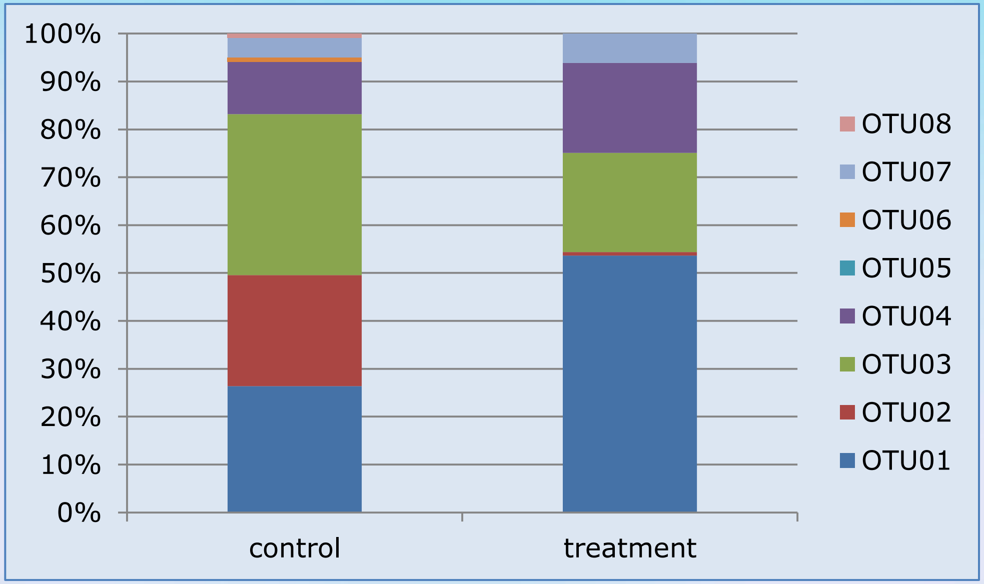
Dwarf vegetation samples

Sparse vegetation samples



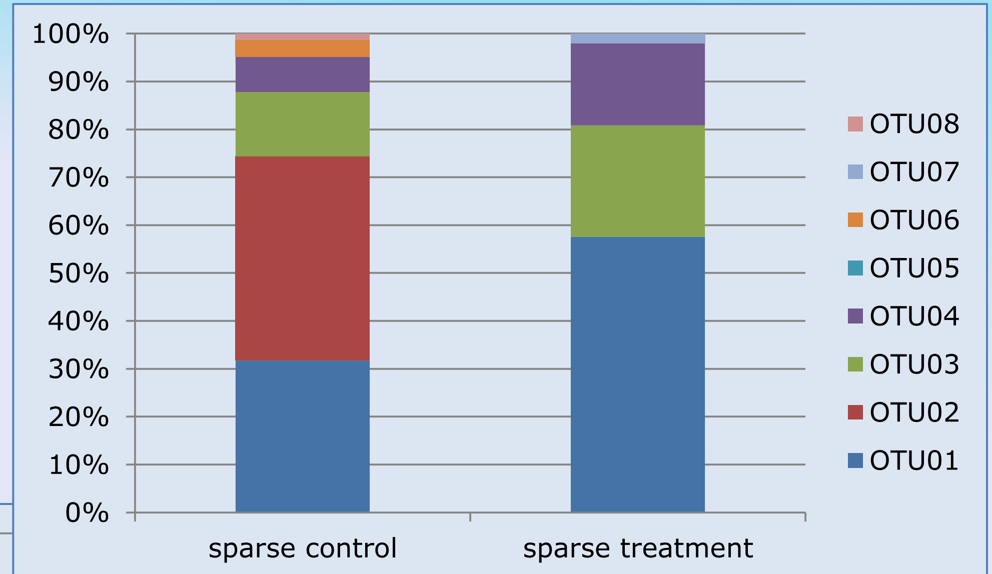
Effect of ammonium plus oxygen addition on the frequency distribution of OTUs of β-AOB

Comparison	**R-value**	**P-value**
Control *vs* treatment	0.149	0.014 *



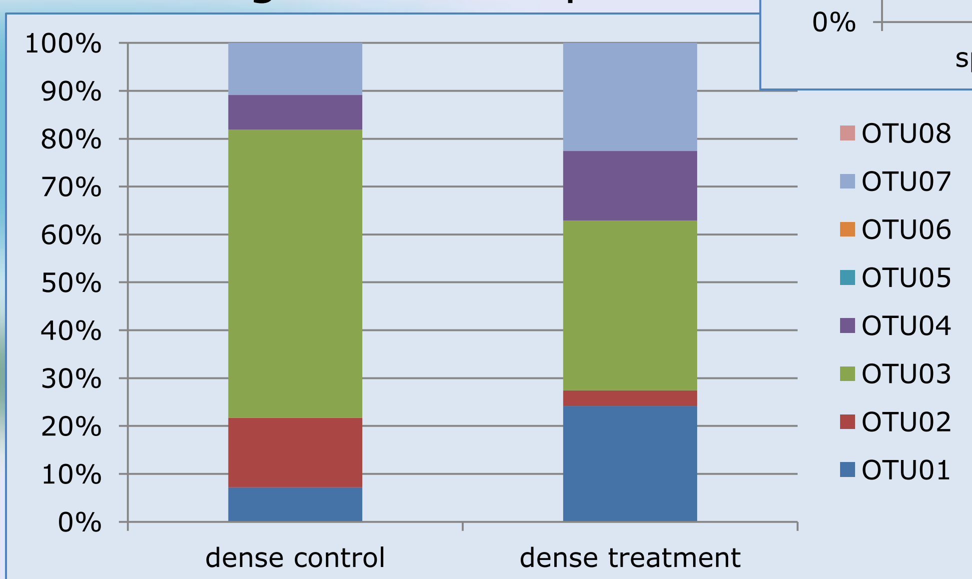
OTU #	**Average abundance**		**Average dissimilarity**	**Contribution (%)**	**Cumulative contribution (%)**
	control	treatment			
01	0.29	0.52	22.27	35	35
03	0.32	0.23	15.07	24	59
02	0.22	0.00	11.03	17	76
04	0.12	0.19	10.47	16	92

Effect of ammonium plus oxygen addition on the frequency distribution of OTUs of β-AOB



Sparse vegetation samples

Dense vegetation samples



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Conclusions

- Only OTUs related to the *Nitrosomonas aestuarii* lineage took advantage of the better growth conditions after supplying ammonium. Aeration stimulated these OTUs even more.
- OTUs related to the *Nitrosomonas* Nm143 lineage and *Nitrosospira* cluster 1 were not stimulated by the better growth conditions.
- *Nitrosospira* cluster 1 almost disappeared as common species in the presence of ammonium and oxygen.

Overall conclusions

1. The distribution of species of β-AOB was not entirely the same for both impoundments. This was largely due to the predominance of OTU01 (*N. aestuarii*) in the dwarf habitat of Impoundment #24 (former salt pans)
2. The distribution of species of β-AOB is hardly affected by flooding; role of the dry winter season and subsequent high pore water salinities in 2009?
3. The distribution of species of β-AOB is affected by Black mangrove habitat conditions; role of moisture and nutrient conditions?

Acknowledgments

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