Community and functional shifts in ammonia oxidizers across terrestrial and marine (soil/sediment) boundaries in two coastal Bay ecosystems

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Originality-Significance Statement

Terrestrial-marine boundaries are very important but overlooked dynamic transition zones from land to sea. They are hotspots of biodiversity and biogeochemical cycling. In addition, they consist of sharp delineated environmental gradients along which niche partitioning of ammonia oxidizers can be tested. In this study, by examining adjoining soil-interface-sediment transects in two coastal Bays via field and laboratory microcosm incubations, we show that AOA are functionally dominant in soil ammonia oxidation, both AOA and AOB contribute at the soil/sediment interface, and AOB are more important in the sediments. Changes in community composition and function of ammonia oxidisers across terrestrial-marine boundaries are important for understanding biogeochemical processes and drivers of the nitrogen cycle across these environments vulnerable to the impacts of climate change, including the increased frequency and severity of storms, to enable better prediction and consequences of climate change.

Summary

Terrestrial–marine boundaries are significant sites of biogeochemical activity with delineated gradients from land to sea. While niche differentiation of ammonia-oxidizing archaea (AOA) and bacteria (AOB) driven by pH and nitrogen is well known, the patterns and environmental drivers of AOA and AOB community structure and activity across soil-sediment boundaries have not yet been determined.

In this study, nitrification potential rate, community composition and transcriptional activity of AOA and AOB in soil, soil/sediment interface and sediments of two coastal Bays were characterized using a combination of field investigations and microcosm incubations. At DNA level, *amoA* gene abundances of AOA were significantly greater than AOB in soil, while in sediments AOB were significantly more abundant than AOA, but at the soil/sediment interface there were equal numbers of AOA and AOB *amoA* genes. Microcosm incubations provided further evidence, through qPCR and DGGE-sequencing analysis of *amoA* transcripts, that AOA were active in soil, AOB in sediment and both AOA and AOB were active at the soil/sediment interface. The AOA and AOB community composition shifted across the coastal soil-interface-sediment gradient with salinity and pH identified as major environmental drivers.

Introduction

Terrestrial–marine boundaries represent the interface between land and sea and are significant sites of biogeochemical activity (McClain *et al.*, 2003). Here, microbially mediated biogeochemical cycling of nitrogen influences the transport of reactive N into coastal waters. Coastal ecosystems are vulnerable to excess nitrogen from anthropogenic terrestrial sources that enter from run-off via streams, rivers and groundwater. Excess nitrogen can be mitigated by microbially mediated denitrification or and/or anammox processes, or enhanced by dissimilatory nitrate

reduction to ammonia (DNRA) (Gruber & Galloway, 2008, Hou *et al.*, 2012, Damashek *et al.*, 2015). Nitrification plays a central role, providing a source of nitrate from ammonia, for subsequent reduction. Coupled nitrification-denitrification has been reported to remove up to 50% of external dissolved inorganic nitrogen inputs to estuaries (Seitzinger *et al.*, 2006). In addition, anammox often occurs with nitrification, resulting in N_2 loss from coastal Bays (Lam *et al.*, 2007) and estuaries (Crowe *et al.*, 2012, Trimmer *et al.*, 2013).

Ammonia oxidation is the first and, usually, rate-limiting step of nitrification. Given the functional and ecological significance of ammonia oxidation, it has received considerable attention, with understanding of ammonia oxidisers evolving with the development of advancing sequencing capabilities. For more than one century, ammonia oxidation was assumed to be driven by autotrophic ammonia-oxidizing bacteria (AOB) affiliated within the beta- and gammaproteobacteria, but is now known to be carried out also by *Thaumarchaea* (Konneke *et al.*, 2005, Prosser & Nicol, 2008). Subsequently, numerous surveys of marine and fresh water, sediments, bioreactors, hot springs and a wide range of soil habitats have demonstrated that AOA are ubiquitous and that AOA usually outnumber AOB, based on quantification of *amoA* genes (encoding the subunit A of the key enzyme ammonia monooxygenase) (Francis *et al.*, 2007, Erguder *et al.*, 2009, Shen *et al.*, 2012). In marine environments, such as the open ocean, AOA are more abundant than AOB and more transcriptionally active, based on abundance of *amoA* transcripts

(Wuchter *et al.*, 2006, Baker *et al.*, 2012, Horak *et al.*, 2013). The isolation of *Nitrosopumilus maritimus* (Konneke *et al.*, 2005) and the marine sponge symbiont *Cenarchaeum symbiosum* (Hallam *et al.*, 2006) have confirmed autotrophic ammonia oxidation by archaea in marine environments. In contrast, several studies suggest that AOB *amoA* gene abundances are greater and increase with salinity in coastal sediments (Mosier & Francis, 2008, Santoro *et al.*, 2008), anoxic sediments (Jiang *et al.*, 2009) and oligotrophic surface sediments (Lagostina *et al.*, 2015). In a recent study, niche differentiation between AOA and AOB in mud deposits of Eastern China Marginal Seas, was attributed to variation in dissolved oxygen, salinity, ammonia and silicate concentrations within the sediments (Yu *et al.*, 2016). Thus, the relative contribution of AOA and AOB to nitrification in marine systems, in particular sediments, is still under debate.

In terrestrial ecosystems, although AOA are numerically abundant in a wide range of soils, evidence from mRNA and stable isotope probing (SIP) in both field surveys and soil microcosm incubations suggests that AOA are functionally dominant in most low-pH and low-N input soils while AOB outcompete AOA in neutral/alkaline or high-N inputs soils (Di *et al.*, 2009, Zhang *et al.*, 2010, Lu *et al.*, 2012, Zhang *et al.*, 2012), indicating niche separation between AOA and AOB (Prosser & Nicol, 2012). Meta-analysis and high-throughput sequencing further indicates that soil pH is the best predictor of AOA and AOB community structure across large biogeographical scales and soil types, and pH-associated selection of

AOA and AOB phylotypes (Fernandez-Guerra & Casamayor, 2012, Hu *et al.*, 2013, Hu *et al.*, 2015). However, multi-factorial influence on AOA and AOB community composition by land use, climate factors and N deposition were highlighted in a national scale soil survey in Scotland (Yao *et al.*, 2013). A meta-survey of -6000 deposited *amoA* gene sequences from 11 different habitats worldwide also indicated the multi-factorial driving and habitat-associated phylogenetic patterns of AOA and AOB communities globally (Fernandez-Guerra & Casamayor, 2012).

To date, the majority of studies on the diversity and functional activity of AOA and AOB have concentrated on soil, riverine or marine ecosystems only and little is known about how AOA and AOB proceed across contrasting but neighboring habitats of terrestrial-marine boundaries. This study therefore investigated two intertidal Bays, Rusheen and Clew, bound by eroding terrestrial soil, on the west Atlantic coast of Ireland. Rusheen Bay, is a sheltered marsh and intertidal mud/sand flat located on the edge of Galway city (population > 75,000). Clew Bay, an intertidal mud/sand flat, 100 km north-west of Rusheen Bay, is surrounded by agricultural land predominated by sheep farming. These two terrestrial-marine boundaries represent strong physical-chemical gradients making them ideal ecosystems over which to test hypotheses on AOA and AOB distribution, activity and the ecological and evolutionary mechanisms underpinning their distribution in various habitats.

In light of previous studies, we hypothesized that AOB will play a more important role within pH neutral intertidal marine sediments while AOA are likely to

prefer the low-pH soils adjoining the Bays. This was tested by analysis of the abundance, community composition and activity of ammonia oxidizers across terrestrial-marine boundaries of soil, tidal-influenced soil-sediment interface, and marine sediments in both the field and controlled microcosms studies, where functional activity was assessed as *amoA* transcript activity. Specifically, the study aimed to address the following questions: 1) What is the distribution pattern of AOA and AOB communities and how are they shaped across terrestrial-marine boundaries, and is habitat (soil-interface-sediment) or Bay (Rusheen versus Clew) the stronger selector for AOA and AOB distribution and diversity? 2) Does the activity and contribution of AOA and AOB to nitrification vary across the terrestrial-marine boundary?

Results

Physiochemical properties and potential nitrification rates in field samples

Samples of soil, interface soil (seawater saturated), muddy and sandy sediment were collected from five sites in each Bay. Generally, pH increased along soil to sediment, and soil samples had markedly lower pH values (5.39 - 6.87) than sediments (7.26 - 7.79; $p < 0.05$; Table 1). Dissolved salt concentration was lowest in two soils (RSoi_1 and CSoi_1), free from regular tidal influence, ranging from 0.05 to 0.1% (g per 100 g fresh sample), but was much higher at the two interface sites (RInt_1 and CInt_1) and seawater saturated grassland soil (CSoi_2) (1.18 - 3.72% (g

per 100 g fresh sample). The five sediment sites had similar *in situ* seawater salinity of 30.7 - 32.1 psu (Table 1). Physical texture varied significantly between soil, interface, sandy and muddy sediment, with values of 42.1 - 98.5% sand (1 mm - 63 µm) and 1.50 - 57.85% mud (< 63 µm). Three sandy sediments (RSed_2, RSed_3 and CSed_2) comprised > 93% sand and < 7% mud, while two muddy sediments (RSed_1 and CSed_1) had > 51% mud and < 49% sand (Table 1). Five soils and interface samples had sand content of 73 - 88%. Ammonia concentration varied within the range 0.73 - 3.64, and 0.81 - 5.32 µg g^{-1} fresh weight in Rusheen Bay and Clew Bay samples, respectively, but nitrate concentrations did not differ significantly between sites, with values of 0.03 - 0.81 µg g^{-1} (Table 1). For both Bays, TOC was highest in soil (13.05 - 14.95%), followed by interface (4.72 - 8.44%) and sediment (0.64 - 3.47%; $P < 0.05$; Table 1). Ammonia concentration was positively correlated with TOC ($p < 0.05$, r = 0.460, n = 30), and both ammonia and TOC showed significant negative correlations with pH ($p < 0.01$, r = - 0.416 for ammonia, r = - 0.868 for TOC, n = 30).

Potential nitrification rates (PNR) in soils and sediments was determined after incubation with PBS and *in situ* seawater, respectively, or, for interface samples, using both PBS and seawater, but values for different solutions did not differ significantly. In Rusheen Bay, PNR was highest (8.71 ± 1.32 NO_2^--N µg g^{-1} d^{-1}) in muddy sediment RSed_1 and, for Clew Bay, in interface samples (1.36 ± 0.46 NO_2^--N µg g^{-1} d^{-1} in PBS; 0.89 ± 0.39 NO_2^--N µg g^{-1} d^{-1} in seawater) (Fig. S1). There was no

significant correlation between any of the measured environmental variables and PNR.

amoA and 16S* rRNA *gene abundance in field samples

For both Bays, AOA *amoA* abundance in soils, ranged between 3.31×10^6 - 2.23×10^7 gene copy g^{-1} fresh soil, and ~100-fold higher than in sediments (3.03×10^4 - 5.66×10^5 gene copy g^{-1} sediment) ($p < 0.05$; Fig. 1). In contrast, AOB *amoA* abundance in soils (1.17×10^5 - 9.33×10^5 gene copy g^{-1} fresh soil) was much lower than in sediments (7.27×10^5 - 7.75×10^6 gene copy g^{-1} fresh sediment) ($p < 0.05$) except for one sediment site, CSed_1, where AOA and AOB abundances were similar (Fig. 1). AOA and AOB therefore dominated in soil and sediments, respectively, with similar abundance in the transition zone from coastal soils to sediments i.e. the interface ($p > 0.05$; Fig. 1). Interestingly, archaeal and bacterial 16S rRNA gene abundances followed different trends, peaking in interface samples and sediments close to land and decreasing in samples away from soils or open Bay (Fig. 1).

Generally, AOA *amoA* abundance was negatively correlated with pH ($p < 0.01$, r = - 0.645, n = 30), but positively correlated with ammonia ($p < 0.05$, r = 0.375, n = 30) and TOC content ($p < 0.01$, r = 0.650, n = 30). In contrast, AOB *amoA* abundance correlated positively with pH ($p < 0.01$, r = 0.525, n = 30), but negatively with ammonia ($p < 0.01$, r = - 0.482, n = 30) and TOC ($p < 0.01$, r = - 0.514, n = 30). Unlike AOA and AOB *amoA* abundance trends, both archaeal and bacterial 16S rRNA gene abundance correlated positively with the percentage of mud ($p < 0.01$, r =

0.781 for archaea, r = 0.644 for bacteria, n = 30). Statistically significant positive correlations were observed between archaeal and bacterial 16S rRNA gene abundances ($p < 0.01$, r = 0.696, n = 30).

Community composition of AOA and AOB across* in situ *soil, interface and sediment

AOA and AOB community composition and diversity in coastal soils and sediments were determined by 454 pyrosequencing. In total, 92,271 bacterial and 84,209 archaeal *amoA* gene reads of high quality, excluding singletons, were recovered from 28 AOB samples and 19 AOA samples, respectively. Eleven AOA samples failed to produce pyrosequencing reads and were subjected to clone library sequencing, and excluded from alpha- and beta-diversity analysis. The total number of classifiable pyrosequencing reads per sample for AOB was 564 - 7924 with a mean of 3295.4, while the number for AOA reads varied from 418 to 9587, with a mean of 4432.1. Thus, sequencing reads were rarified to 564 for AOB and 418 for AOA per sample for subsequent community similarity and diversity analyses. Due to the low abundance of AOA, in particular in sediment sites, alpha- diversity analysis was completed for AOB only. All alpha-diversity indices were significantly lower for AOB in interface samples than in soil and sediment samples for both Bays (Table 2), indicating that only a limited number of AOB phylotypes are adapted to and dominate in the tidal-influenced interface between land and sediment.

Due to the difficulty in amplifying sufficient AOA PCR products for clone library and pyrosequencing analysis, especially from sediments, the community composition of AOA was limited to 23 out of 30 samples, i.e. 19 from pyrosequencing (Fig. 2) and 4 from cloning and sequencing (Fig. S2). All archaeal *amoA* sequences grouped into Thaumarchaeota Group I.1a, I.1a-associated (Clade *Nitrosotalea*) and I.1b. Group I.1b was further defined as Clade A (Fosmid 54d9) and Clade E based on Alves's and our previous classification (Alves *et al.*, 2013, Zhang *et al.*, 2017) (Fig. S3A). Clade A (Fosmid 54d9) exclusively dominated soil samples in Rusheen Bay, with the relative abundance varying between 72.2 - 81.8%, but occupied less than 1.5% of the AOA community in other samples (Fig. 2A and S2). Conversely, Clade E was detected in almost all soil, interface and sediment samples, and dominated the two sediment sites closest to land in both Bays, RSed_2 and CSed_1 (relative abundance 41.6 - 66.5% and 61.9 - 68.7%, respectively, Fig. 2A). Group I.1a was detected in all samples except soil samples from Rusheen Bay and dominated in Clew Bay sediment CSed_2 (relative abundance > 76%). Group I.1a-associated are closely related to Group I.1a in the phylogenetic tree and accounted for 70.8% of AOA sequences in CSoil_1 (pH 5.4; Fig. 2A). The results indicate that adjacent soils and sediments select different dominant AOA phylotypes.

Phylogenetic analysis grouped AOB sequences into two distinct lineages composing of *Nitrosospira* and *Nitrosomonas*. *Nitrosospira*-affiliated sequences were further classified into *Nitrosospira* Nsp2/17 (Cluster 3a.1, C3a.1) and *Nitrosospira* sp.

O13 (Cluster 2, C2) clades based on previous classification (Avrahami & Conrad, 2003, Zhang *et al.*, 2009, Abell *et al.*, 2012). The *Nitrosomonas* lineage was further divided into three clades (Fig. S3B), one containing known *Nitrosomonas* phylotypes, e.g. *Nitrosomonas europaea, N. oligotropha* and *amoA* clones from estuary and Bay sediments, hence named as the "Known *Nitrosomonas*". Another two closely related clusters did not cluster with any AOB isolates and were designated *Nitrosomonas* Group A and *Nitrosomonas* Group B, corresponding partially to groups A and B in O'Mullan's classification (O'Mullan & Ward, 2005). Thus, all AOB sequences could be classified into one of five groups. Interestingly, a distinct AOB community shift was observed among soils, interface samples and sediment sites (Fig. 2B). The two soils RSoi_1 and CSoi_1 farthest away from sea were exclusively dominated by *Nitrosospira* Nsp2/17 (C3a.1) and *Nitrosospira* sp.O13 (C2) clades respectively, with a relative abundance at the range of 72.9 - 85.1% in RSoi_1 and at the range of 78.6 - 82.7% in CSoi_1. Conversely, these two clusters were not detected in any of the interface or sediment sites (Fig. 2B). Among *Nitrosomonas*-affiliated sequences, the Known *Nitrosomonas* group was mainly detected in sediments of Rusheen Bay, with the relative abundance varying between 5.6 - 60.6%. *Nitrosomonas* Group A dominated the seawater-saturated grassland soil close to the interface site in Clew Bay (CSoi_2) with a relative abundance varying between 68.4 - 95.5%. However, Group A accounted for just 0.1 - 35.7% of AOB sequences in the other interface and sediment samples (Fig. 2B). *Nitrosomonas* Group B overwhelmingly dominated all

interface samples with a relative abundance of more than 87.9%, and was detected in all sediment samples ranging from 11.9 - 83.3% (Fig. 2B).

Community dissimilarity of AOA and AOB across soil, interface and sediment boundary and the driving environmental factors

The UPGMA hierarchical clustering procedure was performed to compare the beta-diversity of AOA and AOB communities using the Bray-Curtis distance matrix. Due to the inability of calculating beta-diversity for eleven AOA samples, the remaining nineteen samples roughly clustered according to soils, interface and sediment sites (Fig. 3A). For the AOB community from both Bays, the UPGMA dendrogram revealed a more obvious clustering among soils, interface and sediments regardless of Bay location, with the soil cluster relatively far from sediment and interface clusters (Fig. 3B). Results therefore indicate that both AOA and AOB community composition was influenced by habitat type rather than Bay location, corresponding with the phylogenetic analysis.

To examine the factors driving the dissimilarity in AOA and AOB community composition across the terrestrial-marine boundary, Monte Carlo tests were performed to determine the influence of environmental variables on community composition based on the above classification. Significant influences of ammonia (37.3%), salinity (35.3%), pH (15.7%) and nitrate (5.9%) were found on the AOA community, while TOC (44.6%), pH (32.1%), nitrate (10.7%) and salinity (8.9%) significantly influenced the AOB community ($p < 0.05$). These factors displaying significant

correlations with community structure were therefore extracted for redundancy analysis (RDA). In the resulting RDA plot, the AOA community separated between soil and sediment along the x-axes, while the AOB community in soil and sediment split along the y-axes, with the interface samples in the middle of soils and sediments (Fig. 4). The first two axes explained 91.7% and 92.8% of the variance in the relationship between AOA and AOB community structure and environmental factors, respectively (Fig. 4).

Changes in ammonia and nitrate concentrations during microcosm incubation

To evaluate ammonia oxidizer activity in the coastal soil, interface and sediment gradients, one representative sample from each habitat type was incubated in microcosms following addition of ammonia with and without acetylene (C_2H_2). Acetylene, an inhibitor of nitrification, was added to microcosms to block nitrification activity (Hyman *et al.*, 1995). The ammonia concentration in all C_2H_2-free treatments was significantly lower than in C_2H_2-blocked treatments after incubation for 28 days (Fig. 5A). Ammonia concentrations in the microcosms ranged between 44.3 - 51.5 μg g^{-1} at the beginning of incubation (day 0), and significantly decreased to 2.1- 7.7 μg g^{-1} after 2-weeks of incubation (day 14) in the C_2H_2-free treatments of soil and interface samples ($p < 0.05$; Fig. 5A). After the second ammonia addition (50 μg g^{-1} NH_4^+-N, day 14), ammonia concentration was reduced to 3.0 - 45.2 μg g^{-1} at day 28, which is significantly lower than the corresponding C_2H_2-containing treatments (59.5 - 103.6 μg g^{-1} , $p < 0.05$; Fig. 5A). The ammonia in the C_2H_2-free treatment of

sediment RSed_2 decreased from 51.5 to 28.6 μg g^{-1} NH_4^+-N, and then 1.4 μg g^{-1} NH_4^+-N, at day 14 and 28, respectively (Fig. 5A). This suggests that the added ammonia was actively oxidized in all C_2H_2-free microcosms. Although the ammonia concentration in the muddy sediment CSed_1 increased during the 28-days incubation, high nitrate accumulation (42.4 μg g^{-1} NO_3^--N) was observed in C_2H_2-free treatment at day 28 (Fig. 5A). This implies that ammonia from mineralization might be utilized by ammonia oxidizers in addition to the ammonia added to the microcosm. For all six samples used for microcosms, no significant nitrate accumulation was observed in the C_2H_2-treatments during incubation (<3.5 μg g^{-1} NO_3^--N) compared with C_2H_2-free treatments (Fig. 5B), which further confirmed active nitrification in these samples. Interestingly, nitrate concentration in C_2H_2-free treatments of two interface samples (RInt_1 and CInt_1) and one seawater-saturated grassland soil (CSoi_2), was significantly higher than their initial value but <7 μg g^{-1} NO_3^--N over time. The decrease in ammonia concentration did not correspond with the increase in nitrate which may be a result of N_2 and/or N_2O loss via denitrification coupled nitrification, and probably anammox process.

Abundance and transcriptional activity of archaeal and bacterial* amoA *genes during microcosm incubation

amoA gene and transcript abundances in each microcosm were quantified by qPCR and RT-qPCR to estimate the growth and activity of ammonia-oxidizers. Gene abundances in C_2H_2-free Rusheen Bay treatments responded as in field samples. AOA

amoA genes were significantly more abundant than AOB in soil (RSoi_1, 1.64×10^6 - 1.21×10^7 g^{-1}) and significantly lower in sediment (RSed_2, 2.28×10^4 - 5.97×10^4 g^{-1}; $p < 0.05$), while AOA and AOB abundances in interface (RInt_1) were similar (7.69×10^5 - 5.77×10^6 g^{-1} for AOA, and 4.60×10^5 - 4.27×10^6 g^{-1} for AOB) ($p > 0.05$; Fig. 6A). Gene abundances in Clew Bay samples (CSoi_2, CInt_1 and CSed_1) showed similar trends as those in Rusheen Bay and AOA and AOB abundances were not significantly different during incubation. *amoA* transcripts of AOA, AOB and both AOA and AOB *amoA* gene transcripts were quantified in soil, sediments and interface samples, respectively (Fig. 6B). C_2H_2 did not reduce AOA or AOB *amoA* abundances in any sample, but significantly decreased AOA and AOB *amoA* gene transcripts with the exception of CSed_1 soil at day 14 (Fig 6B). For both AOA and AOB, *amoA* gene and transcript abundance did not change significantly during incubation, indicating that the starting populations and activities of AOA and AOB were sustained throughout the experiment, perhaps due to the use of freshly collected samples in the microcosm incubations.

DGGE profiles of AOA and AOB amoA gene transcripts in microcosms

Changes in active AOA and AOB communities during incubation were characterized by DGGE analysis of *amoA* gene transcripts in microcosms with and without C_2H_2 addition and compared to *amoA* gene profiles at day 0. The dominant bands were excised for cloning and sequencing analysis. As a substantial quantity of PCR amplicons are required for DGGE analysis, transcript profiles for AOA *amoA*

were only obtained from microcosms containing two soils (RSoi_1 and CSoi_2) and a Rusheen Bay Interface sample (RInt_1; Fig. 7A.) and, for AOB, from two sediments (RSed_1 and CSed_1) and a Clew Bay Interface sample (CInt_1; Fig. 7B). DGGE profiles were highly reproducible between the three replicates of each treatment and distinct differences in profiles were observed between C_2H_2-free and C_2H_2-containing treatments, with much weaker or absent band patterns in all C_2H_2-containing treatments (Fig. 7), confirming inhibition of nitrification activity by C_2H_2. DGGE profiles of *amoA* genes from fresh soils and sediments used for incubation were retrieved (marked as "0 d DNA") for comparison to transcript profiles. Generally, both AOA and AOB *amoA* gene and transcript DGGE profiles were similar, while the numbers and relative intensity of DGGE bands in gene profiles were generally greater than in transcript profiles at day 0 (Fig. 7). This suggests that day-0 gene profiles of the AOA and AOB communities (fresh field samples) do not necessarily represent the active ammonia-oxidizer communities.

In the AOA profiles of RSoi_1, more than 10 bands were detected at day 0 and day 14, representing ammonia oxidizer Clade A and Clade E within *Thaumarchaeota* Group I.1b as determined by gel excision and sequence analysis. Bands corresponding to Clade E decreased in relative intensity at day 28 (Fig. 7A). AOA *amoA* transcripts profiles in RInt_1 and CSoi_2 samples did not change significantly during incubation (Fig. 7A), and the bands in low and high gradient areas were identified as *Thaumarchaeota* Group I.1a and I.1b Clade E, respectively, representing

the active ammonia oxidisers in soils and interface samples. As for AOB DGGE profiles, RSed_1 had the most bands, most of which increased in relative intensity with time, while a small number of bands decreased in relative intensity (Fig. 7B). In the AOB *amoA* transcript profiles from the interface sample (CInt_1), four bands were detected, two of which (7-1 and 7-4) dominated the community (Fig. 7B). The DGGE bands of AOB *amoA* transcript in CSed_1 samples were undetectable at days 0 and 14, but significantly increased in relative intensity and displayed a distinct community pattern by day 28, different from the DNA-based profiles at day 0. This indicated active growth of AOB and coincided with an increase in nitrate and gene transcript abundance at day 28. For the two sediments (RSed_1 and CSed_1), all excised mRNA-based bands were phylogenetically placed within the "Known_*Nitrosomonas*" clade even though the excised DNA-based bands were identified as *Nitrosomonas* Group A and Group B (Fig. 7B and Fig. S3), suggesting functional dominance of the "Known_*Nitrosomonas*" in these two sediments. The excised bands from the interface samples (CInt_1) were mainly dominated by *Nitrosomonas* Group_B, followed by Group_A in both DNA and mRNA-based analysis, indicating that there are distinct, active AOB communities between interface and sediment.

Discussion

Environmental factors drive AOA and AOB community shift across coastal soil, interface and sediment habitats

The aim of this study was to investigate the distribution of AO across soil-sediment gradient in two bays and determine if habitat or bay was a stronger selector of AO community composition. We hypothesized that AOA would be more important in low pH soil, and AOB in neutral pH sediments, and that this would be accompanied by a shift in community composition and activity across the gradient. Our results revealed distinct shifts in AOA and AOB communities, due to the influence of multiple environmental variables including salinity and pH across the terrestrial-marine gradient, regardless of the Bay in question. Two soils (RSoi_1 and CSoi_1), free from tidal influence, in Rusheen Bay and Clew Bay were dominated by thaumarchaeal Group I.1b_Clade A and Group I.1a_associated (Clade *Nitrosotalea*), respectively. Group I.1b_Clade A, represented by soil fosmid clone 54d9 (Treusch *et al.*, 2005), are widely detected in soil especially in neutral-alkaline soils (Zhang *et al.*, 2009, Gubry-Rangin *et al.*, 2011). Group I.1a_associated, represented by the cultured *Nitrosotalea devanaterra* from acidic (pH 4.5) agricultural soil (Lehtovirta-Morley *et al.*, 2011), is mainly detected in acidic soils (He *et al.*, 2007, Gubry-Rangin *et al.*, 2011). The dominance of Group I.1b_Clade A in RSoi_1 and Group I.1a_associated in CSoi_1 could be explained by pH differences between RSoi_1 and CSoi_1 with the value at 6.56 and 5.39 respectively, providing further evidence for pH selection of

these AOA phylotypes. In contrast, Group I.1b_Clade E and Group I.1a were detected in almost all soil, interface and sediment samples, and Clade E-affiliating sequences showed high identity with sequences from salt marsh sediments (EU925286, DQ148902), river sediments (KF668774) and soils (EU671395, KM116912) (Fig. S3A), suggesting its widespread distribution in a range of habitats. Group I.1a was represented by a group clustering with AOA isolates *Nitrosopumilus maritimus* SCM1 from the open ocean (Konneke *et al.*, 2005), *Candidatus Nitrosoarchaeum limnia* SFB1 from coastal sediment (Blainey *et al.*, 2011) and *Candidatus Nitrosoarchaeum koreensis* MY1 from agricultural soil (Jung *et al.*, 2011). It is therefore not surprising that these two clades were detected in almost all soil, interface and sediment samples.

AOB communities in the three habitats also varied, with *Nitrosospira* dominating soils (RSoi_1 and CSoi_1) free from tidal influence and *Nitrosomonas* dominating tidal-influenced soil, interface and sediment samples. Among *Nitrosomonas*-affiliated sequences, the Known_*Nitrosomonas* clade accounted for 5.6 - 60.6% of the AOB community in Rusheen Bay sediments. Sequences showed high identity with environmental sequences from salt marsh (JX306633), marine (KM595980,) and estuary (AY702586, HQ888784) sediments, and grouped closely with the known *Nitrosomonas* cultures such as *N. europaea*, *N. halophile* and *N. marina* (Fig. S3B). The majority of AOB in the tidal-influenced soil, interface and sediments clustered with *Nitrosomonas* group A and B were similar to uncultured AOB from other marine environments such as the Baltic Sea (EF222057), San Francisco Bay (EU651496),

Arctic sediment (GU453824), Monterey Bay (AY736932) and deep-sea sediment (KF970501) (Fig. S3B). These results further support the suggestion of O'Mullan and Ward (2005) that *Nitrosomonas* groups A and B prefer marine environments. Similarly, salinity has previously been identified as a strong driver of AOA and AOB community composition in coastal and estuarine sediments (Duff *et al.*, 2017). Studies in San Francisco Bay, Chesapeake Bay, Plum Island Sound and Ythan estuaries reported the shift of estuarine/marine *Nitrosospira*-like *amoA* sequences and *Nitrosomonas*-like sequences between high and low salinity sites (Francis *et al.*, 2003, Bernhard *et al.*, 2005, Freitag *et al.*, 2006, Mosier & Francis, 2008). In this study, a contrasting pattern was seen, with *Nitrosospira* dominating tide-free soils and *Nitrosomonas* dominating tidal-influenced soils and sediments. Nevertheless, it has been reported that the *Nitrosospira* sequences frequently found in marine environments belong to *Nitrosospira* cluster 1, which currently has no cultured representative (Freitag *et al.*, 2006) and was not retrieved in this study. It has also been suggested that all cultured *Nitrosospira* species have low salt tolerance (Kowalchuk & Stephen, 2001, Koop *et al.*, 2006), explaining their dominance in tide-free soils. The AOB community in two soils RSoi_1 and CSoi_1 were exclusively dominated by *Nitrosospira*_Nsp2/17 lineage (corresponding to cluster 3a.1) and *Nitrosospira*_sp.O13 lineage (corresponding cluster 2), respectively, possibly reflecting respective pH values of RSoi_1 (at 6.56) and CSoi_1 (at 5.39), as *Nitrosospira* cluster 3 frequently dominates AOB neutral pH arable fields while *Nitrosospira* cluster 2 dominates acidic pH soil

(Stephen *et al.*, 1996). The results are consistent with our previous observation across a large geographical scale showing that the relative abundance of cluster Nsp2/Nsp17 (C3a.1) and cluster 2 significantly increase and decrease with increasing soil pH, respectively (Shen *et al*., 2012, Hu *et al.*, 2013). These observations therefore provide evidence for pH-selection of clusters Nsp2/Nsp17 (3a.1) and cluster 2. Additionally, samples from the interface site (RInt_1) and the seawater saturated grassland soil site (CSoi_2) had a similar pH, ranging between 6.62 - 6.87 to RSoi_1 (6.56). The AOB community in RInt_1 and CSoi_2 were different from RSoi_1, but similar to the other interface and sediment samples with *Nitrosomonas*-affiliated sequences dominating. The data therefore suggest that the AOB community was firstly regulated by salinity, and then by pH. Taken together, the preference for a certain pH or high/low tolerance to salinity of specific AOA and AOB phyla explained well the separation of AOA and AOB communities across three habitats, supporting our original hypothesis on pH with the added caveat that in coastal environments salinity may be the first regulator.

In addition to salinity and pH, ammonia and TOC correlated significantly with AOA and AOB communities, respectively (Fig. 4). Ammonia is frequently suggested as a major environmental factor influencing AOA and AOB community composition (Shen *et al.*, 2008, Wankel *et al.*, 2011, Zheng *et al.*, 2014, Yu *et al.*, 2016) and determining niche differentiation of AOA and AOB (Prosser & Nicol, 2012), as thaumarchaeal AOA have much lower Km values for ammonia than AOB (Martens-Habbena *et al.*, 2009). However, in this study, ammonia concentration,

ranging between 1.85 - 5.32 μg/g (fresh weight) in soils, between 2.10 - 3.64 μg/g (fresh weight) in interfaces, and between 0.73 - 4.84 μg/g (fresh weight) in sediments (Table 1), showed no significant differences amongst the three habitats. The influence of ammonia and some other factors (such as organic carbon, oxygen, redox potential) may be overridden by that of salinity and pH, due to their slight variation among the three habitats. Significant correlations between the AOB community and TOC were observed, which is consistent with previous data on intertidal sediments of the Yangtze estuary (Zheng *et al.*, 2014). Considering that AOB are obligate chemoautotrophic and cannot grow heterotrophically on organic compounds (Krümmel & Harms, 1982), suggests that TOC may not influence the AOB community directly.

AOA and AOB amoA gene abundance and transcriptional activity in coastal soil, interface and sediment microcosms

In both field samples and microcosm studies, AOA outnumbered AOB *amoA* genes in soils while the opposite trend was occurred in sediments (Fig. 1) while similar abundances were observed at the interface. This observation suggests AOA and AOB may dominate functionally in soils and sediments, respectively, but both groups contribute equally at the interface. This was further supported by the microcosms studies whereby AOA *amoA* gene transcripts were only recovered from soil, both AOA and AOB *amoA* gene transcripts were detected in the soil/sediment interface, and only AOB *amoA* gene transcripts from the sediment microcosms.

Nitrification was active as indicated by the consumption of added ammonia over time (Fig. 5). These results further demonstrated that AOA were functionally active in soil, AOB in sediments and both AOA and AOB in the soil/sediment interface in both Bays. This suggests that habitat differences, particularly seawater-influence or not, made remarkable differentiation on AOA and AOB's activity, in addition to the determining influence on their community structure.

Similar effects of salinity on relative abundances of AOA and AOB have been reported previously in San Francisco Bay (Mosier & Francis, 2008, Santoro *et al.*, 2008) and dominance of AOB *amoA* genes in sediments, with increases in AOB:AOA ratio from freshwater to marine sites, have been observed in the Colne estuary, UK, (Li *et al.*, 2015). Transcriptional activity of AOB was also greater than that of AOA in coastal microbial mats in the North Sea (Fan *et al.*, 2015). In contrast, Zheng et al., (2014) reported strong correlations between nitrification rates and AOA, but not AOB, *amoA* gene abundance in the Yangtze Estuary, with low to medium salinity (0.9 - 14.8 PSU), despite higher AOB gene abundance at most sites. AOB therefore appear to have an important role in nitrification in high saline coastal sediments where salinity is a determining factor driving the separation of AOA and AOB activity.

In contrast to the above findings where AOA are predominant at freshwater and low salinity sites, numerous studies from the North Atlantic and North Sea, Monterey Bay and Hawaii, the Japan Sea, the Gulf of California, have demonstrated that AOA are the numerically dominant ammonia oxidizer (Francis *et al.*, 2007, Smith *et al.*,

2014). Furthermore, good correlations between nitrification rates and AOA *amoA* gene and transcript abundances have been observed in coastal waters (Smith *et al.*, 2014), in the Puget Sound Estuary water column (Urakawa *et al.*, 2014), and sediments in the Oyster ground of North sea (Lipsewers *et al.*, 2014). The inconsistency in the findings on the alteration of AOA and AOB functioning in different marine-influenced systems could be attributed to innate AOA and AOB community composition and variation in physical and chemical conditions among the coastal/marine settings studied, which require further study.

AOA *amoA* genes and transcripts were more abundant than those of AOB in two soils (Rsoi_1 and Csoi_1) which are natural grassland soils, free from tidal influence, with lower pH, 6.56 and 5.39 respectively, and with no additional nutrient input from agriculture. There is evidence for AOA functional dominance over AOB in acidic and low ammonia soils (e.g.Gubry-Rangin *et al.*, 2010, Yao *et al.*, 2011), with direct evidence of nitrification-associated autotrophic growth of AOA provided by stable isotope probing in Scottish grassland soil with high organic matter and low inorganic N input (neutral pH), and a strongly acidic tea orchard soil (pH 3.7) (Zhang *et al.*, 2010, 2012). More directly, an obligate acidophilic thaumarchaeal ammonia oxidizer was cultivated from a nitrifying acidic soil (Lehtovirta-Morley *et al.*, 2011). These results together with the observation of AOB functionally dominating over AOA in nitrogen-rich and alkaline soils (Di *et al.*, 2009, Jia & Conrad, 2009) further confirm

pH and N concentrations are critical factors determining niche separation between AOA and AOB in soil systems.

Both AOA and AOB were observed to be active in the interface sites, with medium salinity due to recurrent saturation by rainwater and seawater. The constant change in salinity created a niche that supported both AOA and AOB. This is one of the few studies that has observed functional activity in nitrification of both AOA and AOB simultaneously.

Active AOA and AOB population in coastal soil, interface and sediment

Sequence analysis of DGGE bands of *amoA* transcripts in microcosm samples identified Thaumarchaeal Group I.1b_Clade A, _Clade E and Group I.1a identified as active AOA in soils and at the interface, as also indicated by their dominance in corresponding field samples (RSoi_1, RInt_1 and CSoi_2 at DNA level). Clade A (represented by fosmid clone 54d9, (Treusch *et al.*, 2005) is the most abundant and widespread AOA phylotype in soils (Bates *et al.*, 2011) but its contribution to nitrification has been questioned. For example, Alves *et al.* (2013) reported low nitrification rate in soil dominated by Clade A in addition, Clade A enrichments from Arctic soils had lowest or little nitrification activity and they suggested that Clade A may not be an obligate NH_3 oxidizer. In this study, AOA communities in grassland soil from Rusheen Bay (RSoi_1) were dominated by Clade A (72.2 - 81.8% relative abundance), while Clade A and Clade E were equally represented as transcripts. This

result indicates that Clade A was not as transcriptionally active in proportion to their DNA abundance further supporting Alves's observations.

In contrast, retrieval of Clade E transcripts in soil and interface samples suggests its broad activity and adaptation to a range of habitats. Despite its broad habitat distribution, the activity and ecophysiology of this clade is poorly defined. Group I.1a transcripts were also detected in Rint_1 and CSoi_2, generating multiple DGGE bands, suggesting active participation in nitrification within tidal-influenced soil and interface samples.

Unlike AOA, dominant AOB groups in field samples did not always correspond to active AOB groups identified from DGGE profiles. For two sediments, all excised transcript bands from microcosms sampled at day 14 in RSed_1 and at day 28 in CSed_1 were represented by the Known_*Nitrosomonas* clade (Fig. 7B), suggesting its activity and contribution to nitrification. However, sequence analysis of field samples (Fig. 3B) and of DNA bands from the microcosm samples at day 0 (Fig. 7B) suggested dominance by *Nitrosomonas* Group B. DNA and cDNA DGGE profiles from day 0 samples of the two sediments also showed distinct differences, which further suggested the difference between DNA- and RNA-based analysis. cDNA profiles shifted with time, and increasing band brightness (Fig. 7B), indicating more intense *amoA* gene expression from the known *Nitrosomonas* clade was induced during the incubation. The sequences affiliated with the Known *Nitrosomonas* cluster were similar to *N. europaea, N. oligotropha* and sequences from estuarine

environments. *N. europaea* is thought to be adapted to high-nitrogen environments, with frequent recovery in high-nitrogen laboratory media and dominance of high-nitrogen wastewater treatment systems (Prosser, 1989, Kowalchuk & Stephen, 2001). *N. oligotropha*-like AOB can adapt to both high and low initial ammonium concentrations (up to 10 mM) (Bollmann & Laanbroek, 2001). The stronger cDNA bands represented by the known *Nitrosomonas* cluster at day 28 in RSed_1 and CSed_1 microcosms result from addition of ammonia (50μM NH_4^+-N) stimulating or enriching the growth of AOB within this cluster.

Nitrosomonas Group B overwhelmingly dominated the two interface sites (87.9% relative abundance), and was exclusively retrieved from both DNA at day 0 and RNA at day 14 in Cint_1, demonstrating numerical and functional dominance, further confirming their contribution to nitrification in coastal ecosystems.

In summary, a combination of field surveys and microcosm incubations were used to test our hypothesis on the distribution and activity of AO across soil-sediment gradient in two bays. We have shown distinct shifts in AOA and AOB communities across terrestrial-marine boundaries regardless of location, indicating habitat and not bay is the stronger selector of AOs. We further showed that both the community composition and activity of AO shifted across this gradient, following similar trends in two geographically separated Bays. Thaumarchaeal Groups I.1b_Clade A and I.1a_associated exclusively dominated the AOA community in soils, while Groups I.1b_Clade E and I.1a dominated interface and sediment sites. AOB communities

shifted from *Nitrosospira*-like to *Nitrosomonas*-like *amoA* sequences across soils free from tidal influence to tidal-influenced soils, interface and sediments. These observations suggest that distinct coastal habitats drive AOA and AOB community shifts across adjacent terrestrial-marine zones. Transcript analysis of microcosm samples further demonstrated that specific AOA/AOB phylotypes switched between soil and sediment dependent on their ecophysiological adaptation to salinity and pH. Quantitative analysis of both genes and transcripts suggested dominance of AOA and AOB in soils and sediments, respectively, and similar abundances and transcriptional activity at the soil/sediment interface. Our results provide fundamental insights into the spatial distribution of AOA and AOB communities and their activities, and the shaping factors across terrestrial-marine boundaries, providing essential, previously missing knowledge for the nitrogen cycle in coastal ecosystem.

Experimental Procedures

Study sites and sampling

Samples were collected across a soil-sediment gradient in Rusheen Bay (53º15´N, 9º6´W~9º7´W) and Clew Bay (53º47´N, 9º36´W~9º37´W) in spring, 2013. Rusheen Bay is sheltered by a mixed sand and storm beach. Soils adjoining the Bay are peat over lithoskeletal acid igneous rock. Clew Bay is sheltered by a sand beach on its seaward side and numerous groundwater upwelling and freshwater streams enter the Bay. Adjoining soils are loamy over sandstone bedrock with high organic matter. Five

samples (top 0-5 cm layer) were collected from each Bay at low tide from five sites including grassland soil close to the shore, tidal-influenced soil-sediment interface and intertidal sediments (Fig. S4). Samples from five points along a ten meter-route were pooled as one replicate and three radical or parallel sampling routes were included as three replicates. After carrying back to the laboratory on ice, samples were subsampled from each replicate into 0.5 - 1 g aliquots and stored at -80 °C for subsequent molecular analysis. Saturated soil and grassland soil samples were dried at room temperature overnight and passed through a 2.0-mm sieve to remove the root tubers, before storage at 4 °C for potential nitrification rate measurements and physicochemical analysis.

Physicochemical analysis and Potential Nitrification Rates (PNR)

Soils/sediment pH was measured with a pH meter using fresh samples and a de-ionized water ratio of 1:2. Salinity of *in situ* seawater was determined with a Coral farm refractometer (Coral farm, Ireland). Dissolved salts were extracted with de-ionized water at a fresh sample to water ratio of 1:5 and the weight was determined after drying at 105 °C until constant weight. Soil and sediment textures were determined by Mastersizer 2000 Laser Particle Sizer (Malvern, UK). Ammonium (NH_4^+) and nitrate/nitrite (NO_3^-/NO_2^-) were extracted with 2 M KCl and concentrations was determined by colorimetric absorbance method using Hach reagent powder pillows and the Odyssey DR/2500 Spectrophotometer (Hach, USA) as described by (Bollmann *et al.*, 2011). Total organic carbon (TOC) was determined

by high-temperature combustion oxidation method at 450 °C for 12 hours with Carbolite furnace (Carbolite Gero, UK).

Potential nitrification rate (PNR) was measured using the sodium azide inhibition method (Ginestet *et al.*, 1998). Briefly, 5 g fresh sample was added to a 500 ml bottle containing 30 ml of site seawater for sediment and phosphate buffer solution (PBS) (g l^{-1}: NaCl, 8.0; KCl, 0.2; Na_2HPO_4, 0.2; NaH_2PO_4, 0.2, pH at the sample pH) for soils with 250 µM $(NH_4)_2SO_4$. Sodium azide (NaN_3, Sigma, Ireland) with a final concentration of 24 µM was added to inhibit nitrite oxidation. PNR measurements at the interface site were made using both *in situ* seawater and PBS buffer. The suspension was incubated in the dark on a shaker at 90 rpm at 15 °C for 24 hours, after which nitrite was extracted with 30 ml of 2 M KCl and determined spectrophotometrically as described above.

DNA/RNA extraction

DNA and RNA were co-extracted from 0.5 g subsamples as described by Griffith (Griffith *et al.*, 2000) and modified by Nicol *et al.* (2005). Briefly, 0.5 - 0.7 g sample was added to a 2-ml screw-cap Blue Matrix tube containing bead mixture (Hybaid, UK) with 0.5 ml 5% CTAB buffer (120 mM potassium phosphate, 5% CTAB in 0.7 M NaCl, pH 8.0), and then bead beaten on a vortex genie (Scientific Industries, USA) at maximum speed for 2.5 minutes to lyse cells. After centrifugation at 16 000 g for 5 min, the aqueous phase was extracted with chloroform-isoamyl alcohol (24:1) and precipitated with 2 volumes 30% (w/v) PEG 6000 in 1.6 M NaCl. Extracted nucleic

acids were re-suspended in 50 µl RNAase-free water and stored at -80 °C until further DNA analysis and RNA transcription (RNA only for microcosm samples as below).

Quantitative PCR (qPCR)

Real-time PCR was conducted on a LightCycler 480 Instrument (Roche Life Science, USA). The abundances of bacteria and archaea in field soils/sediments were quantified using a *Taq*Man assay with the general primers BACT1369F (5`-CGG TGA ATA CGTTCY CGG-3`), PROK1492R (5`-AAG GAG GTG ATC CRG CCG CA) and the *Taq*Man probe TM1389F (5`-CTT GTACAC CGC CCG TC-3`) (Suzuki *et al.*, 2000), and a SYBR Green assay with the general archaeal primers 364aF (5`-CGGGGYGCASCAGGCGCGAA - 3`) / 934b (5`- GTGCTCCCCCGCCAATTCCT - 3`) (Kemnitz et al., 2005), respectively. Each reaction was performed in a 20-μl volume and contained 10 µl Premix Ex Taq^{TM} (Roche Life Science, Ireland) for *Taq*Man reaction and SYBR$^{(R)}$ Ex Taq^{TM} (Roche Life Science, Ireland) for SYBR reaction, 400 nM of each primer and 400 nM probe for bacteria, 200 nM of each primer for archaea, and 10-fold dilution of DNA. The amplification was carried on using the thermal cycle programs: 95 °C for 5 min and 35 cycles of 95 °C for 10 s, 60 °C for 60 s for bacteria and 56 °C for 60 s for archaea. Five-fold serial dilutions of purified PCR product from a clone containing the target gene fragment was used to generate a standard curve with an efficiency of 88.2% an r^2 of 0.999 and a y-intercept of 44.9 for bacteria, 92.3% efficiency, r^2 value of 1 and y-intercept of 42.43 for archaea.

Quantification of bacterial and archaeal *amoA* genes were conducted with primer pairs *amoA*1F/*amoA*2R (Rotthauwe *et al.,* 1997) and Arch-*amoA*F/Arch-*amoA*R (Francis *et al.,* 2005), respectively. The 20 µl-reaction volume contained 10.0 µl SYBR$^{(R)}$ Premix Ex Taq^{TM} (Roche Life Science, Ireland) for DNA and 10.0 µl EVA Green master mix (Biorad, Ireland) for cDNA, 200 nM of each AOB primer or 200 nM each of AOA primer and 2 µl of 10-fold diluted DNA (1-10 ng) or 1µl of cDNA as template. Amplifications were carried out as follows: 95 °C for 5 min, followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C for AOB or 53 °C for AOA, 1 min at 72 °C and plate read at 83 °C for AOB and 81 °C for AOA. Melting curve analysis was performed at the end of PCR runs to check the specificity of the products. Standard curve of *amoA* gene was constructed as for the 16S *rRNA* gene above, with the amplification efficiencies of 84.4 – 95.2% (r^2 = 0.993-0.998) and 82.5.1 – 102.3% (r^2 = 0.995 - 0.998) for bacterial and archaeal *amoA* genes, respectively.

Pyrosequencing and cloning analysis of archaeal and bacterial amoA genes from in situ field samples

amoA sequence composition of archaeal and bacterial across the *in situ* soil, interface and sediment gradient was characterized by 454 pyrosequencing. AOA and AOB *amoA* genes were amplified using primers Arch-*amoA*F/Arch-*amoA*R (Nelson *et al.*, 2010) and *amoA*-1F/*amoA*-2R (Rotthauwe *et al.*, 1997), respectively, in 25-µl reaction volume containing 2.5 µL of 10 X buffer, 400 µM each dNTP, 2 U *Taq* DNA polymerase (SigmaAldrich, Ireland) plus 200 nM of each primer and 0.2 mg/ml of

BSA (Sigma Aldrich, Ireland). A touchdown program was employed for both AOA and AOB, with annealing temperatures from 60 °C to 55 °C for AOA and 62 °C to 57 °C for AOB during the first 10 cycles and 55 °C for AOA and 57 °C for AOB during the following 30 cycles. For each sample, triplicate independent PCR essays (25 μl each) were performed and the resulting PCR products were pooled in a single tube to reduce the bias of the PCR process. Gel-purified PCR products were sent to Molecular Research (MR. DNA) company (USA) for adapter fusion and 454 pyrosequencing in Roche 454 FLX titanium instruments (Roche Diagnostics, USA).

Due to the low abundance of target template in some sediment samples and difficulty in amplifying sufficient PCR products from low pH soil, AOA *amoA* gene PCR amplicon from some samples failed to meet the pyrosequencing standard and were therefore subjected to cloning and sequencing analysis instead. In this case, about 30 positive clones were randomly selected from each clone library for Sanger sequencing and used for further classification analysis.

Processing of pyrosequencing data and community diversity and composition analyses

The raw pyrosequencing data were processed following QIIME pipeline (Caporaso *et al.*, 2010). Prior to clustering, reads with quality score lower than 20, improper primers, or ambiguous bases were removed. The FASTA files of all samples were combined through an add_qiime_labels.py script. Chimeras were identified and eliminated using the identify_chimeric_seqs.py and filter_fasta.py scripts within

QIIME. After quality-control, a total of 84 209 and 92 271 reads were retained for the AOA and AOB *amoA* genes, respectively, and the sequences were clustered into operational taxonomic units (OTUs) at 97% similarity level using uclust command (Edgar, 2010).

The community diversity of AOA and AOB was estimated by calculating OTU richness and Shannon diversity index at the 97% similarity level with QIIME. To eliminate the effects of surveying effort on the estimation of community diversity (alpha and beta diversity), 418 AOA and 564 AOB sequences per sample were randomly selected. Of 30 samples, 28 AOB samples and only 19 AOA samples produced enough reads for alpha- and beta-diversity analysis. The Bray-Curits distance was introduced as the beta diversity metrics. An Unweighted Pair Group Method with Arithmetic Mean Clustering (UPGMA) procedure based on Bray-Curtis distance was performed in QIIME to compare the pair-wise community similarity.

To assign taxonomic classification information to AOA and AOB, one representative sequence of each AOA OTU from the clone libraries and the top 50 AOA OTUs (representing 73.7 - 94.2% of all AOA reads, with a mean of 85.9%) and the top 200 AOB OTUs (representing 70.5 - 98.6% of all AOB reads, with a mean of 85.4%) from the pyrosequencing dataset were selected for phylogenetic tree construction, together with known enrichment cultures of ammonia oxidisers and taxonomy-determined reference sequences from the National Center for Biotechnology Information (NCBI) database. The phylogenetic trees were constructed

in MEGA version 5.0 (Tamura *et al.*, 2011) with bootstrap neighbor-joining method using Kimura 2-parameter distance with 1000 replicates to produce bootstrap values. The taxonomy was assigned to each OTU with robust phylogenetic supports and high bootstrap values.

Soil-interface-sediment microcosms

One soil (RSoi_1, CSoi_2), interface soil (RInt_1, CInt_1) and sediment (RSed_2, CSed_2) site from each bay was selected for microcosm incubations with and without the addition of acetylene (C_2H_2) and then subjected to *amoA* gene and transcript abundance and diversity assays to evaluate the activity of ammonia oxidizers. Microcosms were conducted in 250-ml serum bottles containing 12 g of fresh samples with 50 μg g^{-1} fresh soil/sediment NH_4^+-N (final concentration) added at day 0 and day 14. For grassland and interface soils, $(NH_4)_2SO_4$ was dissolved in sterile water at a suitable concentration and 0.5 - 1 ml of solution was added to adjust the soil water content to 60% - 70% of field moisture capacity. For sediment slurries, 10 ml site seawater containing 100 ppm $(NH_4)_2SO_4$–N was added to each bottle. Each sample had two treatments, in the presence or absence of 0.1% (v/v) C_2H_2 for nitrification inhibition. Serum bottles were sealed with rubber stoppers and aluminum caps and incubated on a shaker at 90 rpm at 15°C in the dark. Aerobic conditions were maintained by opening microcosms to refresh the air in the head space every two days, after which C_2H_2 concentration was re-established. Three replicates of each treatment were destructively sampled at days 0, 14 and 28 and then frozen

immediately at -80°C before further analysis. Ammonium and nitrate were extracted and analyzed as described above.

Transcription activity analysis for microcosm samples

For microcosm samples, DNA and RNA were co-extracted from 0.5 g subsamples as described above. Half-volume of the extracted nucleic acids was used for DNA analysis and half was treated with TURBO DNAase (Ambion, Ireland) according to the manufacturer's instructions. The absence of DNA in RNA templates was confirmed by PCR amplification of bacterial 16S rRNA gene with primers F63 (5'-CAG GCC TAA CAC ATG GCA AGT C-3') and 518R (5'-ATT ACC GCG GCT GCT GG-3') as previously described by Smith (Smith *et al.*, 2006). cDNA was produced using Superscript III reverse transcriptase (Invitrogen, Ireland) with random hexamer primers at a concentration of 50 pmol per reaction. For all reverse transcript (RT) reactions, a control containing RNA template and all RT reagents except for the RT enzyme and another control containing no template were included to ensure that RT reaction was free from DNA and reagent contamination. The first-strand cDNA was further quantified by Q-PCR and amplified for DGGE analysis as detailed below.

DGGE analysis of archaeal and bacterial* amoA *genes transcripts in microcosm incubation

DGGE analysis of archaeal and bacterial *amoA* gene transcripts PCR amplicons from microcosm samples were performed with a DCode Universal Mutation Detection System (Bio-Rad, UK) as described previously (Nicol & Schleper, 2006)

following amplification using primer sets Cren*amoA*23f/Cren*amoA*616r (Tourna *et al.*, 2008) and *amoA*1F/*amoA*2R (Nicolaisen & Ramsing, 2002), respectively. Reaction conditions and thermal programs were as for pyrosequencing except DNA template were replaced by 1 μl of cDNA. PCR products were analyzed with 8% (w/v) polyacrylamide gels containing a 15 - 45% or 20 - 45% linear gradients of denaturant for archaeal and bacterial *amoA* gene assays respectively. DGGE gels were run at 70V at 60 °C for 900 min and stained with ethidium bromide (0.5 μg/ml) buffer.

Statistical Analyses

Statistical analyses were performed using SPSS 19 (IBM Co., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to evaluate statistical difference among samples. Paired-sample *t*-tests were used to compare archaeal and bacterial *amoA* gene copy numbers. Spearman's correlation was used to examine the relationships among PNR, the abundance of *amoA* genes, transcripts and environmental variables. Redundancy analysis (RDA) was employed using CANOCO for window version 4.5 to measure the effects of environmental variables on community structures, and a Monte Carlo test with 999 permutations was carried out to test the significance of relationship between community and environmental matrix.

Nucleotide Sequence Accession Numbers: The representative sequences used in phylogenetic tree construction in this study were deposited in the GenBank database and assigned accession numbers from MG552489 to MG552575 for AOA, from MG580939 to MG581158 for AOB.

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Conflict of Interest

The authors have no conflict of interest to declare.

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Table Legends

Table 1. Sample information and physiochemical parameters of field samples across soil, interface and sediment samples from Rusheen and Clew Bay.

Table 2. Alpha-diversity of AOB and AOA community based on even subsampling of pyrosequencing reads.

Figure Legends

Fig. 1 *amoA* and 16S rRNA gene abundances across soil, interface and sediment samples from Rusheen and Clew Bay. * indicates significant difference between AOA and AOB *amoA* gene abundance at each site tested by independent samples nonparametric tests. The letter indicates significantly different within the same bay at $p < 0.05$.

Fig. 2 Community composition of AOA (A) and AOB (B) across soil, interface and sediment samples from Rusheen and Clew Bay. "Others" denotes pyrosequence reads with low abundance and not selected for phylogenetic analysis.

Fig. 3 The UPGMA dendrogram constructed from Bray-Curtis distance matrix of archaeal (A) and bacterial (B) *amoA* gene sequences from pyrosequencing.

Fig. 4 Redundancy analysis (RDA) on AOA (A) and AOB (B) community versus environmental factors. Hollow symbols denote sites of Rusheen Bay and solid symbols denote sites of Clew Bay.

Fig. 5 Dynamics of ammonia (A) and nitrate (B) during incubation of microcosms in soil (Rsoi_1, CSoi_2), interface (Rint_1, CInt_1) and sediment (RSed_2, CSed_1)

samples. The different letter indicates significantly different between the treatment and across the incubation dates at $p < 0.05$.

Fig. 6 Changes in abundance of archaeal and bacterial *amoA* genes (A) and transcripts (B) during incubation of microcosms in soil (Rsoi_1, CSoi_2), interface (Rint_1, CInt_1) and sediment (RSed_2, CSed_1) samples. Different lowercase letter (for AOA) and capital letter (for AOB) mean significantly different between without- and with- acetylene treatment and across the incubation dates at $p < 0.05$.

Fig. 7 DGGE analysis of AOA (A) and AOB (B) *amoA* gene transcripts in microcosms incubated at 15 °C for 28 days with and without C_2H_2. Each lane represents the profile derived from RT-PCR product of *amoA* gene transcripts from an individual microcosm. Bands indicated with an arrow were excised and sequenced. Results of DGGE band sequencing are indicated in phylogenetic tree (Fig. S3).

Table 1. Sample information and physiochemical characteristics of field samples across soil, interface and sediment samples from Rusheen and Clew Bay

Sample code	Location	Site Description	pH	Salinity		Sand (%)	Mud (%)	NH_4^+-N (µg/g Fresh sample)	NO_3^--N (µg/g Fresh sample)	TOC (% Fresh sample)
				Salinity *in situ* water (psu)	Dissolved salt in fresh sample (%)					
RSoi_1(A\B\C)[a]	Rusheen Bay (N53º15', W9º6´-7´)	Inland Grassland soil	6.56±0.08 c		0.05±0.01	87.67	12.33	1.85±0.10 b	0.21±0.08 a	13.05±1.82 a
RInt_1(A\B\C)		Saturated Soil, interface between land and sediment	6.62±0.06 c		3.72±0.06	82.60	17.40	3.64±0.40 a	0.11±0.03 a	8.44±1.70 b
RSed_1(A\B\C)		Muddy sediment at the bay corner	7.48±0.04 b	32.08		42.13	57.87	0.73±0.17 c	0.14±0.03 a	3.47±0.80 c
RSed_2(A\B\C)		Sediment near to land	7.69±0.04 a	32.08		93.13	6.87	2.74±0.71 ab	0.14±0.03 a	1.87±0.19 c
RSed_3(A\B\C)		Sediment in the center of bay	7.79±0.07 a	32.08		98.50	1.50	1.23±0.18 bc	0.11±0.03 a	1.25±0.06 c
CSoi_1(A\B\C)	Clew Bay (N53º47´, W9º36'-37´)	Bush soil in the center of island	5.39±0.17 c		0.10±0.01	87.70	12.30	5.32±1.64 a	0.81±0.49 a	14.95±1.15 a
CSoi_2(A\B\C)		Saturated grassland soil close to the interface	6.87±0.04 b		1.91±0.03	79.87	20.13	2.04±0.16 bc	0.08±0.05 a	14.56±1.05 a
CInt_1(A\B\C)		Saturated Soil, interface between land and sediment	7.26±0.05 a		1.18±0.14	73.63	26.37	2.10±0.05 bc	0.09±0.03 a	4.72±0.09 b
CSed_1(A\B\C)		Muddy sediment at the bay corner	7.53±0.02 a	32.13		48.10	51.90	4.84±0.32 ab	0.03±0.03 a	2.53±0.03 bc
CSed_2(A\B\C)		Sediment in the center of bay	7.60±0.20 a	30.69		97.00	3.00	0.81±0.17 c	0.08±0.05 a	0.64±0.27 c

a A\B\C mean three sampling replicates in each site.

Table 2. Alpha-diversity of AOB and AOA community based on the even subsampling of pyrosequencing reads

Sample code[a]	AOA[b]				AOB[a]			
	shannon	observed_species	chao1	simpson	shannon	observed_species	chao1	simpson
RSoi_1	1.90 - 3.90[b2]	47 - 72	129.70 - 158.10	0.40 - 0.84	3.48 ± 0.39 a	56.00 ± 8.00 b	71.07 ± 9.16 c	0.81 ± 0.04 a
RInt_1	2.93 ± 0.42[b3]	63.00 ± 7.21	160.03 ± 1.76	0.66 ± 0.07	1.19 ± 0.49 b	28.00 ± 9.71 b	49.87 ± 19.19 c	0.30 ± 0.12 b
RSed_1	~	~	~	~	5.00 ± 0.21 a	152.00 ± 7.51 a	376.45 ± 39.95 a	0.88 ± 0.02 a
RSed_2	3.97 ± 0.20	83.00 ± 2.65	257.96 ± 35.12	0.81 ± 0.03	4.82 ± 0.21 a	140.00 ± 4.51 a	323.16 ± 21.09 a	0.87 ± 0.02 a
RSed_3	~	~	~	~	4.46 ± 0.63 a	117.00 ± 19.73 a	198.50 ± 36.92 b	0.83 ± 0.08 a
CSoi_1	2.13[b1]	39	70.63	0.51	4.29 ± 0.24 a	93.33 ± 3.67 ab	160.26 ± 26.24 a	0.86 ± 0.03 a
CSoi_2	1.07	27	84	0.23	2.09 ± 0.89 b	51.00 ± 14.57 b	88.28 ± 20.16 a	0.44 ± 0.19 ab
CInt_1	2.39 ± 0.58	43.67 ± 7.69	99.00 ± 24.22	0.58 ± 0.15	1.30 ± 0.09 b	36.33 ± 1.45 c	86.88 ± 11.16 a	0.29 ± 0.03 b
CSed_1	3.72 ± 0.07	65.67 ± 7.17	178.59 ± 61.95	0.82 ± 0.02	4.81 ± 0.91 a	132.50 ± 39.50 a	224.96 ± 76.53 a	0.87 ± 0.06 a
CSed_2	2.30 ± 0.16	61.67 ± 7.75	173.79 ± 10.35	0.46 ± 0.02	4.11 ± 0.18 a	102.00 ± 9.85 ab	179.28 ± 21.62 a	0.81 ± 0.02 a

a mean ± SD (n=2 for Rsoi_1 and Csed_1, n=3 for the rest). Values within the same column followed by different letter mean significantly different from the same bay at $p < 0.05$.
b As only 19 samples produced enough pyrosequencing reads, difference significance test was not conducted for AOA community. b1, b2, b3 values are from one replicate, two replicates and triplicate samples, respectively. ~ mean data absent.

Fig. 1 *amoA* and 16S rRNA gene abundances across soil, interface and sediment samples from Rusheen and Clew Bay. * indicates significant difference between AOA and AOB *amoA* gene abundance of each site tested by independent samples nonparametric tests. The letter indicates significantly different from the same bay at $p < 0.05$.

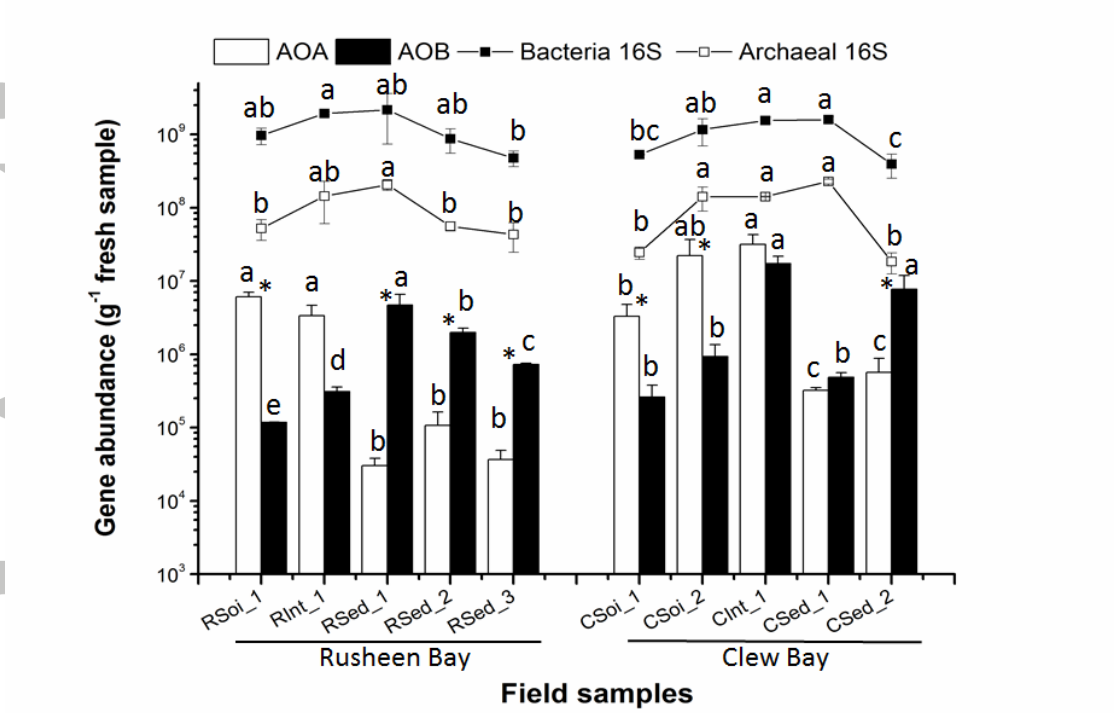
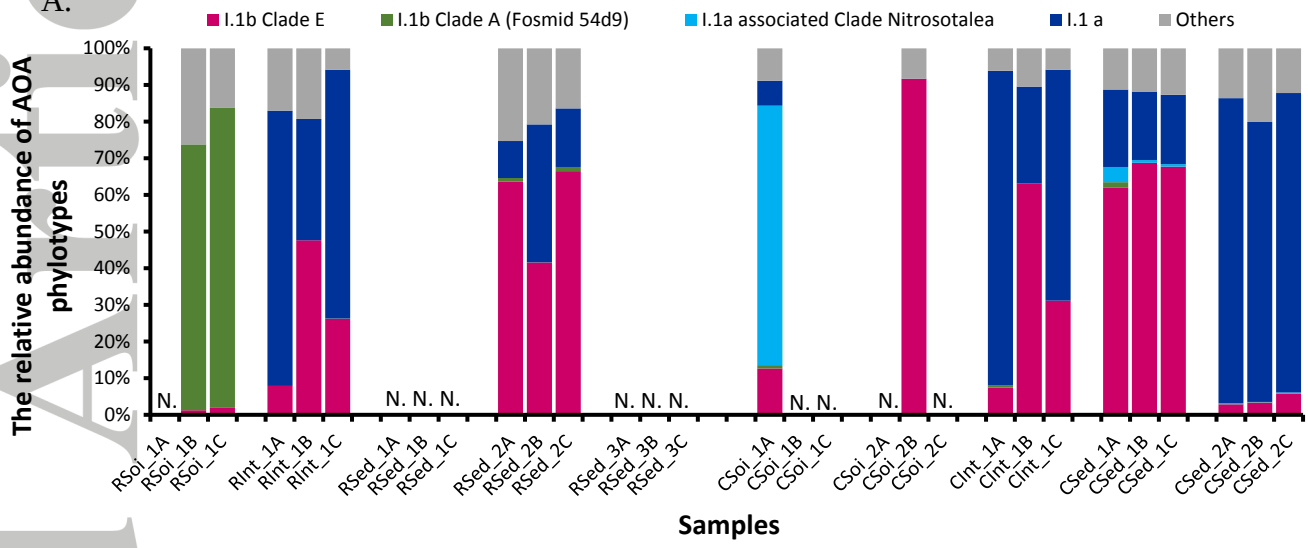
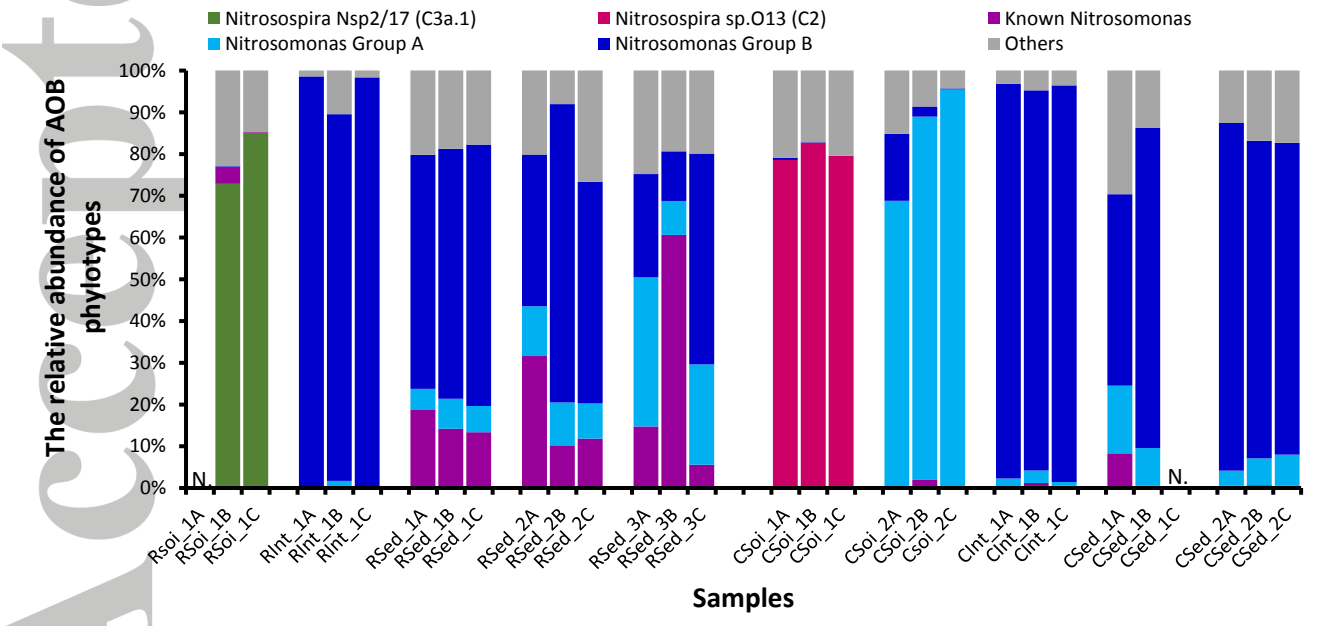


Fig. 2 Community composition of AOA (A) and AOB (B) across soil, interface and sediment samples from Rusheen and Clew Bay. "Others" denotes pyrosequence reads with low abundance and not selected for phylogenetic analysis.

A.



B.



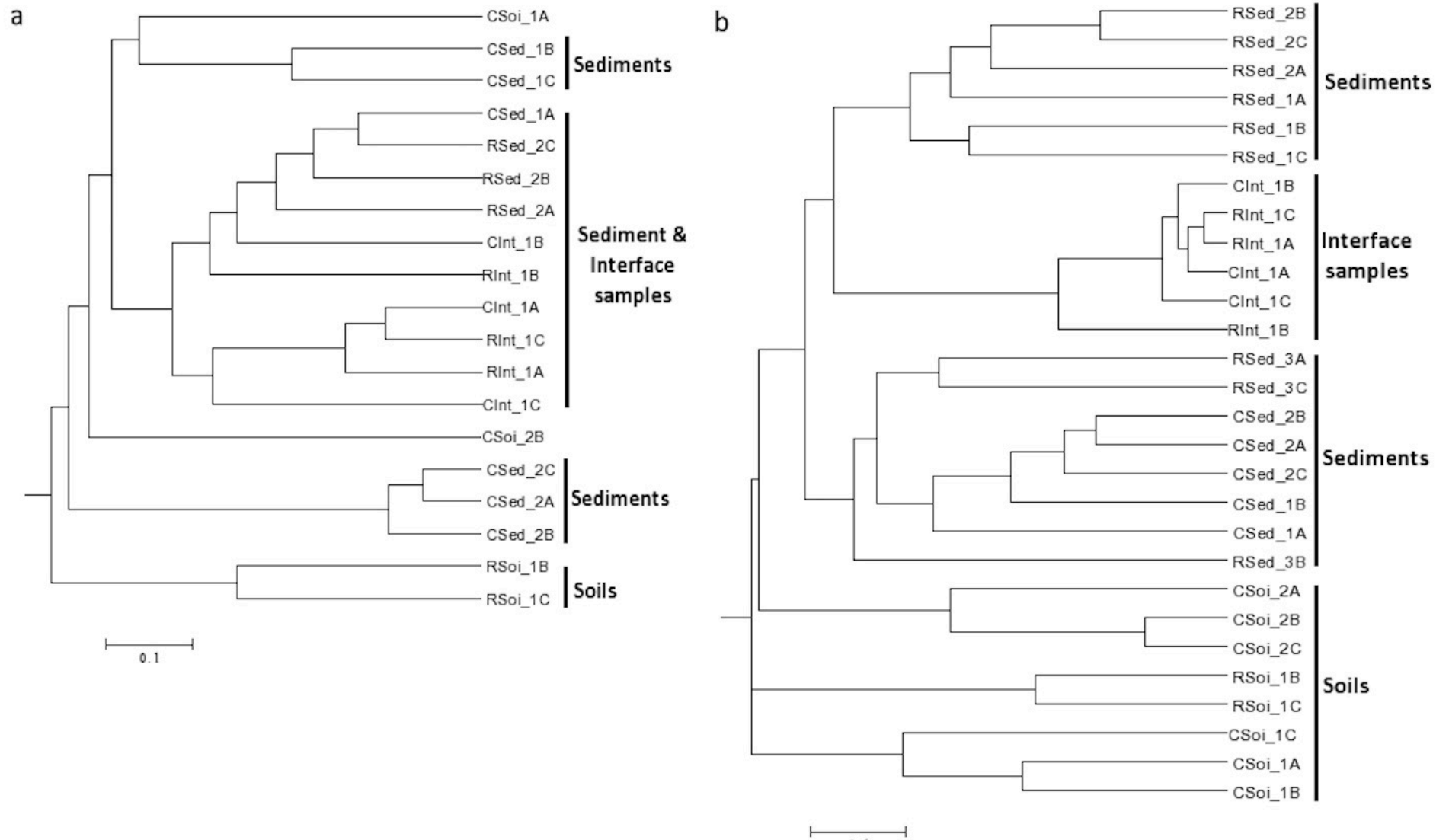


Fig. 4 Redundancy analysis (RDA) on AOA (A) and AOB (B) community verses environmental factors. Hollow symbols denote sites of Rusheen Bay and solid symbols denote sites of Clew Bay.

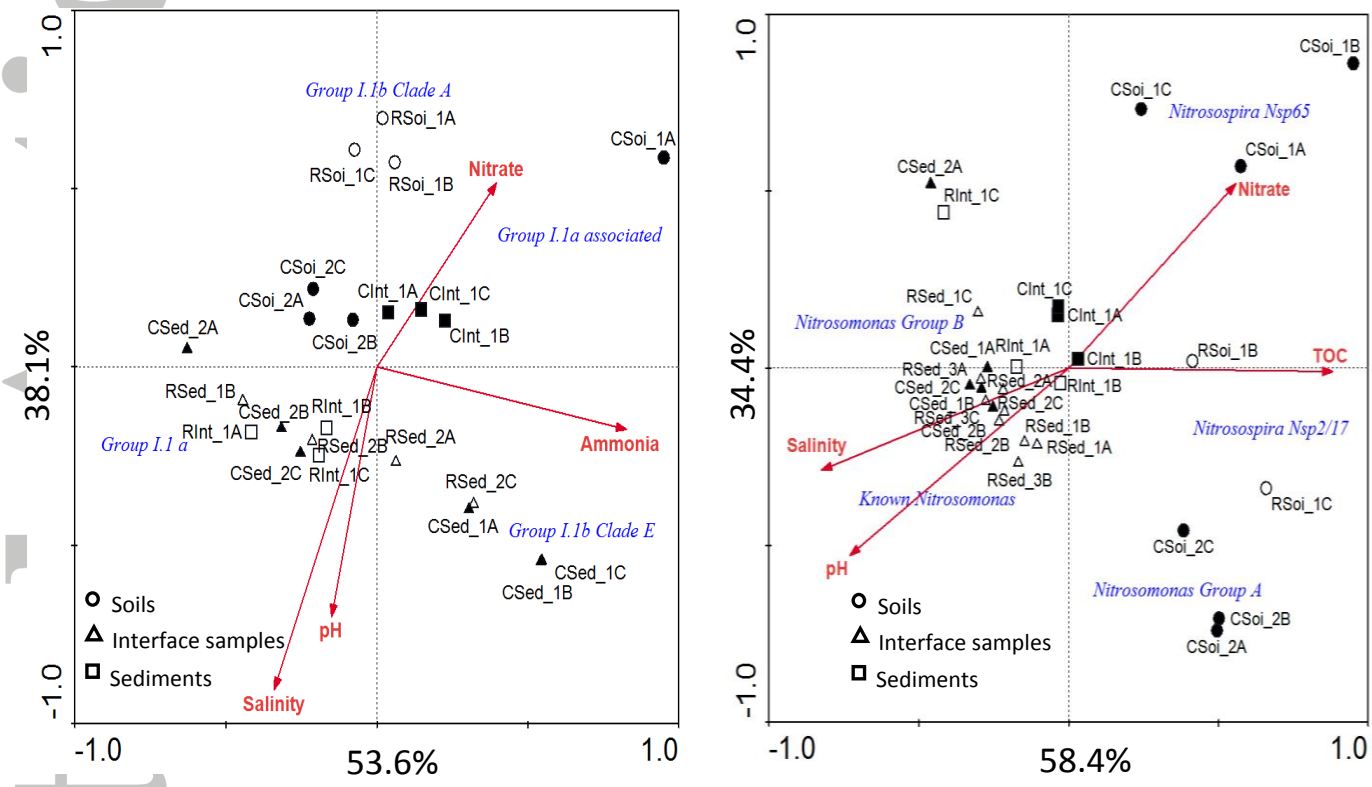


Fig. 5 Dynamics of ammonia (A) and nitrate (B) during incubation of microcosms

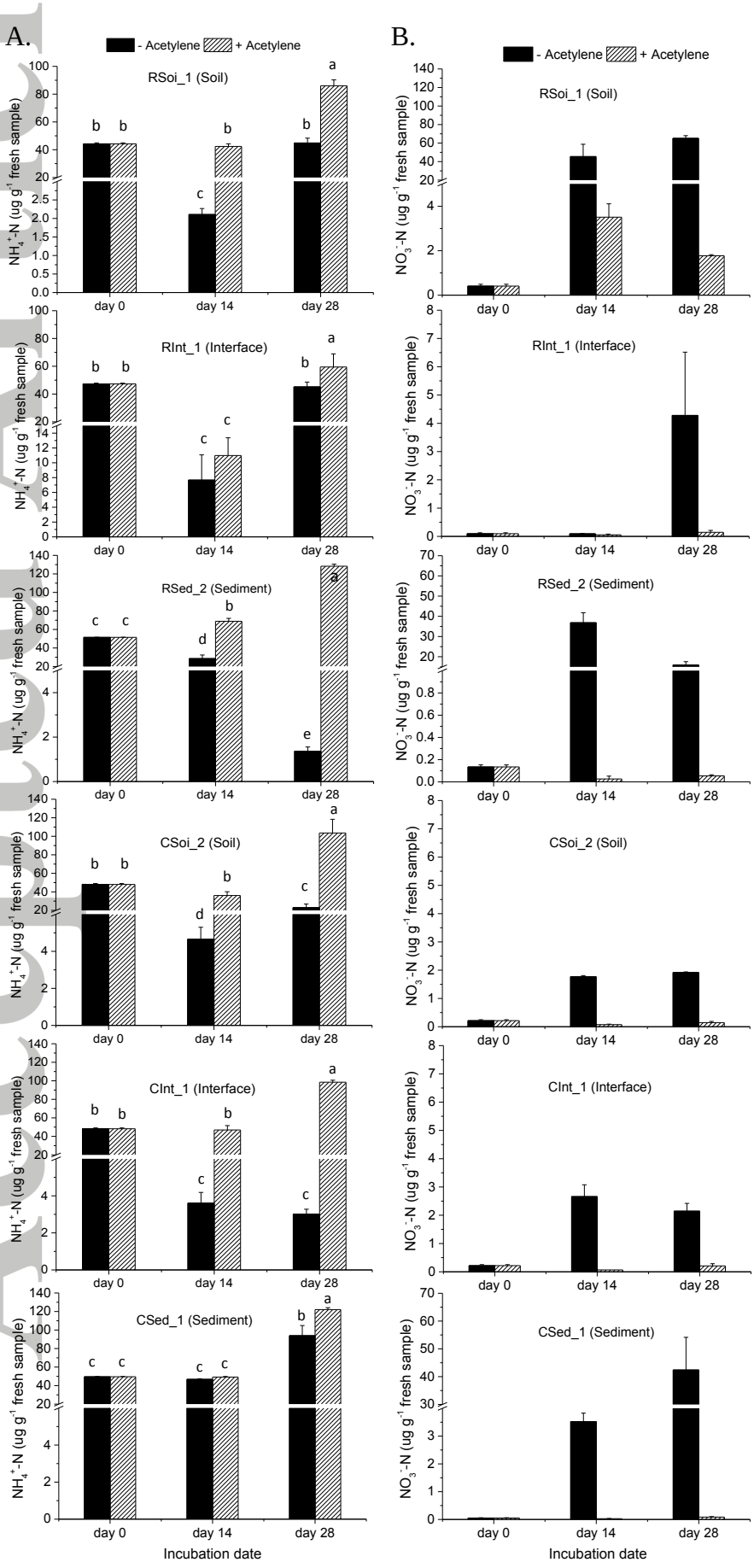


Fig. 6 Changes in abundance of archaeal and bacterial *amoA* genes (A) and transcripts (B) during incubation of microcosms. Different lowercase letter (for AOA) and capital letter (for AOB) mean significantly different between without- and with- acetylene treatment and across the incubation dates at $p < 0.05$.

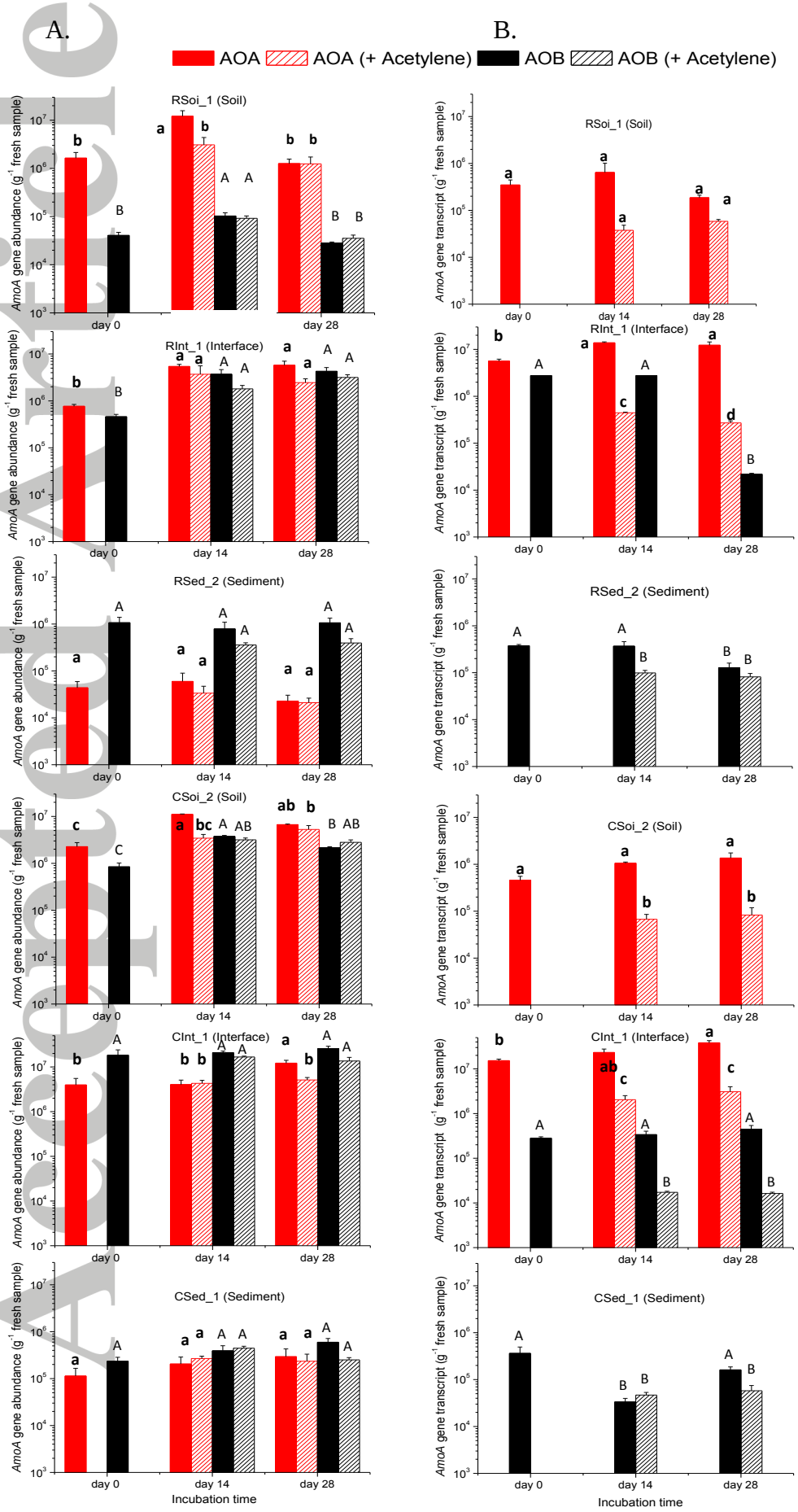


Fig. 7 DGGE analysis of AOA (A) and AOB (B) *amoA* gene transcripts in microcosms