A new conceptual model of nitrification: A key factor in resolving the metabolic state of the ocean

Dña. María Teresa Tamés Espinosa

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Directed By

Dr. Ted Packard

Dra. May Gómez

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

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A new conceptual model of nitrification: A key factor in resolving the metabolic state of the ocean

Mayte Tames-Espinosa[a], May Gómez[a], Ted Packard[a]

[a]Applied Algology and Plankton Ecophysiology Group. Instituto Universitario de Oceanografía y Cambio Global, Universidad de las Palmas de Gran Canaria, España.

ABSTRACT

Until the last decades, nitrification has been considered to occur only in the dark ocean. However, recent studies report nitrification within the euphotic zone, varying with differences in light-sensitivity that favor different groups and strains within the nitrifying community. Here we present a new qualitative conceptual model that not only considers the light as a key factor of the distribution of the nitrifiers, but also stratification, the oligotrophic or eutrophic conditions, and even the ppO_2. This new conceptual model has been developed for the open ocean. Other conditions that involve changes in the community, such as a estuarine zone, with specific pH, temperature, salinity and nutrients gradients, require modifications that are also considered in this study. Integrating the results from the new studies of nitrification, we show that the relative nitrification distribution varies due to the environmental factors, and, occurs within the euphotic zone at higher rates than it was previously thought. This euphotic behavior of nitrification has a direct impact on the metabolic balance of the ocean because it fixes carbon and consumes O_2. Accordingly, it now has to be considered in interpreting measurements of autotrophy and heterotrophy (particularly those based on ppO_2 analysis). Moreover, nitrification within the euphotic zone, along with nitrogen fixation, affects the new and regenerated production classic concepts and measurements. They have to be redefined in line with the changes that have been made with the classic f-ratio, all because of nitrification in the upper ocean. We review the knowledge of nitrification, its community behavior, and the changes in environmental factors that have an influence on it. We examine how the variability in nitrification impacts the N and C-cycles and how it changes the basic concepts used in the understanding of the C-flux. This new level of understanding will be especially relevant in the current global climate change scenario.

Key words: nitrification, carbon cycle, carbon fixation, metabolic state of the ocean

1. INTRODUCTION

The 2013 volume (#5) of the Annual Review of Marine Science devoted special three articles to the dilemma of the metabolic state of the ocean (Ducklow & Doney, 2013).The series was developed as a debate about this question, focused on the oligotrophic ocean. The existing evidence on the prevalence of the autotrophy (Williams et al., 2013), or of heterotrophy (Duarte et al., 2013) were contrasted and ended in stalemate. The authors concluded that more knowledge of the different processes, methodologies, magnitude scales, or agents in needed before a resolution can be achieved. However, this analysis minimizes the contribution of chemoautotrophy to the autotrophic-heterotrophic balance sheet. Here, we reexamine this question, introducing the process of autotrophic nitrification, a ubiquitous process found in all oxygenated ocean waters.

Specifically, we will consider and redefine the following ideas in order to better quantify the chemoautotrophy impact on the ocean balance between autotrophy and heterotrophy:

a. **The influence of nitrification on the current conceptual models of ocean´s metabolic balance**:

The simplified concept of the elemental carbon-oxygen balance in the ocean from Ducklow & Doney, (2013) is:

$$6CO_2 + 6H_2O <-> C_6H_{12}O_6 + 6O_2$$
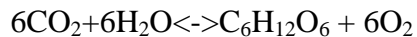
Equation 1: Simplified concept of elemental carbon-oxygen balance in the ocean

while the simplifies balance between photosynthesis and respiration is:

$$GPP = NCP + R$$

Equation 2: Simplified balance between photosynthesis and respiration

Where photosynthesis is the gross primary production (GPP) and Respiration (R) is the difference between GPP and the net Community Production (NCP). In both simplified equations chemoautotrophy is not considered.

Other key concepts need to be reviewed, such as the relationship between the Net Community production (NCP) and the idea of the "New Production", which, in turn, is related to the Autotrophic Productivity associated with the injection of nitrate (NO_3^-) from the aphotic zone (Dugdale & Goering, 1967; Eppley & Peterson, 1979). This idea does not consider the existence of nitrification in the ocean's photic zone and how it could increase the magnitude of New Production or decrease the magnitude of the regenerated Production in the upper ocean.

b. **The involved actors**:

The biological communities involved in nitrification processes are bacteria and archaea that range in size between 0.1 to 10 μm and mainly form part of the so-called picoplankton (Kriger, N., 2005). Due to its size this biological community is especially complex to assess except by biogeochemical analyses.

However, some investigations, both in nitrification and CO_2 fluxes, use the carbon fixation (C-fixation) rates to assess the measurements. The filters used in these techniques cannot retain cells less-than-0,2μm, or even those less-than-0,45μm in the microbial community. Could this size problem be the bias affecting the in vitro results as identified by Williams et al. (2013)?

Furthermore, the nitrifying community is not distributed uniformly throughout the water column. Knowing its specific locations and its abundances allows the scientific community to plan better sampling programs, develop more accurate analytical techniques, and improve data analysis.

c. **Environmental factors**:

Temporal variability of both photosynthesis and respiration in response to storm-related mixing, seasonal temperature shifts, and water-column stratification are causally associated with changes in the gross primary productivity (GPP) and to the balance between autotrophy and heterotrophy.

Normaly, the study of autotrophy in the oligotrophic ocean focuses on the upper ocean, while heterotrophy considers the entire water column, from the sea ocean bottom to the ocean surface. This approach seems logical, not only because light-based photosynthesis does not extend below the euphotic zone, but also because it is the area of atmospheric exchange. Fick's Law of diffusion and Henry's Law of gases argue that the difference between the CO_2 in the upper boundary layer of the euphotic zone and the air immediately above the sea surface defines the ocean as sink or source of CO_2. However, it is assumed that the autotrophy affecting this balance is confined to the existing euphotic layer during the day. It does not consider other autotrophic processes. It does not consider nitrification. Moreover, it does not consider the contributions of particulate organic carbon (POC) and dissolved organic carbon (DOC) from the air, the coast, or from vertical and horizontal fluxes. None of these contributions or fluxes has been quantified definitively, but most studies show that they cannot be ignored (Arístegui et al., 2002; Hansell & Carlson, 1998; Hansell et al., 2009; Hansell & Carlson, 2001).

Does autotrophy, especially nitrification, contribute organic matter to the deep ocean ecosystem, but also in the upper ocean and coastal ecosystems as well? If so, would quantification and inclusion of nitrification help achieve the balance between autotrophy and heterotrophy in these areas? In comparison to other biological processes, such as photosynthesis or respiration, is nitrification influenced by light, stratification, or nutrient availability?

How these and other environmental factors (such as the decline in pH, the increase of the dissolved inorganic carbon (DIC), or the decrease of the ppO_2) affect nitrification is unknown, but are likely to be relevant to the balance question and the final metabolic status of the ocean.

d. **Development comparisons between reported estimations of nitrification rates** :

As in all measurements, biases exist in the methods used in the analyses of the metabolic state of the ocean, both in vitro and in situ techniques (Ducklow & Doney, 2013). The main studies used ppO_2 analyses in the water and C-isotopes in organic matter to determine the metabolic status, but it seems that in some cases, the results of one of the techniques contradict the results of the other. On a theoretical basis nitrification should be involved in this imbalance, however, as with the productivity and respiration measurements, nitrification measurements are also plagued with errors, biases, uncertainties, and data acquisition problems.

Are the in vitro estimates better than in situ estimates of the metabolic studies, the other way round? A proper review in this regard is needed, in which the most suitable techniques would be defined, as well as a specific sampling program based on the goal of this kind of studies.

Although Duarte et al. (2013) have considered these processes in their review of possible organic matter resources, chemoautotrophy was not considered as primary production, even though it creates organic matter from inorganic sources using reduced nitrogen (N) as the energy source instead of light as in photoautotrophy (Ducklow & Doney, 2013).

Throughout this study we plan to verify if chemoautotrophy and, specifically, nitrification could be the missing factor in achieving a metabolic balance in the ocean.

2. THE INFLUENCE OF NITRIFICATION ON THE CURRENT CONCEPTUAL MODELS OF OCEAN´S METABOLIC BALANCE

2.1 The metabolic state of the ocean.

In the debate about the metabolic state of the ocean, a mass balance approach was simplified to a computation of autotrophy relative to respiration in the mixed layer of the surface ocean. In addition, only the oxygenic photosynthesis form of autotrophy was considered (Williams et al., 2013). In this scenario, if the Gross Primary Production (GPP) was equal to sum of the community Respiration (R) plus the Net Community Production (NCP) then the surface ocean ecosystem carbon metabolism was considered to be in balance. If the GPP exceeded R, the ocean was considered autotrophic, with all the catabolic demands of both the heterotrophic and autotrophic communities met. In this case the excess productivity could be exported horizontally or vertically, usually the latter. However, if R plus NCP was greater than the GPP, then the ocean was considered heterotrophic and would require carbon supplies from other organic matter sources to meet the surface ocean's metabolic demands.

To perform this analysis correctly the GPP, NCP, R, and perhaps other processes should be determined independently and with great accuracy over oceanic space and time scales. To date this has not been possible and, given the current state of the oceanographic community's technological capability and resources, this is unlikely to be done in the near future. Still, the problem merits refinement of existing conceptual models of the problem as well as further examination, holistic data analysis, and modeling in the manner of Williams et al. (2013).
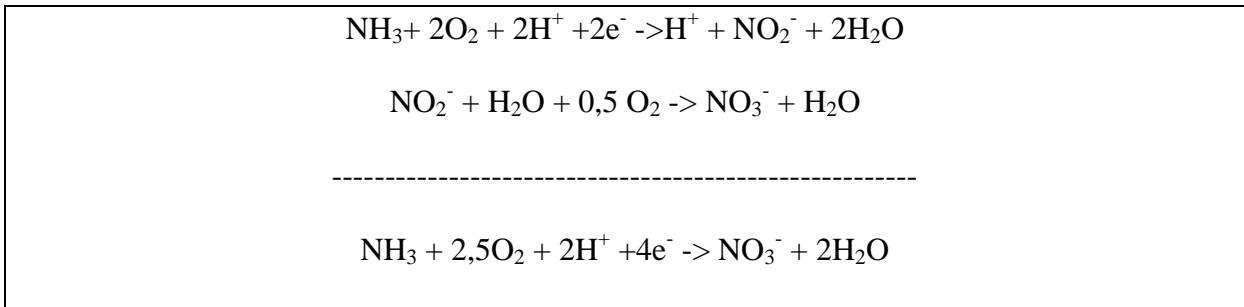
In analyzing the conceptual basis of the balance problem it becomes clear that autotrophic processes that do not involve oxygen (O_2) release have not been considered (Raven, 2009). Some of these processes occur in the euphotic zone concurrently with oxygenic photosynthesis (Hügler & Sievert, 2007) for example, Aerobic Anoxygenic Photosynthesis (AAnP). This process is exclusively confined to the euphotic zone. The AAnP community takes up O_2 from the environment, and fixes carbon by a pathway different from the Calvin-Benson-Bassham (CBB) cycle, very likely the 3-hydroxypropionate cycle. These microbes perform heterotrophic metabolism in the presence of organic matter. They employ light-trapping pigments such as bacteriochlorophyll, carotenoids, or even proteorhodopsin to provide the needed energy (Koblek, 2011; Kolber et al., 2001). Oxidative phosphorylation is partially replaced by photophosphorylation, reducing the loss of CO_2 to the ocean, even when behaving as heterotrophs. Hence, they are able to perform autotrophic metabolism too, as some molecular analyses have observed (Klatt et al., 2007). These pathways allow them to live in either oligotrophic or eutrophic environments. The influence of this mixotrophic AAnP community on the metabolic balance of the ocean differs from that of pure heterotrophs because the mixotrophs do not release CO_2 in the same proportion. It also differs from the pure photoautotrophs because the AAnP community does not release O_2.

When ppO_2, $ppCO_2$, or even chlorophyll-a concentrations are being used to determinate the metabolic state of the ocean, the results could be inappropriately understood without considering the presence of this special community.

Another form of photosynthesis that does not release O_2 is Anaerobic Anoxygenic Phototrophy (AnAnP). This can only occur when oceanographic conditions allow anoxic waters to rise into or to form in the euphotic zone. This can occur under special conditions in upwelling ecosystems (Codispoti et al., 1986) or in anoxic basins (Millero, 2006). The microbes responsible for the AnAnP developed in archaeal anoxic ocean, before the oxygenic photosynthetic organisms evolved. They use Bacteriochlorophyll-a as photon receptors and use hydrogen sulphide or hydrogen gas as hydrogen donnors rather than water (Karl, 2002).

Finally, another form of carbon fixation that does not release O_2 is nitrification. It is the most important form of chemoautotrophy because of its ubiquity. It occurs in the euphotic zone in the dark and in the light (Bianchi et al., 1994; Bianchi et al., 1997; Feliatra & Bianchi, 1993; Merbt et al., 2012) as well as in dark meso and bathypelagic areas at all times (DeLong, 2007; Ward et al., 2007). Furthermore, this community consumes O_2 from the environment. The ammonium (NH_4^+) oxidizers can fix CO_2 via the CBB cycle or via the 3-hydroxypropionate/4-hydroxybutyrate (3-HP/4HB) cycle. The nitrite (NO_2^-) oxidizers can fix CO_2 via the CBB cycle, the reductive tricarboxylic acid (rTCA) cycle and likely by other pathways yet to be discovered (Huyer, 1983). This community takes O_2 from the ppO_2 pool and does not releasing it during the autrotrophic process.

To estimate the O_2 that is consumed during nitrification, the stoichiometry of the simplified equations of the process, developed from the complete reaction equations proposed by Zehr and Kudela (2011), has been studied as is showed below (Zehr & Kudela, 2011):

$$NH_3 + 2O_2 + 2H^+ + 2e^- \rightarrow H^+ + NO_2^- + 2H_2O$$

$$NO_2^- + H_2O + 0,5\ O_2 \rightarrow NO_3^- + H_2O$$

$$NH_3 + 2,5O_2 + 2H^+ + 4e^- \rightarrow NO_3^- + 2H_2O$$

Equation 3: Nitrification process simplified equation. Developed from the complete reaction equations proposed by Zehr and Kudela (2011).

In nitrification, if 1 mole of CO_2 is fixed per 10 moles of NH_4^+ during the oxidation of NH_4^+ (Middelburg, 2011; Wuchter et al., 2006), then 25 mole of O_2 are taken from the environment per 1 mole of CO_2-fixation. 20 moles of O_2 are taken during the first phase of nitrification ($NH4^+$ to NO_2^-), and 5 moles of O_2 are taken up during the second phase of nitrification (NO_2^- to NO_3^-) (Equation 3), which completes the total process (to fix 1mole of C: $10NH_3 + 25O_2 + 10H^+ + 20e^- \rightarrow 10NO_3^- + 20H_2O$).

Within the literature, different ratios between C-fixation and N-oxidation have been reported. In table 1, the most used ratios have been shown, assuming that the C-fixation is developed via the Calvin Benson Cycle. Deeper research in nitrifying behavior, between nitrifying groups and enzymatic pathways, considering the metabolic state of the community, is needed to gain accuracy in the estimations of the C-fixation capacity of this specific community:

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Involved Processes	N-oxidation:C-fixation ratio	N-oxidation:O_2-uptake	C-fixation:O_2production	Total N-oxidation:C-fixation:O_2-uptake
Ammonia Oxidation Calvin Benson Bassham Cycle	8.3:1 [1]	1:2 [4]	1:0	8.3:1:16.6 [5]
Ammonia Oxidation + Nitrite Oxidation Calvin Benson Bassham Cycle	10:1 [2]	1:2.5 [4]	1:0	10:1:25 [5]
Ammonia Oxidation + Nitrite Oxidation Calvin Benson Bassham Cycle (under estuarine conditions)	20.63:1 [3]	1:2.5 [4]	1:0	20.63:1:51.58 [5]
Ammonia Oxidation Calvin Benson Bassham Cycle (under estuarine conditions)	13.15:1 [3]	1:2 [4]	1:0	13.15:1:26.3 [5]
Nitrite Oxidation Calvin Benson Bassham Cycle (under estuarine conditions	28.11:1 [3]	1:0.5 [4]	1:0	28.11:1:14.06 [5]

Table 1: N-oxidation: C-fixation: O_2-uptake ratios. These data have been developed from: (1) Theoretical ratio from Dore and Karl, 1996; (2) theoretical ratio from Middelburg, 2011; Tijhuis et al., 1993; Wuchter et al., 2006; (3) The average of Feliatra and Bianchi, 1993 and Bianchi et al. 1999 (a)´s measurements from an estuarine ecosystem; (4) Stoichiometric estimations from Zehr and Kudela, 2011´s theoretical equations; (5) New developed ratios based on the rations mentioned before.

Middelburg, Tijhuis and Wuchter´s ratio (Middelburg, 2011; Tijhuis, et al., 1993; Wuchter et al., 2006) is the most useful in total nitrification estimations, because it is based in total nitrification : carbon fixation ratios. On the other hand, Dore and Karl and Billen´s one (Billen, 1976; Dore & Karl, 1996) is based on NH_4^+ oxidation ratios. Feliatra and Bianchi calculated both the nitrite (NO_2^-) oxidation and NH_4^+ oxidation ratios (Bianchi et al., 1999(a); Feliatra & Bianchi, 1993), but in the estuarine community, which seems to behave in a different way from open ocean community.

To fix one mole of C is needed oxidize N-compounds. The O_2 consumption ranges from 25 moles to 51.58 based on the different ratios. This may involve an important impact in the interpretation of the results based on ppO_2 measurements.

In summary, without considering these non-oxygenic autotrophic processes, nitrification, AAnP, AnAnP, etc. in the euphotic zone and below any study of the metabolic state of the ocean by ppO_2 analysis, will underestimate carbon fixation. The resulting attempt to assess the balance between autotrophy and heterotrophy in the ocean will fail.

2.1 <u>Other Factors to Consider</u>

In a hypothetical closed ecosystem, the balance in the metabolic state of the ocean would be relatively easy to resolve. Heterotrophy could not exceed primary production and, at the same time, this primary production would be constrained by the remineralized nutrients derived from excretion and degradation processes. The community production would remain in this hypothetical ecosystem, and all the primary production would be considered regenerated production. However, the ocean is an open ecosystem and part of the community production sinks below the sunlit euphotic zone into the dark ocean where it cannot be directly used by the photosynthesizers. This organic matter is remineralized in this dark aphotic zone by the microbes and by all size fractions of the zooplankton community (Arístegui et al., 2002). This regeneration of nutrients could support a phytoplankton carbon fixation if it had occurred near the surface, but in these deep aphotic waters it only supports carbon fixation through nitrification. Still carbon fixation occurs here.

When all the remineralization of N is complete, it remains in the deep water column as NO_3^-. In the huge central ocean gyres these waters are slowly upwelled via thermohaline circulation. In the upwelling zones they are drawn into the euphotic surface waters much more rapidly, but in both cases they support the phytoplankton-based new production (Dugdale & Goering, 1967).

The N that never left the euphotic zone through sinking, mixing, or zooplankton migration is remineralized by excretion or decay to NH_4^+ and urea and almost immediately taken up by the phytoplankton in what Dugdale and Goering (1967) called regenerated production. Note that the new production is generated due to previous sinking of organic matter. Hence, on a long time scaling, vertical carbon flux and new production should be equivalent. The ratio of the new production over the total production is called the f-ratio (Eppley & Peterson, 1979). It allows the estimate the ocean's capacity to serve as a sink for CO_2. It is calculated from the new production and the regenerated production as below.

$$\text{Classic f-ratio} = \frac{\text{New Production}}{\text{New Production} + \text{Regenerated Production}}$$

Equation 4: Classic f-ratio (Eppley & Peterson, 1979)

But how is this f-ratio really measured? Nitrification is classically considered to be confined to the dark ocean, because it was thought to be absolutely inhibited by light. It was thought that all NO_3^- in the euphotic zone originated in the deep ocean. Accordingly the NO_3^- uptake rate was the new production, associated to a previous sedimentation of organic material to depth. Now several studies have shown nitrification in the upper euphotic layers of the ocean. Hence, part of the NO_3^- concentration in the upper ocean must be derived from regeneration of the excretions of the in-situ biological community. This discovery means that not all the NO_3^- available to the phytoplankton is supplied by allochthonous nutrients (Yool et al., 2007). Part of it is clearly regenerated autochthonous.

Futhermore, in the N-cycle microbial community, cyanobacteria fix N from the atmosphere, increasing the particulate organic N (PON) pool. The primary production due to this supply is actual New Production, but it is not consistent with Dugdale and Goering´s (1967) original concept (Clark et al., 2008). This affects the cycle of C in several ways:

1. NO_3^- and NH_4^+ uptake cannot be equated directly with new and regenerated production, respectively, as classically measured (Dugdale & Goering, 1967) or to the export of organic matter to deep ocean (Yool et al., 2007). The overestimation may range from 12% to 32% of the New Production (Yool et al., 2007). Currently, overestimations from 20% to 100% has been reported in the Equatorial Pacific (Raimbault et al., 1999); from 25% to 36% in the California Current (Santoro et al., 2010); a maximum of 60% in the very oligotrophic Mediterranean Sea (Bianchi et al., 1999(b)); from 1% to 37% in the North Pacific (Beman et al., 2012); from 47% to 142% in the Pacific (Dore & Karl, 1996) or from 2% to 16% in the Peruvian Upwelling (Fernandez & Raimbault, 2007).

2. CO_2 fixation in the euphotic zone has a chemoautotrophic component. It is not due solely to the photoautotrophs.

3. Primary productivity calculated from O_2 production (Williams & Purdie, 1991) will underestimate autotrophy. The newly discovered anoxygenic C-fixing processes including nitrification need to be included in the autotrophy calculations.

3. THE INVOLVED ACTORS.

In all ecosystems, living organisms need certain chemical elements or molecules that are necessary for life. For the ocean microbes these nutrients, varying in time and space, are present in the seawater. They consist of macronutrients (found in large quantities), micronutrients (found in low concentrations) and trace elements (found in very low concentrations). All are essential for life. Among the micronutrients, we find N or phosphorus (P) (Millero, 2006). While many chemicals on the planet are in unusable forms for life, the nutrients can be directly assimilated and incorporated into cells. In turn, through cellular metabolism, they are recycled, degraded, and returned to the environment. These state changes constitute the biological side of the biogeochemical cycle. The community of nitrifying bacteria and archaea comprises a set of microorganisms participating in one of the most important biogeochemical cycles of the biosphere: the N-cycle. Fig. 1, taken from Brandes, Devol and Deutsch (2007), shows all of the N-cycle processes catalyzed by microorganisms(Brandes, Devol, & Deutsch, 2007).

Outstanding are the following processes:

1. N-fixation (Fig. 1: (1)).
2. Aerobic NH_4^+ oxidation by bacteria and archaea (Fig. 1: (2)).
3. Aerobic NO_2^- oxidation (Fig. 1: (3)).
4. Denitrification. (Fig. 1: (4)).
5. Anaerobic NH_4^+ oxidation. (Fig. 1: (5)).
6. Dissimilatory reduction of NO_3^- and NO_2^- to NH_4^+ (Fig.1: (6)).
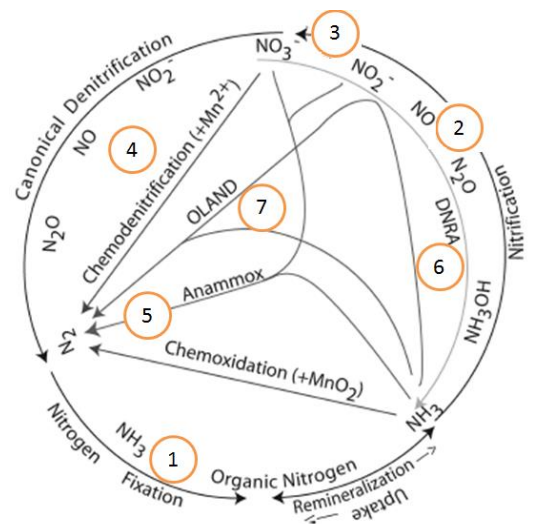7. O_2-Limited Autotrophic Nitrification-Denitrification (Fig. 1: (7)).

Fig. 1: Microbial N-cycle reactions, from Brandes, Devol, & Deutsch (2007)

The N-cycle is essential in both terrestrial and marine ecosystems, which, up to a decade ago, including only processes 1-4 (Fig. 1) was thought to be well understood. However, investigations in the last ten years, with the discovery of processes such as ANAMMOX or OLAND, and the omnipresence of nitrifiers, have shown that the N-cycle, is much more complicated that previously conceived (Brandes et al., 2007; Jetten, 2008; Zehr & Kudela, 2011). Some very significant discoveries have been highlighting these new processes. For example, the oxidation of NH_4^+ in anaerobic situations (anammox) (Devol, 2003; Strous et al., 1999), the aerobic oxidation of NH_4^+ by archaea strains (Könneke et al., 2005; Schleper et al., 2005; Francis et al., 2005; Brochier-Armanet et al., 2008; Agogué et al., 2008; Beman et al., 2008; Santoro et al., 2010) and the discovery of new types of nitrifiers throughout the water column (Wuchter et al., 2006). In addition, the development of molecular techniques (Arp et al., 2007; Strous et al., 2006) has permitted the finding of the great biodiversity and metabolic capacity in this oceanic community of microorganisms (Horak et al., 2013).

Now, the microbiological N-cycle can be outlined as shown in Fig. 1. Within each of the processes, the community of nitrifying bacteria and archaea acquire their energy from aerobic oxidation of NH_4^+ and NO_2^- (routes 2 and 3 in Fig. 1). The combination of both processes is called nitrification and the organisms responsible are the nitrifiers (Nelson & Cox, 2010).

From the energy obtained through nitrification, the nitrifiers extract and fix CO_2 from the DIC in seawater generating their own simple organic compounds.

Breakthrough discoveries in the last decade have identified the responsible deep-sea nitrifiers (Könneke et al., 2005). Until recently it was believed that nitrification was performed by β-proteobacteria and γ-proteobacteria, forming the Ammonia Oxidizers Bacteria community (AOB) (Zehr & Ward, 2002). However, the latest research, suggests that, in marine ecosystems, the Ammonia Oxidizing Archaea (AOA), and more specifically the Crenarchaeota are the main actors in marine nitrification (Agogué et al., 2008; Beman et al., 2008; Brochier-Armanet et al., 2008; Church et al., 2010; Könneke et al., 2005; Santoro et al., 2010) The detection of the AOA, as a key group of nitrifiers in NH_4^+ oxidation was based both on its abundance (determined by the presence of the gene amoA, responsible for the synthesis of ammonia oxidase protein) as well as by its activity (the presence of 16S rRNA, that indicates the actual expression of amoA in the phenotype).

Nitrification consists of two well defined phases, performed by several microorganisms (Beman et al., 2012; Nelson & Cox, 2010). The first is the oxidation of NH_4^+ to NO_2^- performed by AOA or AOB. The second is the oxidation of NO_2^- to NO_3^- in which the Nitrite Oxidizers Bacteria (NOB) are thought to be the organisms responsible. Other microorganisms will likely be discovered in future research: All them will be Nitrite Oxidizing Organisms (NOO). Together this nitrifying community falls within the so-called picoplankton, with cell-size ranging from 0,1 to 10 μm. The presence and diversity of this microbial ocean community has long been suspected but has been particularly difficult to verify, investigate, and quantify (Fournier, 1966; Packard et al., 1971; Pomeroy & Johannes, 1968; Redfield, 1963; Waksman & Renn, 1936). Its small size precluded detailed research, until the development of molecular biology. Previous research had always been hampered by lack of analytical specificity and precision of the biogeochemical and ecological tools used in plankton research.

The recent studies combine different techniques so results that relate the abundances of amoA genes for both AOA and AOB, with the transcription via 16S rRNA (Agogué et al., 2008; Church et al., 2010; De Corte et al., 2008) and nitrification rates (Beman et al., 2012; Newell et al., 2011) are being delivered. The increased accuracy in the results comes not only from the relation between abundances and nitrification rates, but from gene expression via 16S rRNA. The identification of these groups of nitrifiers and the identification of the influence of the environmental factors affecting their distribution and activity has been due to these studies. These studies can be credited with discovering the predominance, by several orders of magnitude, of the AOA over the AOB (Beman et al., 2012; Beman et al., 2008; Wuchter et al., 2006). This predominance is even greater in the upper ocean. Fig. 2, a new qualitative conceptual model, shows the relative trends in the nitrifier distribution within the ocean water column.
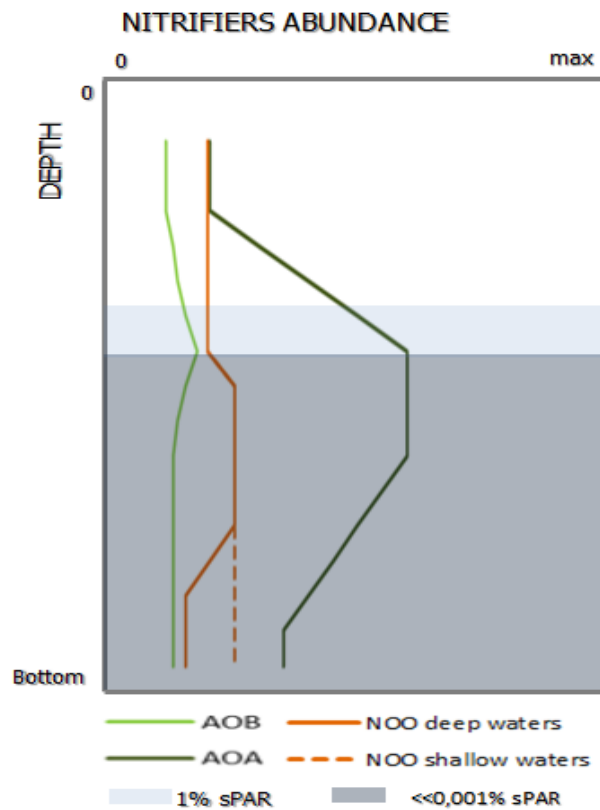

Fig. 2: New qualitative conceptual model showing the relative trends in the distribution of nitrifiers over the ocean water column. This new model integrates the results from Agogué et al. (2008); Beman et al. (2012); Beman et al. (2008); Church et al. (2010); De Corte et al. (2008); and Wuchter et al, (2006).

Usually, the proportion of AOB is lower in these areas, but it increases with depth. Although the AOA are predominant in the euphotic zone, there is an abundance peak at the bottom of euphotic zone associated with the Primary Nitrite Maximum (PNM). Throughout the euphotic zone, in the PNM and above, the expression of the amoA is proportionally high (Church et al., 2010), consistent with the results of carbon isotopes based studies (Hansman et al., 2009; Ingalls et al., 2006). The Crenarchaeota in the upper ocean are thought to be autotrophs (Hansman et al., 2009; Ingalls et al., 2006). AOA abundance and gene expression declines below the PNM and on into the mesopelagic and bathypelagic waters below (Agogué et al., 2008; De Corte et al., 2008). This suggests that bathypelagic archaea are not autotrophic NH_4^+ oxidizers, and live by mixotrophy or even by heterotrophy (Agogué et al., 2008). This is consistent with the schematic diagram of nitrification proposed by Mackey in 2011 where NO_2^- oxidation becomes predominant within the dark ocean. Here, the NH_4^+ oxidation balances the NO_2^- oxidation so that the NO_3^- starts to appear in the deep water column (Mackey et al., 2011). The decline of the AOA in the deep water Crenarchaeota community continues as deep-sea water ages. In old subtropical and equatorial deep waters, this is particularly noticeable (Agogué et al., 2008).

In the smaller population of AOB in the euphotic zone, the β-proteobacteria are the main group and some strains have been identified. In the aphotic zone of the deep-sea γ-proteobacteria are the dominant fraction of the AOB. All of the AOB increase in abundance and relative proportion in the nitrifying community as the latitude increases (Beman et al., 2012).

The connection between the oxidation of NO_2^- and the dark ocean (Mackey et al., 2011) is a characteristic of the NOO niche of activity within the ocean ecosystem (Lomas & Lipschultz, 2006). On the other hand, some studies report that the abundance of NOO is low and does not vary substantially over the water column (Ward & Carlucci, 1985). Also it has been reported that some AOB have the nirK gene, responsible for NO_2^- oxidation, so no only are they able to produce NO_2^-, but they are able to oxidize it further to NO_3^- (Casciotti & Ward, 2001). Thus it appears that the combined action of AOB and NOO below the PNM, and the weak NO_2^- uptake by the phytoplankton, explains why the NO_3^- has a high concentration within the dark ocean.

The nitrifier community behavior seems to follow a certain pattern since equivalent distributions were obtained in Pacific (Church et al., 2010), North Atlantic (Agogué et al., 2008), and Mediterranean waters (De Corte et al., 2008).

More investigations are needed to define the specific nitrification rate by the AOA and by AOB communities (J. M. Beman et al., 2012). The relationship between abundances and nitrification rates has been studied in some publications; however, it seems that this relation is only clear when the community is in a stationary state, due to the permanent supply of nutrients (NH_4^+). When nutrients are limited, the species within the nitrifying community with high affinity for NH_4^+ are rapidly activated even with low NH_4^+ concentrations. On the other hand, when the waters are rich in NH_4^+ the species in the nitrifying community with a low affinity for NH_4^+ reach the stationary state and have lower nitrification rates per cell (Beman et al., 2012).Thus, abundances and nitrification rates are not always well-related (Beman et al., 2012). More studies are needed to know the variability of nitrification rates due to abundances and composition of the nitrifier community, its growth stage and its specific variations in AOA and in AOB groups.

Moreover, a deeper knowledge of specific metabolism of the different groups in the nitrifier community (AOA, AOB, NOO) may improve the understanding of the main biogeochemical cycles. Thus, their significance within them could be assessed, via their C-fixation, O_2-uptake, and organic N-compounds release rates. To gain more accurate knowledge about how the variations in environmental factors may affect this community is really relevant, especially considering the current changing ocean.

The main environmental factors and its influence on the nitrifier community are discussed in the next chapter.

4. ENVIRONMENTAL FACTORS

The nitrifying community distribution and its activity are driven by the environmental conditions in the water column. The light intensity, nutrient availability, the water column stratification, the $ppCO_2$ and acidification, the dissolved O_2 (D.O.), the temperature and the salinity, have a great influence on the distribution, abundance, and the temporal and geographical variability of nitrifying community activity.

The influence of these environmental factors is analyzed in the following sections.

4.1 <u>The light intensity</u>:

In the last decades the euphotic nitrification, and its previous underestimation have been highlighted investigations. This process has been detected within different oceans and trophic conditions (Beman et al., 2012; Bianchi et al., 1999 (a); Bianchi et al., 1999(b); Church et al., 2010; Clark et al., 2008; Mackey et al., 2011; Newell et al., 2011; Yool et al., 2007). These findings oppose the idea of the total photoinhibition of nitrifiers. Recent research has shown that photoinhibition does not uniformly impact the different nitrifiers, or even the different strains within the same group. In particular there are some studies about the variability among two AOB strains and two AOA strains (Merbt et al., 2012). The AOB in this research appear to be less sensitive to light than AOA. These results contradict those based on molecular analysis of Pacific Ocean, North Atlantic Ocean, and Mediterranean Sea water masses (Agogué et al., 2008; Church et al., 2010; De Corte et al., 2008), where AOA seem to be less sensitive to light intensity than AOB. In general, according to Beman et al. (2012), the AOA suffer less photoinhinibition than the AOB (Beman et al., 2012), although there may be some differences between the strains within the group. Thus AOB seems to be confined to the darker layers of the water column, while AOA appear to be more active in the euphotic zone. As for the oxidation of NO_2^-, the NOO seem to be especially sensitive to the influence of light (Lomas & Lipschultz, 2006).

These conclusions are in line with the profile of nitrification proposed by Mackey in 2011 where, based on the intensity of light, different nitrification activity layers may be identified (Mackey et al., 2011). The new conceptual model about nitrification activity distribution considers the combined influence of light intensity, water column stratification and O_2 concentration. This new model is diagrammed in Fig. 3.
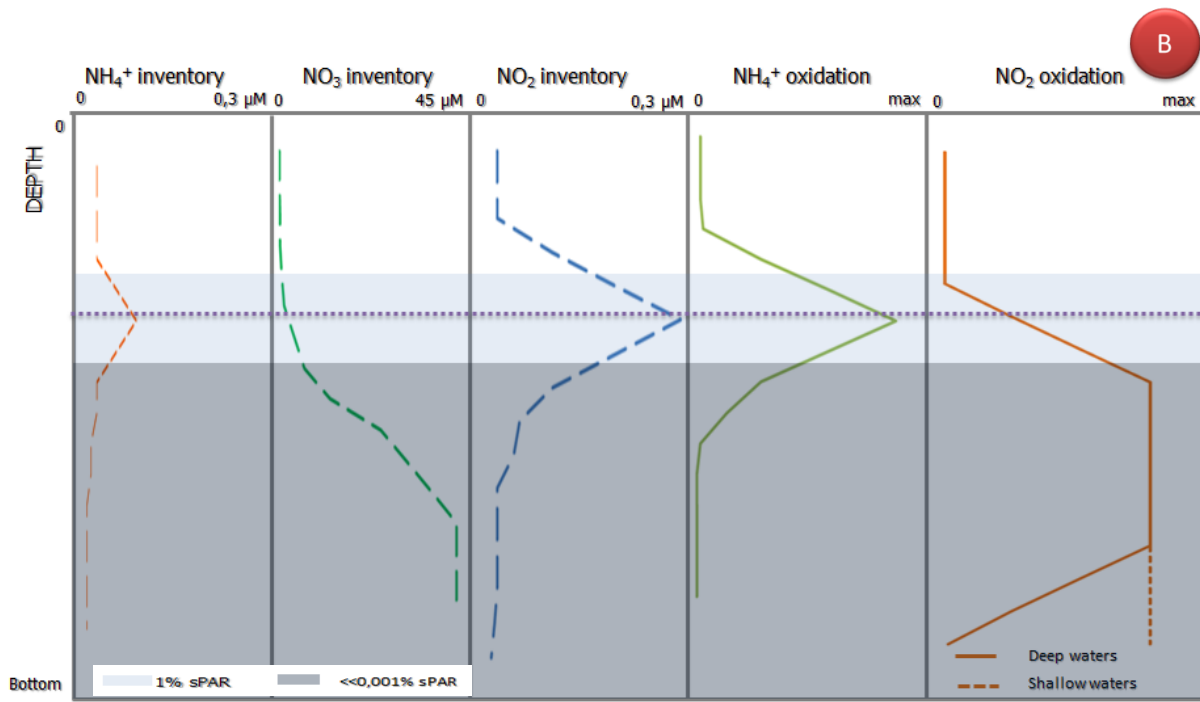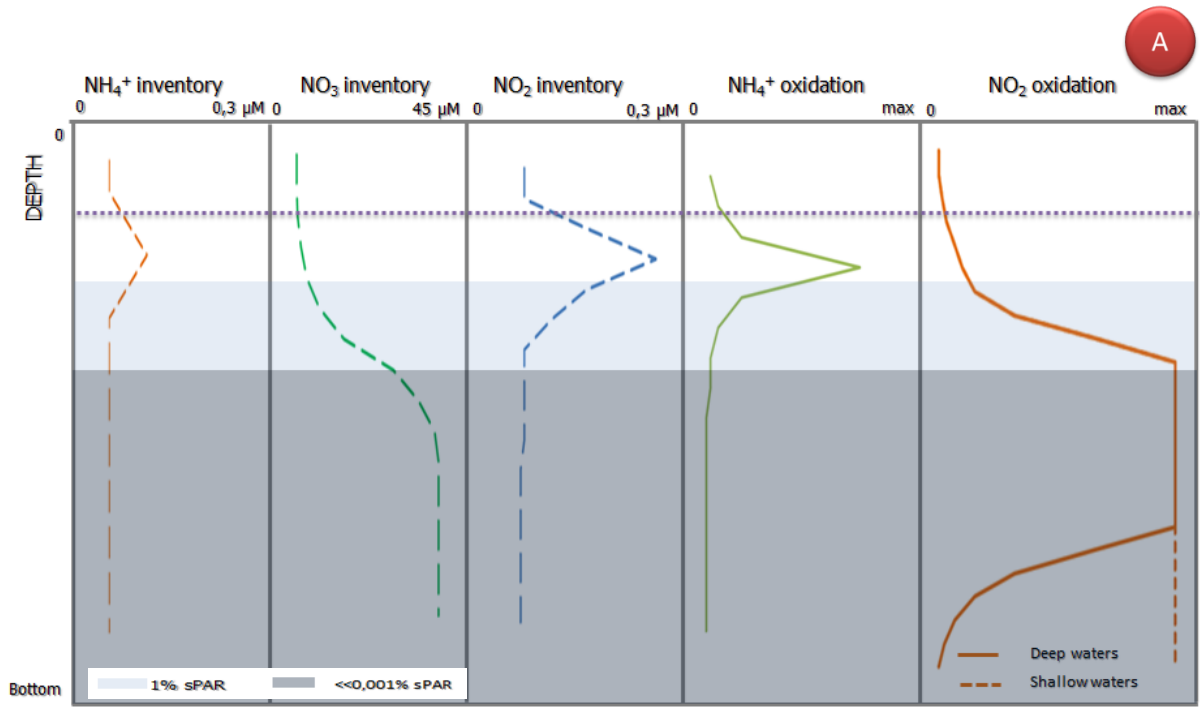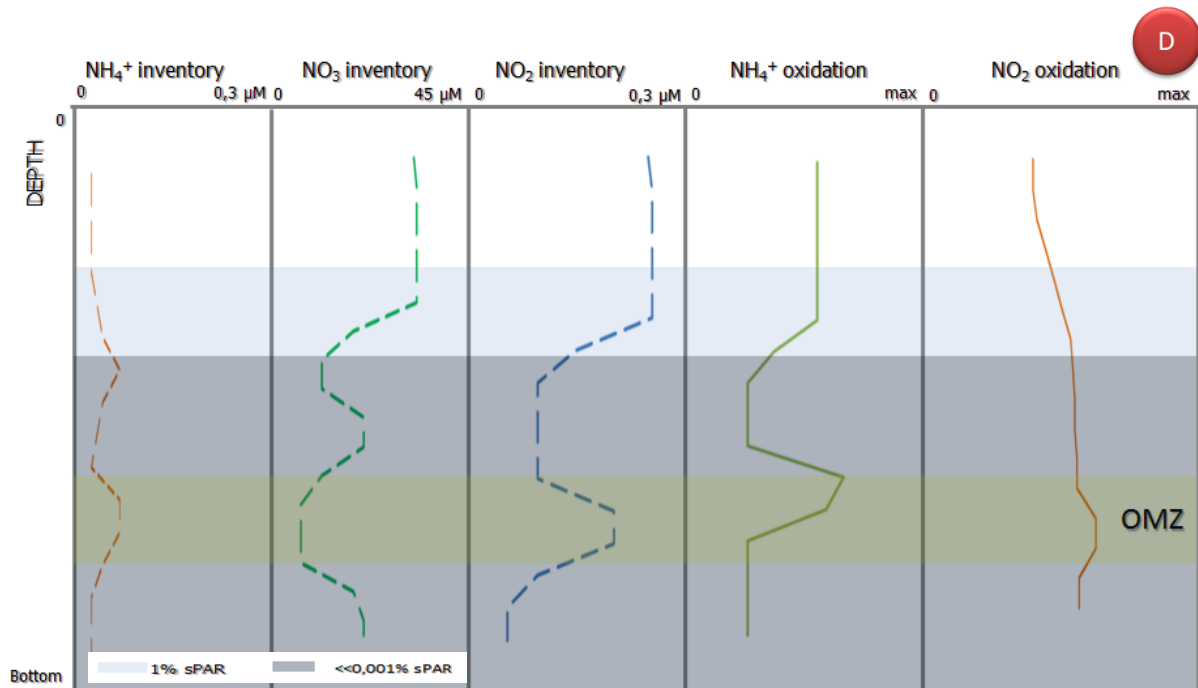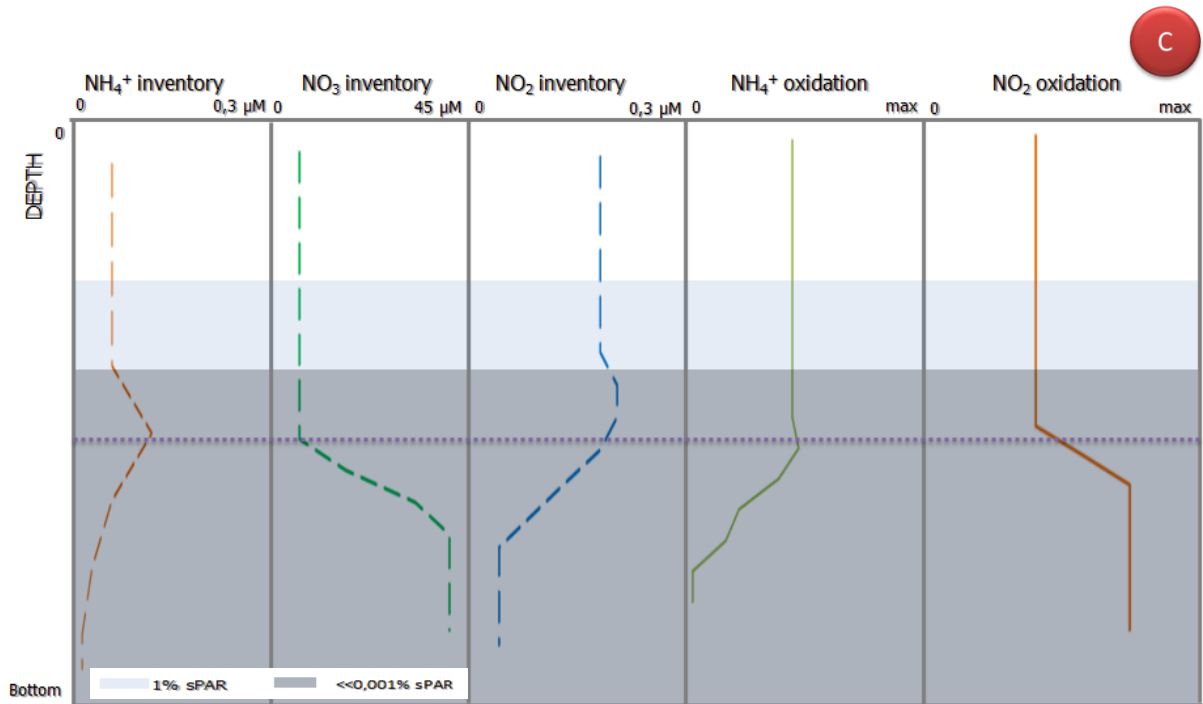
Fig. 3: New qualitative conceptual model based on the integration of the information from Beman et al. (2012), Clark et al. (2008), Lomas and Lipscultz (2006), Mackey et al. (2011), Millero (2006). Schematic diagram showing the relative trends in the distribution of nitrification when: A) The thermocline is shallower than the 1%sPAR depth; B) The 1%sPAR depth and the thermocline match; C) There is no seasonal thermocline or there is a shallow upwelling; D) There is a Coastal Upwelling with an OMZ.

Due to the light intensity variability in the water column, three different layers could be identified:

i. The euphotic zone; above 1%sPAR (surface Photosyntheticly Active Radiation): Nitrification develops within this zone, essentially due to NH_4^+ oxidation (Horrigan et al., 1981; Lomas & Lipschultz, 2006), driven by AOA in marine ecosystems (Church et al., 2010). This process fluctuates daily due to NH_4^+ uptake competition with phytoplankton (Mackey et al., 2011), and light variability during day/night cycles (Bianchi et al., 1999(b)). It even varies seasonality throughout the year (Bianchi et al., 1999(b)). Higher NH_4^+ oxidation rates in light incubations than dark incubations have been reported in some studies (Bianchi et al., 1999 (b)) whereas NO_2^- oxidation rates were always higher in dark incubations in these same studies (Bianchi et al., 1999(b)). Recently, a study with high vertical spatial resolution has been carried out (Beman et al., 2012). The 21% NH_4^+ oxidation, on average, has been reported to develop within the euphotic zone. However, these values are not geographically homogeneous, nor are they maintained throughout the year. NH_4^+ oxidation in the euphotic zone may vary from 1.5% to 42% due to PNM position (Beman et al., 2012). An NH_4^+ maximum peak has been reported near the PNM, being less intensive when the PNM is within the euphotic zone, because the NH_4^+ is used by phytoplankton too (Lomas & Lipschultz, 2006; Woodward & Rees, 2001). Thus, light intensity is not the only main factor in nitrification distribution; nutrient availability has a great influence too (see Nutrient Availability section).

Other factors, such as ocean acidification effect the displacement of nitrification activity to deeper layers in the ocean (Beman et al., 2012; Beman et al., 2011). On the other hand, stratification, that is expected to increase due to global warming, may lead

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to different reactions. In eutrophic conditions, primary production is concentrated within the upper ocean, so the euphotic zone may be reduced due to pigment-mediated light-absorption. In oligotrophic conditions, primary production will be lower (Gruber, 2011). Thus, PNM depth may be affected, causing a parallel decrease in the nitrifier activity. Respectively, nitrification will develop in both the dark ocean (Fig. 3-C) as well as within the euphotic zone (Fig. 3-A). In the following section, the variations in the PNM depth due to stratification are specifically addressed.

ii. In the subeuphotic zone (from 1% sPAR to 0.001%sPAR) the phytoplankton community is under low-light stress enhancing the formation of a chlorophyll-a peak at the DCM, the deep chlorophyll maximum. Stressed phytoplankton release NO_2^-, due to an inefficient reduction of NO_3^-, and are less competitive in extracting NH_4^+ from the NH_4^+ pool (Lomas & Lipschultz, 2006; Mackey et al., 2011). Thus, the NH_4^+ oxidizers (AOB and AOA) are more abundant, and the NO_2^- begins to concentrate. Nitrifier photoinhibition decreases with depth, so nitrification behaves just in the opposite way (Mackey et al., 2011).

As the depth increases, these phenomena (both the decrease in NH_4^+ oxidizing photoinhibition and the increase in low-light stress in phytoplankton) gain intensity, so that the NO_2^- concentration gets higher till the maxima concentration within the PNM. Together NH_4^+ oxidation and limited phytoplankton NO_3^- reduction exceed NO_2^- oxidation.

iii. In the aphotic zone where light levels fall below 0.001% sPAR the AOA abundance decreases, resulting in a relative increase in the AOB proportion in the NH_4^+ oxidizing community. Nitrification to completion is strong at these depths. This is consistent with the increasing NO_3^- concentrations with depth in the dark ocean. In contrast with

the low euphotic zone concentrations of less than 0,05 µM in the open ocean in the

deep North Atlantic these concentrations reach 20-30µM NO_3^-. In the deep North

Pacific they are even greater, reaching 35 to 45 µM NO_3^-. NH_4^+ and NO_2^- normally

maintain very low concentrations throughout the water column. Deep ocean

concentrations of 0.05 µM of NH_4^+ are common, as compared to 0.1 µM at the sea

surface. NO_2^- maintains its concentration throughout the water column at about 0.3 µM

(Millero, 2006), except within the PNM.

Knowing the variability in the distribution of nitrification within the water column due

to the light is important when planning an ocean nitrification sampling program. However, the

influence of other environmental factors has to be considered before sampling depths can be

selected.

4.2 The thermocline depth

Increasing ocean stratification is one of the consequences of global warming (Gruber, 2011). Concurrently, the depth of the mixed layer is reduced, limiting the entry of nutrients from the deep waters to the upper euphotic ocean. Models do not show relevant changes in the overall photosynthetic primary production due to this new situation. On the other hand, both the stratification and the trophic state of the ocean may significantly impact the PNM distribution and the euphotic nitrification (Gruber, 2011):

A strongly stratified ocean (Fig. 3-A)

a) In oligotrophic and permanently stratified water masses: The increase in stratification may lead a decrease in photosynthetic primary production (Gruber, 2011). As a consequence, the boundaries of the euphotic layer may become deeper, as it is represented in Fig. 3-A. Under these conditions, the PNM is located within the euphotic layer, under the thermocline, and linked to NH_4^+ oxidation predominance. These profiles are consistent with the results reported within a dipole eddy system in North Atlantic (Painter, 2011). The NH_4^+ peak, and the increase in oxidized N-compounds $(NO_2^-+NO_3^-)$ are located, in an anticyclone eddy and under the thermocline, but above the boundaries of the euphotic layer, in an intermediate station between a cyclonic eddy and an anticyclone eddy. Assuming that the main component of oxidized N-compounds in the euphotic layer is NO_2^-, based on Mackey´s database (Mackey et al, 2011), it is suggested that the increase in oxidized N- compounds reported by Painter (2011) is due to PNM formation. Fig. 3-A represents this scenario. High NO_2^- oxidation rates have been reported at the base of the euphotic layer (1%sPAR) under oligotrophic conditions (Clark et al., 2008), as it has been represented in Fig. 3-A. Light attenuation is gradual in these zones, allowing NOO

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proliferation even under the presence of light. The gradual changes in the light spectrum enable NOO activity depending on the sensitivity of the different strains (Olson, 1981). Under eutrophic conditions, the strong light attenuation does not allow a gradual transition in the NOO community. In these regions, the NO_2^- oxidation is confined to the dark ocean.

b. <u>Under the conditions of seasonal stratification in a eutrophic zone.</u> Water masses models appear to show stratification stimulating photosynthetic primary production within the upper ocean (Gruber, 2011). This causes euphotic layer boundaries to be shallower and increases in the NH_4^+ oxidation. Light attenuation is due to increases in photosynthetic primary production under these stratified conditions. In these cases the thermocline may be shallower, but never deeper than the euphotic zone (Figs. 3-A and B). Under these conditions, PNM and the maximum NH_4^+ oxidation activity fall within the euphotic zone.

The difference between oligotrophic and eutrophic conditions is that under eutrophic conditions the PNM and nitrification peaks are most likely to be within subeuphotic conditions (from 0.001%sPAR to 1%sPAR) due to the drastic light attenuation as in an intermediate scenario between Figs. 3-A and 3-B. This scenario may become more common in the future due to the warming of the upper ocean.

An intermediate scenario: thermocline and boundaries of the euphotic zone match in depth (Fig. 3-B)

When thermocline and the euphotic zone boundaries match, the PNM may be located within the subeuphotic zone. In this case, both the NH_4^+ oxidation and a declining NO_3^- reduction rate are the processes that generate the PNM (Lomas & Lipschultz, 2006; Mackey et al., 2011). The chlorophyll-a peak, under these conditions could be used as an indicator of the PNM location according to Mackey et al. (2011).

Thermocline is within the aphotic zone (Fig. 3-C).

There are features in the ocean where thermocline is deeper than the euphotic zone. An eddy-induced upwelling or the absence of seasonal thermocline during winter (at North Hemisphere) are examples. Painter (2011) reported such results from a cyclonic eddy induced upwelling in the Iceland Basin. In contrast to strong coastal upwelling, represented in Fig. 3-D, in the Iceland Basin the mixed layer is shallow, and the thermocline occurs below the euphotic zone. Within this mixed layer characterized by episodic high vertical mixing, nutrients maintain their concentrations, independently of the euphotic layer depth (Mackey et al., 2011; Painter, 2011). It can also be noted that, when there is no seasonal stratification, the trend of nitrification is equivalent to that reported from the North Atlantic cyclonic eddy. The distribution in nitrification when thermocline is deeper than the euphotic layer boundaries is represented in Fig. 3-C.

Another noteworthy is the significant C-fixation due to nitrification within the euphotic zone under these conditions. It is even greater than the total C-fixation in the deep waters. Painter (2011) suggested that during upwelling, not only are nutrients upwelled, but with, it is the nitrifier community upwelling.

<u>When there is no seasonal thermocline, or the thermocline is located much deeper than the euphotic zone, as in coastal upwelling (Fig. 3-D)</u>

In this case the nutrient distribution is uniform within the upper layers, with nutrient concentrations elevated because of bottom mixing. Fig. 3-D also shows Oxygen Minimum Zones (OMZs), because they are linked to the coastal upwellings worldwide (Peruvian Upwelling, Namibia Upwelling, etc.). The specific features of the nitrification distribution associated with the OMZ are examined in the Dissolved O_2 section.

Considering the variations in the nitrification distribution throughout the water column, the C-fixation and O_2-uptake related to the NH_4^+ oxidation and NO_2^- oxidation within the euphotic layer may vary significantly. In fact, under oligotrophic conditions, stratification may stimulate nitrification within the euphotic zone. This influence may be significant in analyzing the metabolic state in the upper open ocean, but was not greatly considered in the 2012 debate between Duarte et al (2013), Ducklow and Doney (2013) and Williams et al. (2013). By not considering nitrification and the other forms of chemoautotrophy, the conclusions of this debate are not conclusive.

4.3 <u>Nutrient availability</u>

Previously, the relationship between the PNM and nitrification activity stimulated discussion. The PNM is related to weaker NO_2^- oxidation, under high light conditions where both the NH_4^+ oxidation and the phytoplankton NO_3^- uptake are reduced leading to higher NO_2^- concentrations. Light intensity seems to be the main factor in regulating the distribution of the NO_2^- oxidizers even more than does nutrient availability.

On the other hand, NH_4^+ availability seems to be the main factor in the NH_4^+ oxidation distribution. Potential relationships between this type of substrate availability and nitrification rates are assessed in this section.

Oligotrophic conditions:

As Clark et al., 2008 report in their study of the North Atlantic, before the turn of the millennium, it was thought that nitrification occurred under high NH_4^+ concentrations, subsaturating O_2 conditions, and was restricted to dark waters due to photoinhibition. However, they report significant nitrification rates that enable the regeneration of all the NO_3^- pool in one day, even under oligotrophic conditions. In spite of the significance of nitrification within the euphotic zone, Clark highlights that it is really complicated to assess because of the low concentrations involved. Under these conditions, even "*the low primary production rates that characterize the oligotrophic ocean do not reflect the surprisingly high levels of N cycling activity*" (Clark et al., 2008).

In the open ocean, NH_4^+ supplies are related to NH_4^+ regeneration via decay and excretion as well as to the organic matter fluxes. NH_4^+ is low and uniform within the Dissolved Inorganic Nitrogen (DIN) pool throughout the deep water column.

Under oligotrophic conditions, NH_4^+ oxidation has a hyperbolic relation with NH_4^+ regeneration by the biota (Clark et al., 2008). As the regeneration rate increases, the oxidation rate increases too, until the oxidation rate increase is greater than the regeneration increase. This relationship is more significant within the 1%sPAR zone, due to the less competitive low-light-stressed phytoplankton for the NH_4^+ pool. Nitrifiers have a competitive advantage within this zone. The correlation between NH_4^+-regeneration and NH_4^+-oxidation has been reported by others. Bianchi et al. (1999(b)) reported that when heterotrophic organic-matter remineralization rates decrease due to a wind-induced vertical mixing event, the NH_4^+-oxidation rates decreased in parallel (M. Bianchi et al., 1999 (b)). The presence of deep peaks of chlorophyll-a (DCM) also leads to a nitrification increase due to the release of detrital NH_4^+ (M. Bianchi et al., 1999(b)).

A new hypothesis about the processes involved in carbon cycle is being developed from this dependence of nitrification and organic matter remineralization (Newell et al., 2011). It starts with the heterotrophic community being linked to the organic matter fluxes. Nitrification, as a chemoautotrophy process contributes to this organic matter supply for the heterotrophic community (Herndl et al., 2005). However, at the same time, nitrifiers need that organic matter flux and its remineralization, to supply NH_4^+ via decay (Newell et al., 2011). Probably, the different metabolic pathways that allow the development of microbial communities within the deep ocean will be better known from future research. For example, Hansman et al (2009) and Ingalls et al. (2006) have already begun to determine the autotrophic or heterotrophic characteristics of archaeal metabolism in the deep ocean. Within the nitrifier community, the mixotrophic AOA may have a special role in this influence in the deep autotrophy or heterotrophy, because they may develop both the remineralization of

organic matter and the NH_4^+-based chemoautotrophy (Walker et al., 2010). In epi and mesopelagic waters the AOA abundance is high although the 16sRNA transcription decreases with depth (Church et al., 2010). This community seems to have an especial affinity for NH_4^+ (Martens-Habbena et al. 2009). Even when NH_4^+ concentrations are very low, after a new and sudden injection, the nitrification rate per cell is unusually high. The more permanent the supply, the higher the growing rate, so the community is able to reach steady state (Beman et al., 2012). The abundance of AOO (and the 16S rRNA trasnscription) is correlated with the nitrification rates at this stage. This scenario is developed in biogeochemically stable or stratified systems whereas in physically turbulent systems, with new N supplies, the nitrifier community may vary its nitrification rates per cell (Beman et al., 2012). The hypothesis related to the changes in nitrification rates per cell has still to be tested. Assuming that it were verified, this variability may lead changes in nitrification-related C-fixation rates and in O_2-uptake rates per cell. A better knowledge of the NH_4^+ oxidizers behavior under different nutrient-availability conditions may significantly improve our understanding of the oligotrophic ocean.

As said above, NO_2^- oxidizers are mainly influenced by light intensity, photoinhibition, and to a lesser extent, by nutrient availability. Thus, all of these factors have a direct influence on the nitrifier community. Under upwelling conditions the NO_2^- oxidizing community rises into the euphotic zone and kept active (Painter, 2011). When considering the differences in light sensitivity (Lomas & Lipschultz, 2006), Bianchi et al. (1999(b)) found that NO_2^- oxidation activity developed in the euphotic zone at lower rates than in the dark below.
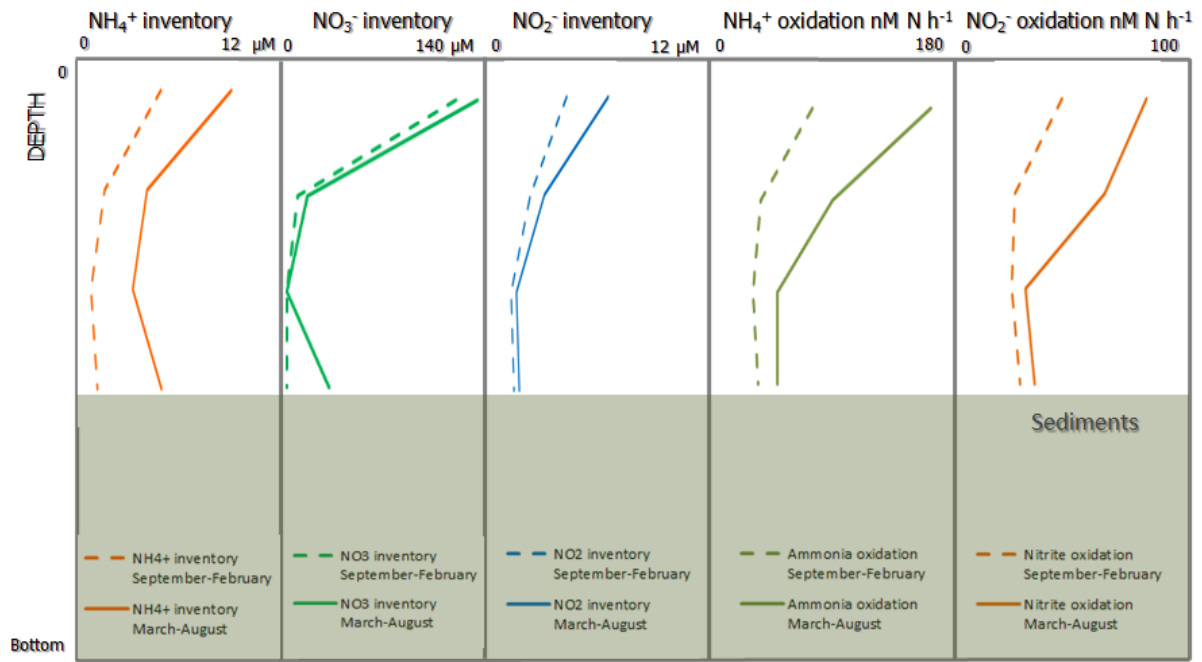
At the bottom of the euphotic layer under oligotrophic conditions, there is a NO_2^- oxidation-rate increase, where both the light spectrum and a rise in the PNM-related NO_2^- availability allowed NOO proliferation. Nitrification is developed within the euphotic zone in

these zones, so the regenerated NO_3^- may stimulate regenerated photosynthetic primary production. If this process is not considered in the assessment of the carbon exportation rate, via f-ratio measurements, an overestimation of the vertical carbon flux in the open ocean would be made (Clark et al., 2008; Yool et al., 2007).
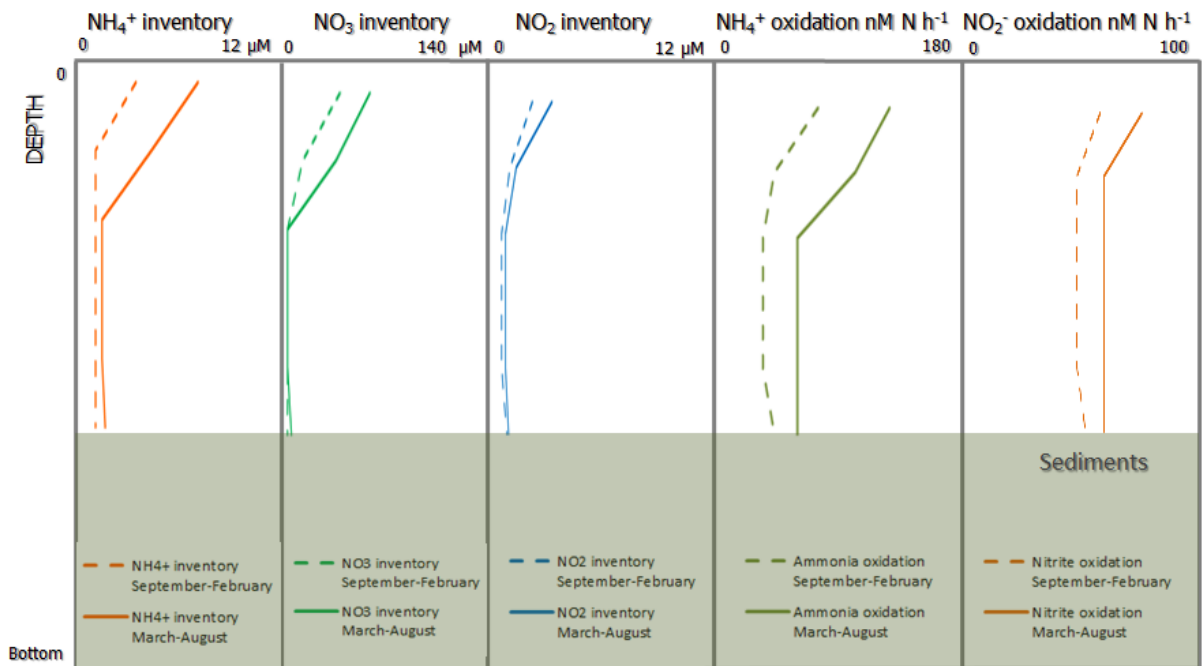
Eutrophic conditions:

Under eutrophic conditions in upwelling zones NH_4^+ reaches high concentrations within the DIN pool (Clark et al., 2008). Considering these profiles is important because inhibition of phytoplankton NO_3^--uptake has been reported under high NH_4^+ levels in upwelling waters (Harrison et al., 1996), leading to greater competition between phytoplankton, bacteria, and archaea for the NH_4^+ pool. NH_4^+ regeneration and zooplankton NH_4^+ excretion rates are higher in these zones due to the large dynamic phytoplankton community. Under eutrophic conditions in estuarine zones, mixing conditions ensure highly variable NH_4^+ concentrations throughout the water column. High NH_4^+ supplies occur in the upper layers. Changes in salinity, temperature, or pH occur also, leading to variations in both the nitrifying community and its activity. Water columns will have higher nutrient concentrations of NH_4^+, NO_2^-, and NO_3^- near river mouths (Fig. 4-A) (Bianchi et al., 1999 (a)). The nearer the river plume, the higher nitrification rates become, even within the euphotic zone (Fig. 4-A).

A

NH₄⁺ inventory — NO₃⁻ inventory — NO₂⁻ inventory — NH₄⁺ oxidation nM N h⁻¹ — NO₂⁻ oxidation nM N h⁻¹

Low salinity: plume water

B

Intermediate salinity : edge of the plume water
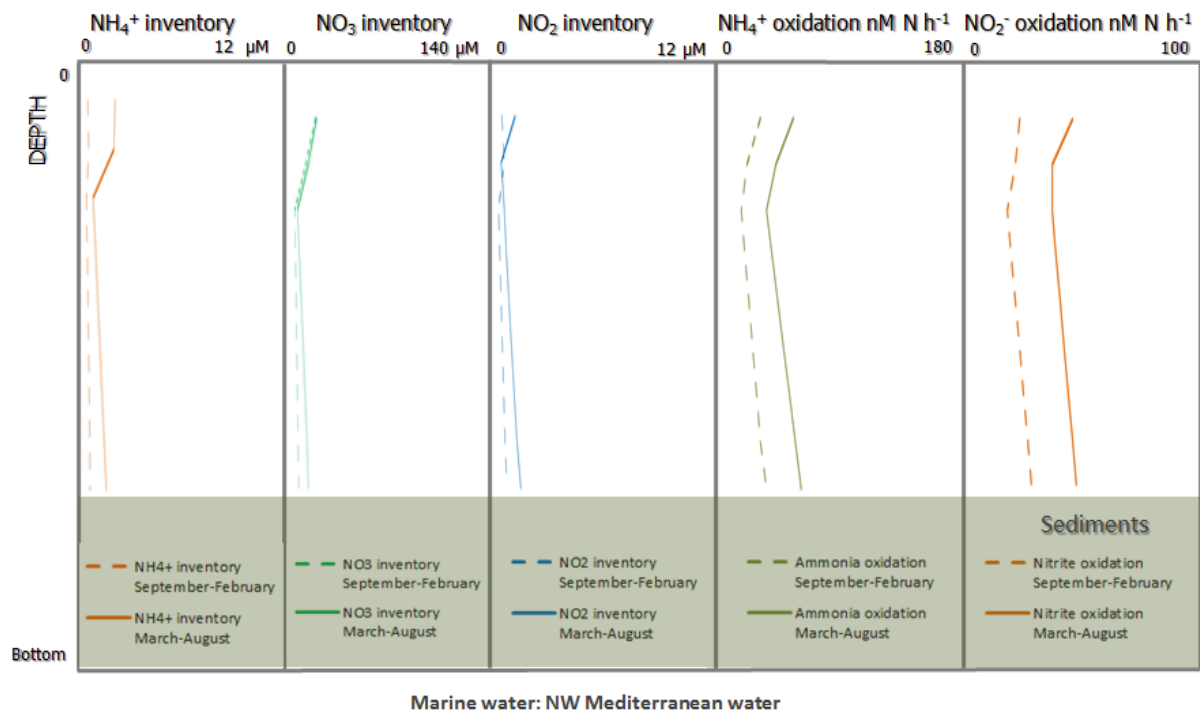
Fig. 4: Schematic diagram showing the main trends in the distribution of nutrients and nitrification activity under the influence of estuarine conditions: A. under low salinity conditions within the plume, B. under intermediate salinity conditions at the edge of the plume, C. under marine conditions out of the plume (Recalculated and redrawn from Bianchi et al, 1999 (a)).

In an estuarine zone with shallow waters, nitrification rates, both the NH_4^+ and NO_2^- oxidation phases, are higher in the surface waters where nutrients are available. Both rates of oxidation are lower at intermediate depths (Bianchi et al., 1999(a)). Within the plume, NH_4^+ oxidation rates are higher than NO_2^- oxidation rates (Fig. 4-A and Fig. 4-B). These rates decrease in parallel while moving away from the plume (Fig. 4-C). The seasonal thermocline in these zones leads to higher nitrification activity in surface waters, decreasing sharply with depth, until a constant value is reached (Fig. 4-A and Fig. 4-B). Outside the plume, the profiles show different features. If the bottom is less than 100m both phases of nitrification increase near the sediment-water interface (Bianchi et al., 1999(a)).
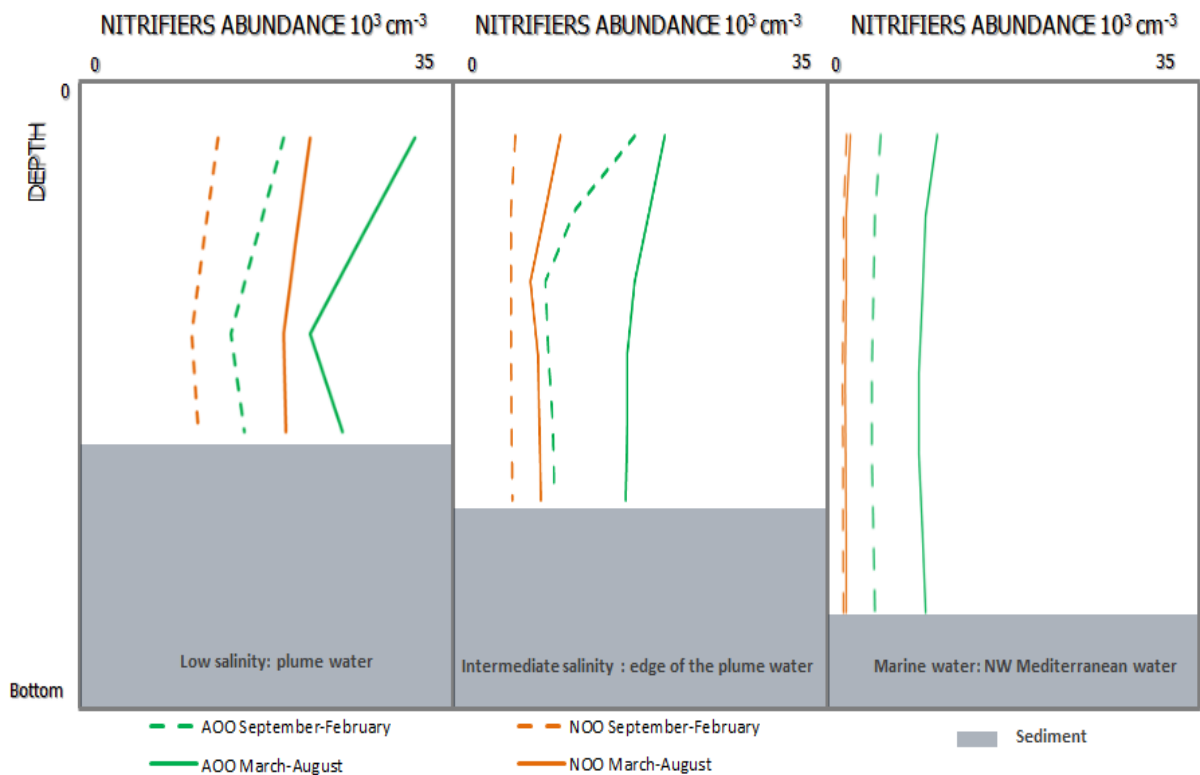

Fig. 5: Schematic diagram showing the main trends in the distribution of nitrifiers under the influence of the estuarine conditions: (A) under low salinity conditions; (B) within the plume, under intermediate salinity conditions at the edge of the plume, and (C) under marine conditions out of the plume (Selected data, reinterpreted, and redrawn from Bianchi et al, 1999(a)).

In summary, nitrification is linked to substrate (reactants) availability in the ocean. The supply of these substrates differs in coastal, estuarine, upwelling, and open ocean environments. However, in all cases, wherever high NH_4^+ levels occur, regardless of the depth, nitrification rates will increase. In addition, other factors will impact these rates, the nutrient supply, as well as the nitrifying community itself (Fig.5). These include salinity, temperature and pH (Ward et al., 2007). Their influence will be addressed in the following sections.

4.4 Dissolved Oxygen (D.O.)

The presence of an electron acceptor is required for the oxidation processes to function in nitrification. Different O_2 concentrations effect changes in the species composition in the nitrifying community; different strains are adapted to different environmental conditions. Nitrifying bacteria are particularly sensitive to low O_2 concentrations. Archaea, on the other hand, are adapted to suboxic conditions (Beman et al., 2008; Bouskill et al., 2012). If this were the only environmental factor influencing in the community, nitrifying bacteria would be dominant in the euphotic supersaturated oxic surface waters. Actually, AOA are predominant within the nitrifying community throughout the water column. Archaea have a wide range of tolerance to different O_2 partial pressures (ppO2) and are especially competitive under suboxic conditions. The highest values in abundance have been reported within the Oxygen Minimum Zone (OMZ) (Bouskill et al., 2012).

The oxicline is a common feature deep in the water column, where ppO_2 decreases from the supersaturated oxic upper layers to deep subsaturated waters. Within intermediate waters from the Pacific and Atlantic Oceans, this feature arises from both the heterotrophic organic matter remineralization and the advection of deep O_2-enriched waters (Millero, 2006). The archaeal predominance in nitrifying community is even higher when the ppO_2 decreases.

<u>NH_4^+ oxidation within the OMZ</u>

The first phase of nitrification has a special role within the OMZ. It produces NO_2^- that permits denitrification and anaerobic NH_4^+ oxidation (annamox) to proceed. Lam & Kuypers, (2011) noted elevated NH_4^+ oxidation in the upper layer of the OMZ (Fig. 3-D). During this first phase in nitrification, the NO_2^- released by AOO exceeds the requirements of the NOO, so a NO_2^- peak is formed. This feature is formed by both the AOA and AOB within the OMZ. Unlike the rest of the water column, in the OMZ the transcription of amoA gene is high within the ɣ-proteobacteria, although AOA keeps being the predominant group by abundance (Francis et al., 2005). Also in these areas there is strong competition for NH_4^+ between AOA and anammoxidizers. The high affinity in the Archaea for NH_4^+, as found in the group, Thaumarchaeota (Martens-Habbena et al., 2009), may give them an adaptive advantage to sequester NH_4^+ and outcompete other groups within this environment (Lam & Kuypers, 2011). Another factor is the AOO adaptation to low ppO$_2$ which enables them to oxidize the NH_4^+ with other electron acceptors, different from O$_2$. An example is the so-called nitrifying denitrification, which uses NO_2^- or N_2O_4 as final electron acceptors. During the process, NO_2^- is finally reduced to N_2 and N_2O (Schmidt & Bock, 1997). Furthermore, under suboxic conditions, archaea release N_2O, which is consistent with the idea of nitrification under low ppO$_2$ conditions being the main process generating N_2O (Newell et al., 2011). Correlations between higher N_2O emission rates and archaeal amoA gene abundances have been reported (Löscher et al., 2012). Estimation of global N_2O emissions shows higher concentrations within the upper layers (where the sea-air flux occurs) in upwelling zones (Peruvian Upwelling, Namibian Upwellin, etc) where the OMZs are permanent. The nitrification under suboxic conditions is estimated to generate 93% of the global N_2O emissions (Freing et al., 2012).

NO$_2^-$ oxidation within OMZ

NOB are also adapted to low ppO$_2$ conditions. This group seems to be able to use NO$_3^-$ as an electron acceptor, releasing NH$_4^+$ as well as other N-oxides (Bock et al., 1988; Freitag et al., 1987; Lam & Kuypers, 2011). Thus, in the Pacific Ocean, deep in the OMZ, NO$_2^-$ oxidation rates are higher than NH$_4^+$ oxidation rates (Lipschultz et al., 1990; Ward, et al., 1989). This process retards the loss of N from the OMZ by returning it to its highest oxidation state (Lam & Kuypers, 2011). Even though, NO$_3^-$ concentration keeps decreasing, due to its use the main electron acceptor for metabolism within these anoxic layers, if there were a deeper layer with higher ppO$_2$ in the water column, NO$_3^-$ concentrations will rise because O$_2$ will be used instead within that layer. This scenario has been reported in Arabic Sea (Millero, 2006; Newell et al., 2011).

Improving our understanding of the N- cycle, the behavior of the nitrifiers, and their impact on the production rates of the N oxides within these suboxic zones, should be a goal of the scientific community in this time of global warming (Beman et al., 2012; Keeling et al., 2010)

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4.5 <u>Salinity</u>

Recent studies about environmental factors that influence nitrification have shown that AOB are more abundant in low saline waters (S‰ < 30 PSU), whereas AOA are predominant at normal oceanic salinities (S‰ = 35 ± 3 PSU) (Bouskill et al., 2012; Ward et al., 2007). Nevertheless, other environmental factors may promote other distributions of nitrifiers. For instance, within estuarine aquifers, the dissolved O_2 is the most important factor, leading directly to a distribution of archaea and bacteria completely opposite to the distribution proposed by Bouskill et al. (2012) (Erguder et al.,2009). The AOA appear less abundant in saline and oxic waters within the aquifer in this study, while predominated over β-proteobacteria in less saline but suboxic waters. Under these conditions, archaeal amoA abundances are relatively constant within a salinity gradient from 0,5 y 33 PSU and an O_2 gradient from 100-200 μM. So the AOB is the group that seems to be more sensitive to the variability of salinity and ppO_2, and this leads to changes in the distribution of the nitrifying community (Erguder et al., 2009). AOA seems to have high salinity tolerance (Santoro et al., 2008). Concrete ecotypes confined to specific salinity ranges are thought to exist, for instance, those which live in hypersaline conditions (>36,6 psu) (Venter et al., 2004).

Estuarine zones

Apart from the estuarine aquifers, the water column under estuarine conditions merits special attention in the analyzing the nitrification distribution. The distribution of nitrifying organisms is shown in Fig.5, as well as the distribution associated with plume-influenced activity (Fig. 4). These figures have been drawn from the data collected by Bianchi et al. (1999 (a)).

It should be noted that in both Fig. 4 and Fig. 5, the whole water column is considered the upper ocean, due to the shallower basin (the bottom does not exceed 90m in any case) (Bianchi et al., 1999 (a); Feliatra & Bianchi, 1993). Surely, nitrification within deeper layers would be influenced by the nitrification in the sediments.

As reported, both NH_4^+ and NO_2^- oxidation rates seems to be higher at low salinities. Equivalent results for a low salinity coastal system, find nitrification rates increasing as pH decreases (Fulweiler et al., 2011). Similar higher nitrification rates are found near river mouths, where nutrients, such as NH_3, NO_2^-, NO_3^-, are more available (Bianchi et al., 1999 (a)). Additional studies showing the impact of salinity and pH gradients on nitrifying communities can be found in (Bouskill et al., 2012; Feliatra & Bianchi, 1993; Fulweiler et al., 2011; Ward et al., 2007). Overall, the influence of the nutrients availability seems to be greater than that of the salinity and pH gradients.

4.6 <u>Temperature</u>

Within all ecosystems the archaeal amoA gene has been found over a wide range of temperatures, from 0.2 ºC (at a depth of 2956m) to 97 ºC (within a terrestrial hot spring) (Erguder et al., 2009; Nakagawa et al., 2007; Reigstad et al., 2008). Any particular nitrifying community is expected to be related to a special temperature range. Some studies from a saline wastewater have found activity variations over a wide temperature range. As temperature increases, an increase in the activity is reported, reaching a maximum activity from where it begins decreasing (Sudarno et al., 2011). This is expected from Arrhenius theory (Arrhenius, 1889, 1915; Sizer, 1942 Moore, 1955; Pauling, 1958; Eggers et al., 1964; Fruton & Simmons, 1958; Giese,1963; Prosser & Brown, 1961; Mahler & Cordes, 1971; Segel, 1976; Nelson et al., 2010; Hochachka & Somero, 2002).

Global warming has a significant influence on all biogeochemical processes, due to the temperature-linked physiologic and enzymatic pathways. As Gruber published, a 2° C increase in temperature is expected to increase the physiologic processes between 7 and 33%.

Among them, organic matter turnover rates will be increased in the ocean, although the effects in photosynthetic primary production and the export rates to the deep ocean are still unknown (Gruber, 2011). While nutrients remain available, nitrification rates are expected to increase initially. Therefore, under eutrophic or estuarine conditions, nitrification activity will probably reach higher values. Meanwhile, this increase will be linked to NH_4^+ regeneration rates due to the in situ biological community under oligotrophic conditions.

Besides the direct influence on biogeochemical processes, it is worth mentioning that an increase in temperature leads to stratification and the particular effects of stratification on nitrification have been discussed before.

4.7 <u>Environmental acidification and CO_2 partial pressure (ppCO$_2$)</u>

Global warming is one of the consequences of an increase in atmospheric CO_2 concentrations. These higher values of atmospheric CO_2 lead to an increase in the $ppCO_2$ in the ocean and to a decrease in the pH. A potential consequence of an ocean $ppCO_2$ increase is fertilization of the autotrophic CO_2-fixing community (Denecke & Liebig, 2003). However, this seems to be occurring until the $ppCO_2$ reaches a high value, from when the nitrification activity is inhibited (Denecke & Liebig, 2003). Both AOA and AOB seem to develop mechanisms that avoid the influence of environmental changes. Potential mechanisms for carbon concentration (Badger & Bek, 2008), as well as new enzymatic pathways for different inorganic carbon compounds uptake (Koblek, 2011) have been suggested. This suggests that, currently, AOA, AOB and cyanobacteria do not use their entire CO_2-fixation potential.

Assuming an increase ppCO$_2$, this may lead to a reduction in the N-oxidation:C-fixation ratio. As a consequence, nitrification´s CO$_2$-fixation efficiency may improve, leading to a reduction in the O$_2$-uptake per mole of fixed C.

The second consequence of the ppCO$_2$ increase, the pH reduction, causes ocean acidification and would reduce marine nitrification rates through substrate limitation by decreasing seawater NH$_3$ proportions, the ammonium-compound they have more affinity for, due to its reduction into the NH$_4^+$ form (Beman et al., 2011).

Another consequence of the ppCO$_2$ increase and ocean acidification is the chemical reduction of Fe compounds, rendering them more available to microorganisms and facilitating their uptake. As a consequence, the cyanobacteria (N-fixing community) may proliferate, improving the ammonia (NH$_3$+NH$_4^+$) supply into the upper ocean (Arrigo, 2005). Overall, nitrification rates are expected to decrease between the 3% and 44% as a consequence of a pH decrease of 0.1 units. Both, these lower rates and the increase in N fixation, would lead to the following (Beman et al., 2008):

- A decrease in N$_2$O emissions from the ocean, equivalent to those related to all the fossil fuel combustion and other industrial processes (from 0.06 to 0.83 Tg N y^{-1}).

- A decrease in the available NO$_3^-$ concentrations within euphotic zone. This supply may be replaced by NH$_4^+$. From 1% to 25% of the global photosynthetic primary production would be supported by NH$_4^+$ regeneration.

- Decrease the mean size of photosynthetic organisms since the small sizes are more competitive in taking up NH$_4^+$ than big ones. An increase in N-fixation as well as a decrease in nitrification may lead to higher abundances of cyanobacteria within the photosynthetic community (Beman et al., 2011).

-	Diatoms and other big primary producers that specialize in NO_3^--uptake, may decrease within the phytoplankton community. As a consequence, the food web, fisheries, and the C export fluxes to the deep ocean will be impacted (Hutchins et al., 2009). The later may be influenced due to different particle sinking rates.

Apart from the anthropogenic acidification of the ocean, there are natural conditions that lead to low pH values in marine ecosystems. In an opposite way, some of them are linked to an increase in nitrification (Fulweiler et al., 2011). This is the case in estuarine zones. Freshwater has a lower pH than seawater. A pH gradient, from low values near a river mouth to high values in the open ocean, is a typical feature in estuarine zones. Associated with this gradient are gradients in nutrient concentration (Fig. 4) (Bianchi et al., 1999(b)), salinity (Bianchi et al., 1999 (b); Fulweiler et al., 2011), and in the nitrifying community (Fig. 5) (Bouskill et al., 2012). Recent studies have probed higher AOB abundances within well-oxygenated cold waters, with lower salinity, and higher environmental concentrations of nutrients (NH_4^+ and NO_2^-). On the other hand, AOA seem to be more abundant in high salinity conditions, under lower values of NH_4^+ and ppO_2 (Bouskill et al., 2012). Although there no pH analyses within Bouskill´s results, their results suggest that the nitrifying community within an estuarine zone is different from the pure marine ecosystem one due to pH changes and other special conditions (Bouskill et al., 2012). The estuarine community needs adapt to lower pH values, to lower salinity and to higher nutrient supplies than the marine community.

As a consequence of this, Fulweiler´s results in 2011 appear inconsistent with Beman´s assumptions in 2010, due to the different nitrifying communities adapted to different ecosystems. In pure marine waters, a decrease in pH may lead to a decrease in nitrification as

discussed above. This condition may be positive if it reduces ocean N_2O emissions; on the other hand, it may be negative if it reduces the vertical carbon flux.

In summary, nitrifying activity does not occur homogeneously in the ocean. The rates vary geographically, temporally, and vertically throughout the ocean water column. Many environmental impact nitrification to cause this variability on different temporal and spatial scales. It is important to consider this variability during field experiments as well as during data analyses.

The euphotic zone depth, the stratification, the trophic state of the study zone, the dissolved O_2 profiles, the possible nutrients supplies, or salinity gradients all must be taken into account if our understanding of nitrification and its community is to be improved. Furthermore, a deeper understanding of the ecological response of the ocean ecosystem to changes in temperature, pH or $ppCO_2$ is critical in the current scenario of a changing ocean, where changes in nitrifying activity and its distribution within the water column are likely to occur.

5. DEVELOPMENT COMPARISONS BETWEEN REPORTED ESTIMATIONS OF NITRIFICATION RATES

One of the aims of this study was to make a comparison between the distribution of nitrification and its impact on carbon cycle in different oceans by applying the hypotheses and findings disclosed in previous chapters on the current databases from: the Atlantic Ocean (Clark et al., 2008; Fernandez & Raimbault, 2007; Painter, 2011; Rees et al., 2006), Pacific Ocean (Beman et al., 2012; Dore & Karl, 1996; O'Mullan & Ward, 2005), Mediterranean Sea (Bianchi et al., 1999(a); M. Bianchi et al., 1997; M. Bianchi et al., 1999(b); Feliatra & Bianchi, 1993; Fernandez & Raimbault, 2007), Arabian Sea (Newell et al., 2011), Baltic Sea (Enoksson, 1986), and Red Sea (Mackey et al., 2011). While trying to achieve this objective, some problems with the databases have been found and these make it difficult to directly compare them (Table 2). It is revealing to examine some of these problems:

5.1 <u>Techniques for nitrification rates estimation and measurements.</u>

Many studies have estimated the nitrification activity by assessing the C-fixation. This technique uses ^{14}C-labelled inorganic compounds to measure the difference between total C-fixation and the C-fixation after inhibiting the nitrification activity. Then, a N-oxidation:C-fixation ratio is used to estimate the nitrification. Dore and Karl´s proposal in 1996, using Billen´s ratio and some conclusions from Feliatra and Bianchi´s publications, is an example of this kind of estimation (Billen, 1976; Dore & Karl, 1996; Feliatra & Bianchi, 1993).

The results from this approach have some biases:

a) Estimations via ^{14}C have a high standard deviation and that becomes the standard deviation of the nitrification estimations. It is usually about the same order of magnitude as the average values.

b) Dore and Karl´s ratio of 8.3 is a theoretical estimation, based on NH_4^+ oxidation. In addition, the N-oxidation:C-fixation ratio may vary, as has been discussed in previous chapters, due to the metabolic state of the community, its composition (NO_2^- oxidizers have a greater N-oxidation:C-fixation ratio than NH_4^+ oxidizers) and, probably, due to the variability of this ratio between different nitrifying microbial groups and even between different strains within these groups.

c) This method requires sample filtration. Commonly, 0,2 μm, or even 0,45 μm, filters are used to separate out the organic matter suspended in the sample. Part of the archaeal community could be as small as 0,1 μm size, so part of the nitrifying community is lost to the measurements.

Some of the studies considered here are based only on these measurements. These results are interesting for assessing trends in the nitrification distribution, but are not so useful for developing comparisons with data from other publications.

When ^{15}N isotope techniques are used, the standard deviation seems to be lower, possibly because the bias due to the filtration does not occur. These techniques are based on the evolution of dissolved N inorganic compounds in the water sample in which filtration is not employed. However, there are other special deficiencies:

a. When adding the high ^{15}N-labelled concentrations needed in the assay, the results yield a potential rate, not the actual (with the environmental ^{15}N-labelled concentrations) nitrification rate. It is important to consider this difference before comparing the results with other measurements.

b. When attempting to assess the actual nitrification rate, oligotrophic conditions make it particularly difficult due to the low concentrations of N-compounds in the environment.

To sum up, different studies appear to have equivalent results, but they are not. They cannot be compared because some are about the potential rate, and others about the actual nitrification rate.

Besides these techniques, there are other methodologies based on chemical assays. These measure the nitrification rates via the NO_2^- or NO_3^- released during an incubation period. In these assays the sensitivity of the measurement decreases in oligotrophic conditions, due to the lower environmental nutrient concentration. In addition to the ^{15}N isotope techniques, there are other ways to assess potential nitrification (employing nutrients saturation in the assay) as well as other ways to assess the actual nitrification rate (employing seawater nutrient concentrations). All these techniques are based on sample incubations, under unique conditions depending on the aim of the study (dark-light, day-night periods, under environmental nutrients conditions, under nutrient-saturation conditions, etc). Assays that required incubation have been discussed elsewhere, as in those related to the metabolic state of the ocean (Duarte et al., 2013; Ducklow & Doney, 2013; Williams et al., 2013). It is worth mentioning the bottle-effect, with its bias due to confinement. This problem impacts both photosynthetic autotrophic biomass (Calvo-Díaz et al., 2011), and heterotrophic biomass. It is accentuated by changing light conditions (Bender et al., 1987), as well as by shifting trophic condition of the water mass (Llabrés et al., 2011).

Finally, some studies have estimated nitrification rates via N_2O emissions and vertical fluxes. These results seem to be about the same magnitude order than other isotopic methods (Dore & Karl, 1996). Nevertheless, as discussed previously, the validity of this kind of estimation is limited to OMZ conditions, because the N_2O emissions are related to the archaeal nitrification under suboxic conditions.

5.2 <u>Nitrification phases.</u>

Within recent research on nitrification, three approaches may be found.

a) Independent NH_4^+ oxidation or NO_2^- oxidation assays, using different inhibitors of the two phases of nitrification and using different ^{15}N-labelled substrates.

b) NH_4^+ oxidation measurements, via the analysis of the N inorganic compounds evolution, usually by ^{15}N-labelled substrates (NO_2^- oxidation not measured.)

c) Total nitrification measurements, via the ^{15}N-tracer methods, or even the ^{14}C-fixation, matched with the use of N-oxidation:C-fixation ratios, that are mainly related to the NH_4^+ oxidation.

When analyzing and drawing conclusions based on these results from these three approaches it is important to consider the nitrification phase that is being assessed, otherwise mistakes will be made due to the comparison of incomparable results.

5.3 <u>Nitrification rates units</u>:

The most of the studies present their results in µmol L^{-1} d^{-1}, or nmol L^{-1} d^{-1} or the equivalent, µM d^{-1} or nM d^{-1}. In these cases, the issue is rather whether these nitrification rates are concerned about:

a) A specific depth.

b) An average between some samples from several depths.

c) A range between the results from different depths.

The accuracy in the vertical scale is especially significant while comparing different studies results. The number of sampling depths may vary, and the results may be more or less representative of the water column, depending on the case. Nitrification distribution varies with depth, as it has been discussed in previous chapters, so it is especially important to determine the depth of sampling with the aim of identify the main nitrification activity layer. Be able to identify this depth or do not, could lead to different final nitrification values, both in average, and in range.

On the other hand, some studies present their results integrated by depth, in nmol m^{-2} d^{-1}. Knowing the integrating depth is relevant, because these results are not directly comparable if the layer thickness or the depth range is not the same. Integrated nitrification rates from 20 to 40m are found within the bibliography, as well as rates from the seasurface to 175m. Knowing the maximum nitrification rates within the studied layer, its distribution, as well as the euphotic layer bottom or even the thermocline, is essential to understanding those integrated values and using them to calculate relevant and comparable results.

5.4 <u>Sampling Depths</u>:

Nitrification distribution and its impact within the euphotic zone are influenced by different environmental factors, as is said in chapter 3. Different criteria in determining the sampling depths have been used in the analyzed studies, based on different starting depths.

a) The 1%sPAR depth.

b) The 0,001% sPAR depth, because of the importance of the subeuphotic zone in nitrification activity under many conditions.

c) The Primary Chlorophyll-a Maximum.

d) The Primary Nitrite Maximum.

All of them are very similar, especially under eutrophic conditions, and well related to the nitrification activity. But, under oligotrophic conditions, the lower attenuation of the light intensity may separate them. Other factors such as stratification, or when relevant, the oxycline depth, need to be considered to determine the correct depth in assessing the nitrification activity.

5.5 <u>Spatial frequency of sampling depths</u>.

When it comes to characterizing nitrification, variability in the accuracy of published results needs to be considered. Comparison requires special attention to the sampling depths used.

Within the literature, studies based on only two relevant depths (for instance, samples from 55%sPAR and 1%sPAR depths) or, on the other hand, multiple depths, may be found. These may represent samples from within the euphotic zone, below it, or they may represent multiple values from an experiment tangent to nitrification. The accuracy, the representativeness, and the influence of these results in the improvement of the knowledge about nitrification are different, so obtaining significant conclusions from direct comparisons is complex.

5.6 <u>Temporal scale</u>.

Finally, although some of the studies allow seasonal trend estimations, most of them show results from the sampling stations only once a year. Therefore, these results may be from different seasons, or under different environmental conditions, in different oceans, adding to the difficulty in making direct comparisons.

From all this analysis we can conclude that the development of a common protocol must be followed in order to determine the most effective way to characterize the nitrification activity within the water column as well as to allow the generation of a directly comparable data base from different ocean basins.

Nitrification actitivity varies seasonally and temporally as does other biological processes. The development of a common protocol where the main influential environmental parameters were well characterized, could lead to a stronger data base. The results would be more comparable leading to a deeper understanding of the nitrification process.

Also, the development of a common simple measurement technique for nitrification, that would allow direct comparison between water samples and would help to define the impact of the nitrifying community on biogeochemical cycles, climate change, and the autotrophic-heterotrophic balance question.

References	Ocean	Trophic State	Nitrification phase	Rate Measurements	Filtration Mehods	Units	Depth	Vertical Resolution	Temporality
Painter, 2011	North Atlantic Ocean(Iceland basin)	Both eutrophic and oligotrophic due to dipole eddie system	Ammonium oxidation	^{14}C/allylthiourea inhibitor method x Dore and Karl N-oxidation: C-fixaton ratio	0,2 µm	nmol L^{-1} d^{-1}	Upper 125m	3 depths within the euphotic zone 3 depths within the aphotic zone	late summer 2007
Clark et al., 2008	Atlantic Ocean (from North Atlantic Drift Province to South Atlantic Gyral Province)	Both eutrophic (African Upwelling) and oligotrophic (Central gyres)	Ammonium oxidation Nitrite oxidation	^{15}N isotope dilution method in conjunction with sensitive gas cromathography/mass spectrometry methods	N/A	nmol L^{-1} d^{-1}	55%sPAR 1%sPAR	2 depths: 55%sPAR and 1%sPAR	september and october 2003
Rees et al., 2006	Atlantic Ocean between 60ºN and 50ºS	Both eutrophic (African Upwelling) and oligotrophic (Central gyres)	Ammonium oxidation	^{14}C/allylthiourea inhibitor method x Dore and Karl N-oxidation: C-fixaton ratio	0,2 µm	µmol L^{-1} d^{-1}	Upper 125m	2 depths within the euphotic zone, 3 depths within the aphotic zone	From UK to Falkland Islands in september and october 1997 From South Africa to UK in May and June 1998 North East Atlantic in June and July 1996
Fernández and Raimbault, 2007	NE Atlantic Ocean	Pre-bloom conditions	Total nitrification (more potential than actual activity)	^{15}N isotope dilution method in conjunction with mass spectrometry methods	N/A	µM d^{-1}	Upper 100m	8 depths	Winter (February to March) and Spring (March to May) 2001
Beman et al., 2012	Pacific Ocean: Gulf of California and Eastern Tropical North Pacific Ocean. OMZ	Eutrophic	Ammonium oxidation (more potential than actual activity)	^{15}N isotope dilution method in conjunction with mass spectrometry methods	N/A	nmol L^{-1} d^{-1}	Upper 200m Additional depth at 400m	1 to 3 depths above PNM and NH$_4^+$ maxima, 5 to 7 spaced every 5 m and several additional depths spaced every 10m below	July and August 2008
Beman et al., 2012	Pacific Ocean: Gulf of California and Eastern Tropical North Pacific Ocean. OMZ	Eutrophic	Ammonium oxidation (more potential than actual activity)	^{15}N isotope dilution method in conjunction with mass spectrometry methods	N/A	mmol m^{-2} d^{-1} integrated within above 1%sPAR	1%sPAR	1 to 3 depths above PNM and NH$_4^+$ maxima, 5 to 7 spaced every 5 m and several additional depths spaced every 10m below	July and August 2008
O´Mullan and Ward, 2005	Pacific Ocean: Monterrey Bay-California	Eutrophic	Total nitrification	^{15}N isotope dilution method in conjunction with mass spectrometry methods	0,2 µm for DNA assay	nmol m^{-2} d^{-1} integrated within the euphotic zone	Above 1%sPAR	3 depths through the euphotic zone	Bimonthly samples from February 1998 to October 1999: Seasonal pattern analyse allowed
Dore and Karl, 1996	Pacific Ocean (station ALOHA)	Oligotrophic	Ammonium oxidation When available, nitrite oxidation	^{14}C/allylthiourea inhibitor method x Dore and Karl N-oxidation: C-fixaton ratio Chemical assay: changes in NO$_2$ an NO$_3$ in abscense of light	0,2 µm	µmol m^{-3} d^{-1} near the bottom of the euphotic zone	From 100 to 175m	From 2 to 4 depths	October 1991 April 1992 October 1993
Dore and Karl, 1996	Pacific Ocean (station ALOHA)	Oligotrophic	Ammonium oxidation	N$_2$O production based estimations	N/A	mmol m^{-2} d^{-1} integrated within the upper 175m	Upper 175m	Sea-air and vertical N$_2$O fluxes	September 1992 October 1992 February 1993 October 1993 September 1994

References	Ocean	Trophic State	Nitrification phase	Rate Measurements	Filtration Mehods	Units	Depth	Vertical Resolution	Temporality
Dore and Karl, 1996	Pacific Ocean (station ALOHA)	Oligotrophic	Ammonium oxidation Nitrite oxidation	Chemical assay: changes in NO_2 an NO_3 in abscense of light	N/A	mmol m^{-2} d^{-1} integrated from 100 to 175m	From 100 to 175m	From 2 to 4 depths	October 1993
Newell et al., 2011	Arabian Sea	Eutrophic-OMZ	Ammonium oxidation	^{15}N isotope method with and without ampicillin to meassure the contribution of AOB	N/A	A range, in nmol L^{-1} d^{-1}	Upper 135m and a deep one: between 900 and 1500m	3 depths: PNM, Oxycline at the top of OMZ, below the lower boundary of the OMZ Additional depth within the OMZ core	Monsoon/Intermonsoon transition in September and october 2007
Bianchi et al, 1999-a	Mediterranean Sea	Oligotrophic and Mesotrophic	Ammonium oxidation Nitrite oxidation	Chemical assay: changes in NO_2 with and without allylthiourea	N/A	nM d^{-1}	Below the peak of the in situ fluorescence to 150m or 250m	3 depths below the peak of in situ fluorescence. 1 addition depth within the Rhône Plume	Spring (April, May and June) 1995
Bianchi et al, 1999-b	Mediterranean Sea: Rhône River Plume: Proximal waters to the river mouth, distal and seawater samples	Eutrophic	Ammonium oxidation Nitrite oxidation	Chemical assay: changes in NO_2 with and without allylthiourea and $NaClO_3$	N/A	nM d^{-1}	From surface to bottom (max depth 90m)	Surface 1,5m 10m bottom	Monthly from November 1991 to October 1992: Seasonal pattern analyse allowed
Feliatra and Bianchi, 1993	Mediterranean Sea: Rhône River Plume: Salinity gradient	Eutrophic	Ammonium oxidation Nitrite oxidation	Chemical assay: changes in NO_2 with and without allylthiourea and $NaClO_3$ ^{14}C-allylthiourea and $NaClO_3$ inhibitor method	0,2 µm	µM d^{-1}	Surface	1 per station	May 1992
Bianchi et al, 1996	Indian Sector of the Southern Ocean	Eutrophic	Ammonium oxidation Nitrite oxidation	^{14}C-allylthiourea and $NaClO_3$ inhibitor method	0,2 µm	mmol m^{-2} d^{-1} integrated within the upper 100m	Upper 100m	Surface 50m 100m	April-May 1993
Enoksson, 1985	Baltic Sea	Eutrophic OMZ	Total nitrification	^{14}C- Nserve inhibitor	0,45 µm	NO_2 + NO_3 µmol L^{-1} d^{-1}	Upper 100m	6 depths in 10 to 30m intervals	June 1982 and November 1980
Enoksson, 1985	Baltic Sea	Eutrophic OMZ	Total nitrification (more potential than actual activity)	^{15}N ammonium oxidation isotope method by emission specrometry by the method of Fiedler and Proksch	N/A	NO_2 + NO_3 µmol L^{-1} d^{-1}	Upper 130m	7 depths in 10 to 30m intervals	June 1982 and November 1980
Enoksson, 1985	Baltic Sea	Eutrophic OMZ	Total nitrification	^{15}N nitrate dilution isotope method	N/A	NO_2 + NO_3 µmol L^{-1} d^{-1}	Upper 130m	7 depths in 10 to 30m intervals	June 1982 and November 1980
Mackey et al., 2011	Red Sea	Oligotrophic (stratification)	Ammonium oxidation	^{15}N tracer experiment	N/A	nmol L^{-1} d^{-1}	Surface	Surface water (1m)	Monthly from January to December 2003 and January to December 2008. Seasonal pattern analyse allowed

Table 2: Characterization of the current nitrification data bases.

6. SUMMARY

A new conceptual model of nitrification has been developed from the latest research. The main trends in the distribution and the defining parameters of oceanic nitrification are identified here.

A. Stratified oligotrophic seawater: Contrary to previous understanding, the highest NH_4^+ oxidation rates are within the euphotic zone. NO_2^- oxidation also begins to increase within the euphotic zone.

B. Stratified eutrophic seawater: Here NH_4^+ oxidation rates are highest in the subeuphotic zone. NO_2^- oxidation begins to increase within this zone too, below the euphotic zone.

C. Shallow Upwelling: NH_4^+ and NO_2^- oxidation maintain activity levels within the mixed layer, independent of the light. Below the deep thermocline, NH_4^+ oxidation decreases, but NO_2^- oxidation increases.

D. Deep Upwelling with OMZ. Within the mixed layer, the nitrification profile is the same as in the shallower upwelling case. At depth, the NH_4^+ oxidation peaks at the beginning of the OMZ. Below this peak, the NO_2^- oxidation begins to increase.

This new model determines the conditions where the nitrification is high within the euphotic zone where it would make a special contribution in the upper ocean autotrophy. Note that this influence is significant in an oligotrophic and stratified ocean. Nitrification fixes CO_2 and consumes O_2, so it has to be considered in assessing the metabolic state of the ocean, particularly from ppO_2 measurements. Moreover, nitrification and N-fixation in the euphotic zone impacts the New Production and Regenerated Production concept of Dugdale and Goering (1967). Both concepts need to be redefined. Also, the f-ratio of Eppley and Peterson (1979) is influenced by the magnitude of the euphotic nitrification, according to Yool´s

publication. New Production is specially overestimated under oligotrophic conditions because of nitrification.

The ocean distribution of nitrification varies temporally due to the influence of environmental factors. Light, temperature, and ppO_2 have to be considered while planning sampling programs. A new protocol in sampling planning should be developed taking in account this new conceptual model.

Nutrients, salinity, temperature and pH gradients influence the nitrifying community's composition and its metabolic state. This is especially significant under estuarine conditions, where the nitrification profiles are different from those in the open ocean.

Currently, direct comparison between reported nitrification rates is difficult due to the variability in methodologies and sampling. This difficulty increases when the goal is to estimate the influence of nitrification on the carbon cycle. Both the carbon fixation and the biases in ppO2 measurements need to be considered. The development of a new nitrification assay, that would allow direct comparison between water samples and would avoid the incubation process, will improve our understanding of nitrification, the impacting environmental factors, and the different organisms involved.

Future research

1. Calculate, using this new conceptual model, the quantitative influence of nitrification on the carbon cycle in the open ocean.

2. Develop an enzymatic assay of nitrification, as outline in the Innova Canarias 2020 scholarship Program. This will allow measurements of potential nitrification in water samples.

3. Application of this new conceptual model to an ocean field program to improve the assessment of nitrification rates, in Canary Island waters.

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