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Abstract

Nitrate, phosphate, and silicate concentration profiles were measured at monthly frequency at the DYFAMED time-series station (central Ligurian Sea) between 1991 and 2011. The resulting data set, which constitutes the longest open-ocean time-series in the Mediterranean Sea, underwent quality control. A reproducible climatological pattern was observed with an

unprecedented resolution, confirming the typical seasonal cycle of mid-latitudes. In summer and autumn, when the water mass is well stratified, *i.e.* the mixed layer depth (MLD) is shallow, nutrient concentrations in surface are very low or under the detection limit. In winter, as a result of the MLD extent, nutrients are supplied to the surface layer. Then, nutrient concentrations progressively decrease during spring. MLD appears to play a key role in controlling nutrient availability in the surface layer, but a direct, quantitative relationship between MLD and nutrient concentrations is difficult to establish due to undersampling. Regarding nutrient molar ratios (N:P, Si:N, and Si:P), results show anomalous values compared to those of other oceanic regions, presumably due to strong influence of external sources. As a consequence, nutrient molar ratios exhibit a seasonal pattern, with, in particular, an increase of the N:P ratio in condition of stratification. Over the period 1991-2011, the DYFAMED data set reveals decadal trends in nitrate and phosphate concentrations in deep waters (+0.23% and -0.62%, respectively) resulting in increasing N:P and Si:P ratios (+1.14% and +0.85% per year, respectively). Such a long-term variability is presumably related to changes in water mass and/or changes in external sources, even if is difficult to assess due to not enough concomitant data from atmospheric and riverine inputs.

1. Introduction

The distribution of nutrient concentrations is driven by a complex combination of biotic (*i.e.* phytoplankton production, excretion and remineralisation) and abiotic forcing (upwelling, thermohaline circulation, atmospheric and terrestrial inputs). Among the abiotic factors, the mixed layer depth (MLD, which indicates on the depth reached by turbulent mixing in a recent past; Brainerd and Gregg 1995; Kara et al. 2003; de Boyer Montegut et al. 2004) is probably the most important. MLD variability has important repercussions on nutrient distribution, as it is known to play a key role in controlling the nutrient re-distribution in the

water column and, therefore, driving their availability to phytoplankton (Mann and Lazier 1996). In temperate regions, primary production is critically controlled by nutrient availability, which, in turn, is strictly dependent on MLD dynamics (Obata et al. 1996; Wilson and Coles 2005).

Compared to other temperate seas, the Mediterranean Sea exhibits extremely low nutrient concentrations (McGill 1965; Krom et al. 1991), with a significant geographical variability characterised by a west-to-east gradient (Pujo-Pay et al. 2011; Moutin and Prieur 2012). At climatological scale, the gradient is often considered as a consequence of the anti-estuarine circulation of the basin (Crispi et al. 2001). Few nutrients available in the Atlantic surface inflow entering the Mediterranean Sea are consumed along the way to the eastern basin, and exported to deep water (Crise et al. 1999; Lazzari et al. 2012). A major part of these nutrients do not cross the Sicilian strait and are stored into the western basin. It results in an outgoing Mediterranean nutrient-enriched water flow at Gibraltar, and a loss of nutrients (10% for N and P, and up to 50% for Si; Durrieu de Madron et al. 2011). A compensation from external sources is commonly postulated (Béthoux et al. 1998; Ribera d'Alcalà et al. 2003).

Another specificity of the Mediterranean Sea is the anomalous value of nutrient molar ratios, compared to those of other oceanic regions (Herut et al. 1999; Kress and Herut 2001; Pujo-Pay et al. 2011; Ribera d'Alcalà et al. 2003). In particular, N:P ratios in the stocks are high, compared to the typical value of 16, but the Redfield model (Redfield 1934) may not be relevant for semi-enclosed seas such as the Mediterranean (Takahashi et al. 1985; Geider and La Roche 2002; Wang et al. 2013). Basically, it is explained either by the strong influence of external sources, which are generally N-enriched, relative to P (Krom et al. 2004; Bartoli et al. 2005; Markaki et al. 2010; Ludwig et al. 2009), or to dinitrogen fixation (Béthoux et al. 1998; Sachs and Repeta 1999; Pantoja et al. 2002), although this process might not be significant in the Mediterranean Sea (Ibello et al. 2010; Sandroni et al. 2007; Yogev et al. 2011).

Consequently to this high N:P ratio, it is commonly admitted that phytoplankton and heterotrophic bacteria in the western basin are limited by phosphate (PO_4^{3-}) during the stratification period (e.g., Thingstad et al. 1998; Migon and Sandroni 1999; Marty et al. 2002), while phytoplankton growth in the euphotic layer is limited by N during the spring bloom period (e.g. Béthoux et al. 1992; Marty et al. 2002; Durrieu de Madron et al. 2011).

From this point of view, the northwestern Mediterranean area plays a crucial role in the functioning of the whole basin. In winter, submitted to a strong physical forcing (due to the action of cold and dry winds, combined with low temperatures) surface waters are cooled and their evaporation is enhanced, increasing their salinity. This regularly initiates the process of convection, which conducts, sometimes, to dense water formation events (Marty et al. 2002; Millot and Taupier-Letage 2005; Marty and Chiaverini 2010). Strong MLD events drive the thermohaline circulation of the whole northwestern Mediterranean (MEDOC Group 1970), and also contribute to re-distribute nutrients within the water column. The role of intense convection events (and MLD in general) on the Mediterranean nutrient distribution is still debated. Modeling exercises (Crise et al. 1998; Crise et al. 1999) identify deep convection events as the primary factor in determining the seasonal nutrient availability in the surface layer. Recently, autonomous platforms smoothed this statement by observing a nitrate increase two months before the deep convection event (D'Ortenzio et al. 2014). Only based on a one-year sampling, the results obtained from autonomous platforms confirmed, however, that only a seasonal monitoring may shed light on the interactions between MLD and nutrient distribution in a complex area such as the northwestern Mediterranean.

However, despite several studies already carried out in the Mediterranean Sea (e.g. Béthoux et al. 1998; Kress and Herut 2001; Marty et al. 2002; Ribera d'Alcalà et al. 2003; Krom et al. 2004; Durrieu de Madron et al. 2011), seasonal and inter-annual dynamics of nutrient concentrations remain poorly known. In this context, measurements from the DYFAMED

time-series sampling station (located in the northwestern Mediterranean area) constitute an invaluable data set to study evolving Mediterranean biogeochemistry. The present paper firstly proposes to address the climatological pattern of the seasonal cycle of nutrients and molar ratios. Our results were directly compared to the pioneer study of Marty et al. (2002), who worked on part of the same time-series: we analysed more deeply the link between nutrient seasonal variability and abiotic forcing. In addition, the seasonal shift from N-limitation during winter and spring to P-limitation during the oligotrophic period, observed by Marty et al. (2002) was re-examined. In the second part of the paper, we also analysed longer time scales, by exploiting the 21-year data collected at DYFAMED. It is likely that rapid environmental, climatic and meteorological changes around the Mediterranean basin are capable of altering the stocks and the molar ratio of nutrients at decadal time scales (Turley 1999; Marty and Chiaverini 2010). Due to relatively reduced dimensions of the basin (2.51 x 10^6 km^2) and very short residence time of Mediterranean waters (50-100 years; Millot and Taupier-Letage 2005), compared to those of the global ocean (500–1000 years), the Mediterranean area could be then considered as a "sentinel" to identify the effect of climate change on ocean biogeochemistry (Siokou-Frangou et al. 2010). In this paper, we addressed more specifically, and for the first time in the western Mediterranean, the decadal evolution of nutrient concentrations, in relation to environmental variables (MLD, external inputs etc.).

2. Experimental

2.1 Site description

The DYFAMED site is located in the central Ligurian Sea (28 nautical miles off the continental French coast, 2350m deep, 43°25'N, 7°52E; Fig. 1). Cruises were conducted every month, apart from impossibility due to bad meteorological conditions or ship maintenance. CTD casts were obtained on each cruise (Marty and Chiaverini 2010).

Additional data were collected in 2004 during nine MELISSA (MEditerranée LImitationS SAisonnières) cruises, in January and February, and from April to October. The DYFAMED station is surrounded by the permanent geostrophic Ligurian frontal jet flow that results from the cyclonic circulation of the Northern Current (Millot 1999). This front creates a band (~ 30 km width, more than 250m depth) that presumably separates the sampling area from coastal lateral inputs by a strong horizontal density gradient (Niewadomska et al. 2008). The Northern Current also isolates the sampling area from direct riverine inputs, such inputs being in addition particularly poor in the Ligurian Sea (Migon 1993). Episodic, infrequent intrusions of waters from the Northern Current may occur in winter (Millot 1999). However, the site is believed minimally affected by lateral inputs. Acoustic Doppler Current Profiler (ADCP) measurements have shown that the DYFAMED station is sheltered from the Northern Current (Andersen and Prieur 2000). Three major water masses are evidenced in the area (Béthoux et al. 1998; Millot 1999; Marty and Chiaverini 2010): the surface water (~ 0-200m-depth), strongly affected by local climatic conditions (Schröder et al. 2006), the subjacent layer (approximately between 200m and 600m depth at the DYFAMED site) characterised by maximum temperature and salinity due to the intrusion of Levantine Intermediate Water (Millot 1999; Marty and Chiaverini 2010), and the deep layer characterised by low salinity, and low temperature.

2.2. Mixed layer data

Temperature and salinity profiles from 1991 to 2011 were post-calibrated (using the standard Sea-Bird Electronics method) and all spikes and questionable data were removed from the data set. The potential density was calculated using the Thermodynamic Equation Of Seawater - 2010 (TEOS-10).

The intensity of mixing was assessed through the variation of the MLD. Here, the MLD was calculated as the depth where the difference of density from the surface reference, fixed at 10m-depth, is 0.03 kg m^{-3} (de Boyer Montegut et al. 2004; D'Ortenzio et al. 2005). Density profiles were calculated from temperature and salinity profiles and linearly interpolated at 1m-depth interval. If no observation was available at 10m, the shallowest measurement was used as the surface reference if it was above 20m, otherwise MLD was not computed.

2.3 Nutrient analysis

From 1991 to 2011, time-series nutrient concentrations have been measured at the DYFAMED site. Nutrient samples were collected using 12 clean 12L Niskin bottles (General Oceanics 1010X) mounted on a carrousel water sampler (SeaBird SBE32) with CTD sensors (Seabird SBE911). The bottles were generally closed at 12 depths (10, 30, 50 or Deep Chlorophyll Maximum, 80, 120, 200, 300, 500, 800, 1000, 1500 and 2000m), and samples were transferred to 60mL polyethylene flasks. These samples were poisoned with 100μL HgCl$_2$ (*i.e.*, 20μg L^{-1}; Kirkwood 1992), and stored in a dark room at 5°C until analysis (until 2009), or placed directly to a freezer (from 2009 to 2011). After Dore et al. (1996), low inorganic nutrient concentrations (basically oligotrophic seawater) can be adequately maintained for several months if samples are frozen immediately after collection. Only low concentrations of phosphate may be affected by the freezing process. However, the consequence is to increase variability, but not to bias the result in one direction or the other. Furthermore, the effect on phosphate concentration is small, only detectable using sensitive low-level techniques, and would be included in the normal analytical variability of standard techniques (Dore et al. 1996). The same applies to nitrate concentrations. Samples with silicate concentrations >120 μM may also be biased by freezing, due to silicate polymerisation, but this does not apply to the concentration range of the present data set.

All nutrient samples were analysed by standard automated colourimetry system, using a Technicon AutoAnalyser II (from 1991 to 2008) and since 2009 with a Seal Analytical continuous flow AutoAnalyser III (AA3) at the Observatoire Oceanologique de Villefranche-sur-Mer. Nitrate (NO_3^-) ions were analysed according to the protocol of Bendschneider and Robinson (1952) with a detection limit of 0.05μM and 0.01μM (since 2009). Phosphate (PO_4^{3-}) ions were analysed according to the protocol of Murphy and Riley (1962) with a detection limit of 0.02μM. Silicate ($Si(OH)_4$) ions were analysed according to the protocol of Strickland and Parsons (1972) with a detection limit of 0.05μM and 0.02μM (since 2009). For the present work, the total number of raw data was 3579 (nitrate), 3290 (phosphate), and 3699 (silicate).

2.4. Quality control of nutrient data

Owing to the strong scattering of results, caused by various types of possible errors (uncertainty on measurements, approximations, change of operator, etc.), nutrient data underwent a quality control. As a first step, data were pooled per month to avoid biases due to seasonal variations. Then, data were pooled by depth class (Table 1), in order to get homogeneous data within each class (surface water was typically better sampled than deeper water). For each depth and each nutrient, a central trend and a scattering index were computed. The median has been selected as the central trend, because it is statistically more robust and little influenced by outliers compared with, e.g. the mean, and the median absolute deviation (MAD) was used to measure the scattering of data. For a given nutrient, and for each depth, MAD is computed as follows:

$$MAD = mdn(|x_i - mdn(x_i)|) \qquad (1)$$

where mdn is the median, and x_i the ensemble of measured concentrations within a given depth class. To render MAD a consistent estimator for the estimation of the standard deviation (σ), a scale factor b is defined as:

$$\sigma \simeq b.MAD \qquad (2)$$

b is determined from the quantile function of the x_i, and is usually taken to be 1.4826 for normally distributed data. From the above, 1.4826.MAD is approximately equal to the standard deviation. Then, for each nutrient, a median profile and confident intervals have been determined. Measurements are considered as wrong if

$$|x_i - mdn(x_i)|/(b.MAD) \geq 3 \qquad (3)$$

Measurements are considered as questionable if

$$3 \geq |x_i - mdn(x_i)|/(b.MAD) \geq 2 \qquad (4)$$

Measurements are considered as good if

$$|x_i - mdn(x_i)|/(b.MAD) \leq 2 \qquad (5)$$

After the quality control processing and considering only data above detection limit, 87%, 86%, and 90% of the data for nitrate, phosphate, and silicate, respectively, were considered as good data. It should also be mentioned that values available once the quality control process is applied are not necessarily correct, they are just considered as non-aberrant. This procedure

may delete some low-frequency extreme events, but it result in a limited effect on the study of seasonal processes and decadal trends. In the following, only data that fulfill equation (5) are taken into account. Distribution of sample number per year and per month is given in Fig. 2. Years 1991 and 2004 were well sampled due to the monthly double casts performed at the DYFAMED site (1991) and to additional data from the MELISSA project (2004). In 2009-2011, nutrient samples were stored in a freezer instead of using poison ($HgCl_2$), and analysed at the Marseilles Institute of Oceanography. This change in sample preservation could explain the lower quality of analyses, hence the lower number of data from this period, once the quality control was performed. At monthly scale, the sampling was fairly regular, August being the least regularly sampled (annual maintenance of the ship). Raw DYFAMED and MELISSA nutrient concentration data, and the associated controlled values, can be found at ftp://oceane.obs-vlfr.fr/pub/laurcopp/

3. Results and Discussion

3.1. MLD dynamics

At climatological scale, MLD exhibits the typical pattern of temperate seas (Fig. 3a). In autumn and winter, deepening of the MLD is observed, with maximum median values around 70m-depth in January and February. This period is characterised by strong scattering of MLD values (Fig. 3a), and MLD deeper than 100m-depth are often observed. In spring, there is a gradual increase of surface density, leading to a progressive stratification of the water column. Consequently, the MLD becomes thinner and rarely exceeds 60m (median values around 30m in March, falling around 10m in May). During summer, MLD values and scattering are minimal, indicating low inter-annual variability. Our data confirm the climatological patterns already described in the past (Marty and Chiaverini 2010; Heimbürger et al. 2013). MLD time-series (Fig. 4a) also confirm a strong variability in winter, at inter-annual scale. In

particular, years 1999, 2000, 2005 and 2006 were characterised by strong MLD, *i.e.,* deeper than 300m (Fig. 4a). In particular, the MLD was very intense in winter 2006, with values greater than 2000m, which was observed for the first time at DYFAMED, and evidenced using modelling approach (Heimbürger et al. 2013). Conversely, from 2006 to 2011, no significant events were measured at the DYFAMED station. Over this period, the MLD never exceeded 200m-depth.

The analysis of MLD time-series is particularly problematic when winter events are analysed. Median values of MLD deduced from monthly cruises are underestimated because mixing events can be shorter than the temporal interval of the measurements. Moreover, cruise sampling are cancelled when extreme weather conditions occur (*i.e.,* during convection). In the following, we will discuss the possible impact of these results, by comparing them with nutrient data. Despite this limitation, MLD data at DYFAMED provide enough information to improve our comprehension of nutrient dynamics, which will be described in the next section.

3.2. Seasonal variability of nutrient concentrations

Composite years built from 1991-2011 data for each nutrient (Fig. 3b-d) exhibit clear seasonal patterns in relation to physical processes (Fig 3a). Contrasted seasonal situations may be outlined in the surface layer, where maximum variability is generally observed (Béthoux and Tailliez 1994), in relation to nutrient dynamics. From December to February, when maximum values of MLD are observed, nutrients accumulated in depths are brought to the surface layer, and increasing concentrations along the first one hundred metres are observed. Median concentrations range around 2.70 μM (nitrate), 0.13 μM (phosphate), and 2.0 μM (silicate) (Fig. 3 and Table 2). In spring, concomitantly with a progressive stratification of the water column, one can observe a decrease in nutrient concentration in the surface layer. Median values of nitrate concentrations decrease from 2.70 μM in February to 0.28 μM in May (from

0.14 µM to 0.04 µM for phosphate). This decrease is less apparent for silicate (Fig. 3 and Table 2). Summer period typically starts in June-July. During this period, the water column is stratified (MLD is minimal), and depleted surface waters are isolated from underlying layers (Marty et al. 2002; D'Ortenzio and Prieur 2012). Nutrient concentrations are always <1 µM along the 0–50m-depth water column, and close to (or under) the detection limit in very oligotrophic situations (Fig. 3 and Table 2). The thickness of the depleted surface layer progressively increases. During autumn, the MLD is, on average, still shallower than the depleted layer, and not yet important enough to allow the homogenisation of the water column (Fig. 3a). Depleted layers are roughly at their deepest level, reaching 70m-depth for nitrate concentrations, and 90m-depth for phosphate concentrations (Fig. 3). During this period, wind episodes may partly break the stratification of the water column and, therefore, allow the injection of nutrients in surface waters (DiTullio and Laws 1991; Andersen and Prieur 2000). However, no nutrient concentration increase was observed in surface from the present data set. This is presumably due to the monthly frequency of the sampling, which does not allow to observe short scale variations before November, when destratification is actually observed, on average (Fig. 3). Nutrients are re-injected in surface water once the MLD exceeds the depth of the depleted layer, which occurs typically in winter (Fig. 3 and Table 2).

This annual cycle is characteristic of the northwestern Mediterranean, despite possible inter-annual variations. A two-way ANOVA indicated a significant influence of seasonality on nitrate, phosphate and silicate surface concentrations (P-value <0.01), relative to inter-annual variability. Detailed results of the ANOVA are given in Annex. Below 200m-depth, no clear seasonal variability can be observed (Table 2). Whatever the nutrient, intermediate and deep water appear insignificantly affected by seasonal variations. Median values of nutrient concentrations in intermediate and deep water (Table 2) are relatively constant whatever the season (around 8 µM for nitrate, 0.3 µM for phosphate, and 7 µM for silicate), and

characterised by low deviation. However, as the DYFAMED zone is an area where deep water formation occurs, one would also expect seasonal changes in deep water concentration. The relatively small variations observed among concentration profiles are more likely due to natural fluctuations (inter-annual variability, change in water mass, etc.), and hide the potential impact of seasonality on deep nutrient concentration. In fact, assuming a shift from an extreme summer condition (surface water depleted from 0 to 100m-depth) to a theoretically rectilinear profile in winter, the expected change in deep water would be around 0.3 µM for nitrate concentrations, which is generally below the standard deviation associated with intrinsic variability due to physical processes and the whole procedure to determine nutrient concentrations (Table 2). In addition, the readjustment of deep nutrient concentrations following these events might be so rapid that it is not possible to detect concentration variations through monthly sampling. A two-way ANOVA confirms that seasonality has no significant effect on nutrient concentrations in intermediate and deep water in the present data set (see Annex).

The MLD cycle largely constrains nutrient concentrations in surface layer but a direct relationship between MLD magnitude and nutrient concentrations may be complex and not linear (Fig. 4b-d). It is clear that deep MLD, generally observed in winter and early spring, induces high nutrient concentrations (Box 3 on Fig. 4b-d). On the contrary, shallow MLD are associated with very low nutrient concentrations (Box 1, summer situation). Intermediate MLD (ranging from 20 to 100m-depth; Box 2) are observed in autumn, spring and winter, and have different effects depending on the season. In autumn, whatever the depth reached by the MLD, nutrient concentrations remain low (MLD shallower than the depleted layer). However, it is striking that high nutrient concentrations can be observed in winter and spring even though MLD values are not exceptionally great. In other words, deep convection, which is often believed to trigger nutrient supply to the surface layer, is not necessarily needed to

observe high nutrient concentrations. Such a situation (shallow MLD and high nutrients concentrations) was already observed in the past (D'Ortenzio et al. 2014; Manca et al. 2004), and related to the cyclonic circulation of the northwestern Mediterranean that uplifts the nutrient deep reservoir (Crise et al. 1999). However, this statement should be moderated, due to the monthly sampling. Indeed, observed nutrient concentrations in surface result from the past weeks of MLD dynamics and do not directly emerge from the MLD measured at the time of the sampling. The duration of winter mixing events should be taken into account, but this point cannot be here elucidated any further.

3.3. Seasonal variability of molar ratios

To avoid errors in the computation of molar ratios (calculated as the quotient of two elemental concentrations), essentially when nutrient concentrations are close to the analytical precision, the theoretical relative error was calculated from the partial derivative method:

$$\frac{\Delta R}{R} \sim \left[\frac{|\Delta N_1|}{N_1} + \frac{|\Delta N_2|}{N_2} \right] . 100 \tag{1}$$

where R is the ratio between the two elements N_1 and N_2, and ΔN_1 (resp. ΔN_2) the uncertainty on the measurement of N_1 (resp. N_2). Then, $\Delta R/R$ is the relative error on the ratio. Ratios with an error greater than 30% were therefore discarded. For example, results for N:P ratio showed that, in surface (< 50m-depth), the mean relative error value is around 75%, and can reach more than 130% in summer, when NO_3^- and PO_4^{3-} concentrations are low (assuming an uncertainty on nitrate and phosphate measurement in the order of 0.02 µM; Aminot and Kerouel 2007). In comparison, the maximum relative error under 200m-depth never exceeds 10%. This results in two types of bias: scattering of the data and convergence towards low values in surface (Fig. 5), those values being generally considered not reliable (e.g. Krom et

al. 1991; Marty et al. 2002). The number of N:P ratio values considered as not reliable (error greater than 30%) represents 10.5% of the whole data set, and 75% of these questionable values lie above 50m-depth.

The DYFAMED data set was splitted into four calendar-based seasons: Winter (December-February), spring (March-May), summer (June-August), and autumn (September-November). Seasonal median profiles were obtained by calculating median values into 17 depth classes (Table 1). All year long, ratios are constant in deep and intermediate layers, and the scattering of the data is low. Median values are around 20:1 for the N:P ratio, slightly below 1 for the Si:N ratio, and around 19 for the Si:P ratio (Fig. 6). As for nutrient concentrations, seasonal changes are detected in the surface water. In winter, when the process of vertical mixing occurs, the N:P ratio exhibits a quasi-rectilinear median profile, from the bottom to the surface. The relative abundances of nutrients in surface water mirror the nitrate-to-phosphate ratio in the nutritive material stored in depths. Strong variability is observed in the surface water (down to 200m-depth), with N:P values equivalently distributed around the median. It results in a T-shaped envelope of points, centred on 20:1 (Fig. 6a). In spring, the N:P ratio remains constant, as for winter situation. In summer, as soon as the stratification of the water column develops, the N:P ratio in surface evolves towards higher values (median value around 30, with possible maxima up to 70). Autumn may be viewed as the continuation of the summer period. During this period, the water column is still stratified, and N:P ratio values are maxima (Fig. 6a).

Marty et al. (2002) obtained similar results with respect to N:P ratio and inferred there is a N-limitation during winter and spring (because all the N:P ratio values below 20 were observed during this period). We recommend caution when addressing the complex issue of the limiting factor since it depends on both phytoplankton physiology and resource competition (Klausmeier et al. 2004). First, in winter, both nitrate and phosphate are abundant in the

surface layer (Fig. 3b-d), suggesting that nutrients are probably not the limiting factor. Then, the T-shaped envelope of points observed in both winter and spring (Fig. 6a) is characteristic of ideal (*i.e.*, non-fractioned) situation of nutrient co-variation (see figure 1 in Fanning 1992). This suggests that the net removal of nitrate and phosphate by biota occurs in proportions close to the molar ratio in the stock (around 20:1), with alternation between periods of N-depletion, relative to P (right portion of the T) and periods of P-depletion, relative to N (left portion of the T). In any case, this suggests that N and P, overall, may play a similar role in the control of phytoplankton growth in blooming conditions. Therefore, N does not appear clearly as the limiting factor in winter and spring. By contrast, high values of N:P ratio observed in summer and spring suggest an incomplete removal of nitrate once all phosphate is consumed by phytoplankton (typical of P-limited oligotrophic configurations). With respect to Si:N and Si:P ratios, we observe increasing values from spring to autumn because surface water becomes strongly depleted in nitrate and phosphate compared to silicate (Fig. 3b-d). It is noteworthy that profiles of N:Si ratio and, more markedly, profiles of Si:P ratio display a permanent curvature in the intermediate layer. Indeed, silicate concentration profiles exhibit a different shape, compared with those of nitrate and phosphate: $Si(OH)_4$ concentrations increase progressively from surface to depth, whereas nitrate and phosphate concentration profiles are generally characterised by a strong gradient between the depleted layer and the enriched deep layer. The behaviour of Si is different from that of other nutrients insofar as its recycling is slow, because it essentially depends on the dissolution of diatom frustules (Kamatani 1982; Tréguer et al. 1989). Even if our results suggest that, basically, Si does not play any role in the limitation of primary production, the profiles recorded on Fig. 6b and 6c do not permit to discard the potential episodic control of diatom production by Si during the early bloom period, as suggested by Marty et al. (2002).

3.4. Decadal trends in nutrient concentrations in deep layer

Decadal variability of nutrient concentrations and molar ratio was studied by considering only samples acquired at depth (below 800m), where the variability is the smallest (Béthoux and Tailliez 1994). Trend analysis was conducted using the non-parametric Mann-Kendall test (Mann 1945; Kendall 1975), which determines whether a time-series exhibits a monotonic pattern. To support the findings, regression analysis was also performed. According to both of these tests, the null hypothesis assumes that there is no trend, and this is tested against the alternative hypothesis that assumes that there is a trend. The non-parametric Pettitt test (Pettitt 1979) was applied to the de-trended data (Vincent et al. 2011; Bates et al. 2012) to find a significant break in the data series. The null hypothesis of stationarity was tested against a shift in the average value. This test provides the indication of when a potential break point occurs. All tests were run at 5% significance level.

Over the 1991-2011 period, increase of nitrate concentration, and decrease of phosphate concentration were observed in deep waters (Fig. 7), and were statistically significant (p-values <0.05; Table 3). The slopes of the regression line show an increase of 0.23% and a decrease of 0.62% per year on average for nitrate and phosphate concentrations, respectively. By contrast, silicate concentrations do not exhibit any trend over the considered period (p-value >0.05). The Pettitt test also shows that abrupt changes were detected in 2005-2006 in all time-series (*i.e.*, nitrate, phosphate and silicate), characterised by a slight but significant increase in nutrient concentration (Fig. 7 and Table 3). A similar analysis was achieved for molar ratios (Fig. 7 and Table 3). According to the trends displayed for nutrient concentrations, we observed a significant increase by 1.14% per year for N:P deep molar ratio, which represents an increase of 4.2 in the value of N:P ratio over the 20-year period. In the same way, the increase of Si:P molar ratios (0.85%) is mainly due to the decrease of phosphate concentration. Si:N ratio does not show any trend, even if a slight increase was

observed in 2005-2006. No tipping points were detected at significant level ($p < 0.05$) in N:P and N:Si time-series, probably because all nutrient concentrations sharply increased in the same time, *i.e.,* around 2005-2006.

Compared with the historical study performed by Béthoux et al. (1998) (nitrate and phosphate increased by 0.56 and 0.53% per year, respectively, over the 1961-1994 period, while silicate concentrations remained stable), the present study shows a slowing down of nitrate concentration increase by a factor of two, and a reversal of the trend for phosphate concentration. Several reasons may account for the differences in nitrate and phosphate trends.

(1) The statistical analysis realised by Béthoux et al. (1998) may not be directly comparable to our work. Historical data were acquired upon low frequency sampling and the spatial variability was not taken into account (e.g. gathering data from Ligurian and Alboran Seas).

(2) Changes in external sources (atmospheric and riverine inputs) may have occurred. Particularly, an increase in nitrate flux in rivers was observed from the 1970s to the 1990s, before remaining stable in recent years (Ludwig et al. 2009). Similarly, phosphate loads increased in the 1970s (two times the nitrate increase), but a strong decrease was observed in the 1990s thanks to the ban on phosphate in detergents, and/or the upgrade of waste water treatment plants (Ludwig et al. 2009). Results observed at the DYFAMED time-series station might mirror this trend. Regarding changes in non-riverine emission sources (*i.e.*, mainly atmospheric deposition), we have not enough post-1990s data at present time on the regional atmospheric deposition to conclude.

(3) Changes in water mass may have occurred. An increase in the frequency and the intensity of winter convection events was recorded from 2003 to 2006 (Fig. 4), and was attributed to a significant warming and salinification of the water column (Millot et al. 2006; Schröder et al. 2006; Skliris et al. 2007), which supposedly permitted to trigger the diving process with the

smallest heat loss (Leaman and Schott 1991; Schröder et al. 2010b). At the DYFAMED site, a regular increase of temperature and salinity was recorded in deep water (+0.005°C yr^{-1} and +0.0022 psu yr^{-1} during the 1995-2005 period), and in intermediate water (Schröder et al. 2006; Marty and Chiaverini 2010). The propagation of the Eastern Mediterranean Transient has been suggested as a possible explanation for the increase in temperature and salinity observed in intermediate waters since this event strongly affected the water flux at the Sicilian strait with an enhanced salt export from the eastern Mediterranean Sea to the western Mediterranean Sea (Klein et al. 1999). A cumulated deficit in fresh water budget over years 2003/2004/2005, and a concomitant increase in surface salinity were also observed (Stott et al. 2004; Marty and Chiaverini 2010). This peculiar situation led to the intense winter convection observed in 2006 (Fig. 4). New warmer and saltier western Mediterranean deep water was formed (Smith et al. 2008) as already observed in winter 2004-2005 in the Gulf of Lions and in the Catalan basin (López-Jurado et al. 2005; Font et al. 2007; Schröder et al. 2006; Schröder et al. 2010b). This yielded the uplift of the old western Mediterranean deep water by several hundreds of metres (Schröder et al. 2008, 2013), which usually occurs roughly below 2000m-depth (Van Haren and Millot 2004). Thus, an increase in nutrient concentration over the 800m to 2000m-depth range that indicates an increase in water age (Kress et al. 2014; Schröder et al. 2010a), could be the consequence of the presence of the new deep water. The abrupt change occurring in 2005-2006 (linked to the deep mixing event) is believed to strongly contribute to the increase of deep nitrate concentration over the 1991-2011 period. As well, the decrease in phosphate concentration over the 1991-2011 was probably reduced by this event. The increase in silicate concentration occurring in 2005-2006 resulting from the same event might counterbalance a gradual decrease over the 1991-2011 period and, therefore, hide any trend.

4. Conclusions

For the first time, a quality-controlled 20-year data set of nutrient concentrations in the northwestern Mediterranean was presented. Such a rich data set, almost unique for the open Mediterranean Sea, was analysed from different perspectives. In this work, we discussed the variability of nutrient dynamics by evoking the seasonal patterns of the MLD, and we estimated the possible temporal evolutions of nutrient stocks. Phosphate controls primary production in conditions of stratification (summer and autumn oligotrophy), but our results also suggest that PO_4^{3-} and NO_3^- play a similar role in blooming conditions. This contradicts the reigning paradigm of limitation driven by N in such conditions (e.g. Marty et al. 2002).

Over longer time scales, an important change characterised by an increase in nutrient concentration in the 800 to 2000m-depth layer occurred in 2005-2006, presumably driven by a deep water uplift. This increase is believed the main cause in the increasing trend observed for nitrate concentration during the whole period (1991-2011), even if the contribution of external sources cannot be excluded. The same increase is superimposed upon a regular decrease in phosphate concentration. As a result of the strong decrease observed in nitrate concentration, N:P and Si:P ratios significantly increase. The trends observed suggest that the role of P as limiting factor of primary production may be currently increasing, as already suggested (D'Ortenzio and Ribera d'Alcalà 2009; Durrieu de Madron et al. 2011). The perpetuation of projects such as DYFAMED time-series (as well as similar projects, as HOT or BATS) is certainly a priority for future oceanography. However, our results demonstrate that several processes are difficult to characterise with the available temporal resolution of data. Some statements of this paper should be then moderated by taking into account the bias introduced by monthly sampling. Indeed, for the case of processes that occur over a short duration (e.g. winter convection), such periods necessarily gather several sampling configurations that do not strictly correspond to the process in question. Increasing the

temporal resolution of ship-based sampling is costly and logistically very difficult. However, even in optimal conditions, with unlimited ship-time and resources, meteorological conditions should prevent a long-term high-frequency sampling. The only realistic alternative is a combination of ship-based operations and autonomous sampling. The estimation of nitrate concentrations from moorings and profiling floats has been already achieved in the past (Johnson et al 2006, 2013), strongly improving our capacity to detect and understand the chemical dynamics of the ocean at unexplored temporal scales. A nitrate sensor will be added to the DYFAMED mooring in the next months, while a network of floats equipped with nitrate (plus florescence, coloured dissolved organic matter (CDOM), optical backscattering coefficient (bb) and photosynthetically available radiation (PAR)) sensors is already operational in the northwestern Mediterranean (NAOS project). The combination of all the components of the future DYFAMED observing system (ship, moorings, profiling floats) will strongly enhance our capability to understand the nutrient dynamics (including chemical limitation) of the northwestern Mediterranean. It will also provide pertinent data to confirm the present results.

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Table captions

Table 1: Distribution of available data according to depth. Reference depths and associated intervals are given (in db) for each nutrient.

Table 2: Monthly median nutrient concentrations integrated over the surface layer (0-50m), the intermediate layer 200-600m, and the deep layer (>800m). The deviation is given by equation (2).

Table 3: Statistical analysis for nutrients and nutrient ratios. P-values less than 0.05 indicates a strong presumption against null hypothesis, meaning that result obtained from tests can be considered as significant.

Table 1

depth classes		Nitrate	phosphate	silicate
reference [db]	Interval [db]	number of sampling		
5	0-10	198	252	326
20	10-30	239	260	342
40	30-50	328	277	342
60	50-70	221	195	213
80	70-90	207	183	199
100	90-110	196	179	188
130	110-150	326	294	310
175	150-200	232	211	217
250	200-300	156	138	148
350	300-400	152	136	148
450	400-500	162	143	157
550	500-600	143	125	139
700	600-800	279	245	272
900	800-1000	174	158	166
1150	1000-1300	163	141	149
1550	1300-1800	229	201	215
2000	>1800	174	152	168
Total sampling		3579	3290	3699

Table 2

Months	Nitrate [μM]			Phosphate [μM]			Silicate [μM]		
	0-50m	*200-600m*	*>800m*	*0-50m*	*200-600m*	*>800m*	*0-50m*	*200-600m*	*>800m*
Jan	2.68 ± 0.97	7.85±0.89	8.24±0.29	0.12 ± 0.09	0.37±0.11	0.39±0.044	2.15 ± 0.82	6.25±1.11	7.58±0.31
Feb	2.70 ± 1.05	7.56±0.75	8.08±0.59	0.14 ± 0.09	0.34±0.07	0.40±0.07	1.95 ± 0.79	5.41±1.17	7.80±0.95
Mar	2.60 ± 1.71	7.34±0.99	8.13±0.86	0.11 ± 0.05	0.38±0.04	0.41±0.03	1.75 ± 0.97	5.69±2.01	7.99±1.28
Apr	0.85 ± 0.77	7.90±0.81	8.16±0.34	0.06 ± 0.04	0.33±0.02	0.37±0.05	1.16 ± 0.82	5.67±0.96	7.17±1.10
May	0.28 ± 0.33	8.14±0.52	8.23±0.50	0.04 ± 0.03	0.38±0.05	0.39±0.04	1.25 ± 0.62	6.16±0.83	7.71±16
Jun	0.64 ± 0.80	8.10±0.65	8.18±0.33	0.03 ± 0.01	0.35±0.06	0.37±0.04	0.95 ± 0.54	6.74±1.04	8.23±0.48
Jul	0.65 ± 0.83	7.89±0.50	8.11±0.27	0.03 ± 0.01	0.37±0.03	0.40±0.04	1.21 ± 0.63	6.22±0.85	7.61±0.38
Aug	0.78 ± 0.72	8.24±.049	7.75±1.1	0.04 ± 0.02	0.32±0.03	0.33±0.03	0.85 ± 0.31	6.13±1.58	6.70±1.85
Sep	0.16 ± 0.12	7.92±0.82	7.88±0.75	0.03 ± 0.01	0.38±0.04	0.37±0.03	0.86 ± 0.87	6.171.34	7.67±1.11
Oct	0.19 ± 0.15	8.22±0.95	7.76±0.80	0.03 ± 0.01	0.36±0.07	0.38±0.04	0.90 ± 0.50	6.42±1.73	7.95±1.42
Nov	0.38 ± 0.31	7.72±0.52	7.66±0.77	0.04 ± 0.01	0.34±0.06	0.36±0.07	0.69 ± 0.36	5.38±1.74	7.96±1.74
Dec	1.20 ± 0.98	7.67±0.77	8.04±0.62	0.05 ± 0.03	0.36±0.04	0.40±0.03	1.12 ± 0.68	5.02±1.88	7.16±1.88

Table 3

	Linear regression			Mann-Kendall		Pettitt	
	trend	*R^2*	*p-value*	*Z-statistic*	*p-value*	*p-value*	*year*
N	+ 0.23%	0.03	$4.2\ 10^{-2}$	2.60	$9.0\ 10^{-3}$	$9.0\ 10^{-3}$	2006
P	- 0.62%	0.11	10^{-4}	-3.55	$3.8\ 10^{-4}$	$4.2\ 10^{-2}$	2005
Si	+ 0.11%	$2\ 10^{-3}$	0.60	0.39	0.7	$1.6\ 10^{-4}$	2005
N:P	+ 1.14%	0.26	$<10^{-5}$	5.75	$<10^{-5}$	0.54	-
Si:P	+ 0.85%	1.6	$1.5\ 10^{-3}$	3.53	$4.0\ 10^{-4}$	0.20	-
Si:N	- 0.3%	0.02	0.1	-0.52	0.6	$4.0\ 10^{-4}$	2005

Highlights

1 – An unique northwestern Mediterranean nutrient time-series spanning from 1991 to 2011 is quality controlled and studied

2 – The seasonal variability of nutrient concentration and availability is described in relation to physical forcings, in particular the mixed layer depth

3 – The seasonal succession of chemical limitation is re-visited

4 – For the first time, the decadal evolution of nutrient stocks is assessed

Figure captions

Fig. 1: Location of the DYFAMED time-series sampling site. The black arrows figure the North Current circulation in surface.

Fig. 2: Distribution of sample number per year and per month.

Fig. 3: Monthly box plot of the mixed layer depth (MLD) at DYFAMED (a). The central red mark is the median, and the edges of the box are the 25th and 75th percentiles. Composite years for nitrate (b), phosphate (c), and silicate (d) concentrations, within the 0-200m layer at DYFAMED, drawn over a period of 20 years (1991-2011), and built using triangle-based cubic interpolation method.

Fig. 4: MLD time-series over the 1991-2011 period (a). Scatter plot of mean concentration in the MLD *versus* MLD for nitrate (b), phosphate (c) and silicate (d).

Fig. 5: Distribution of N:P ratios in surface (0-50m), considering ratios with high (> 30%) and low (< 30%) errors.

Fig. 6: N:P, Si:N, and Si:P molar ratios (a, b and c, respectively). Black lines are the median profiles. Data are separated for the four seasons. Mind the scale distortion on the y-axis.

Fig. 7: Evolution of nutrient concentration (a) Nitrate, (b) phosphate, (c) silicate and molar ratio (d) N:P, (e) Si:P, (f) Si:N in deep water at the DYFAMED site from 1991 to 2011. Data are averaged over the 800m-bottom layer and the error bar are 2 time the standard deviation length. Regression lines are in black and triangles indicates the date of change, determined from the Pettitt test, if significant.
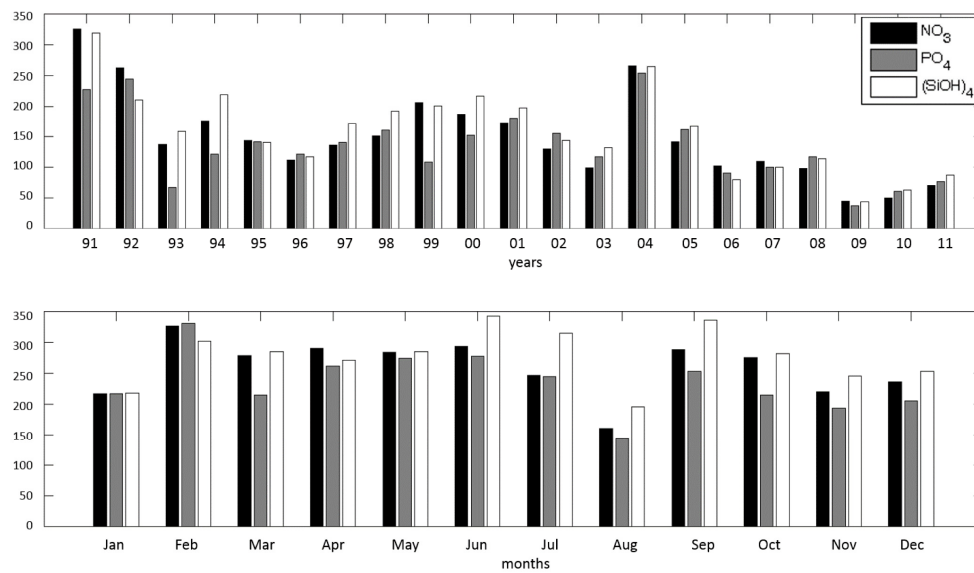
Fig. 1

Fig.2

Fig.3

Fig.4
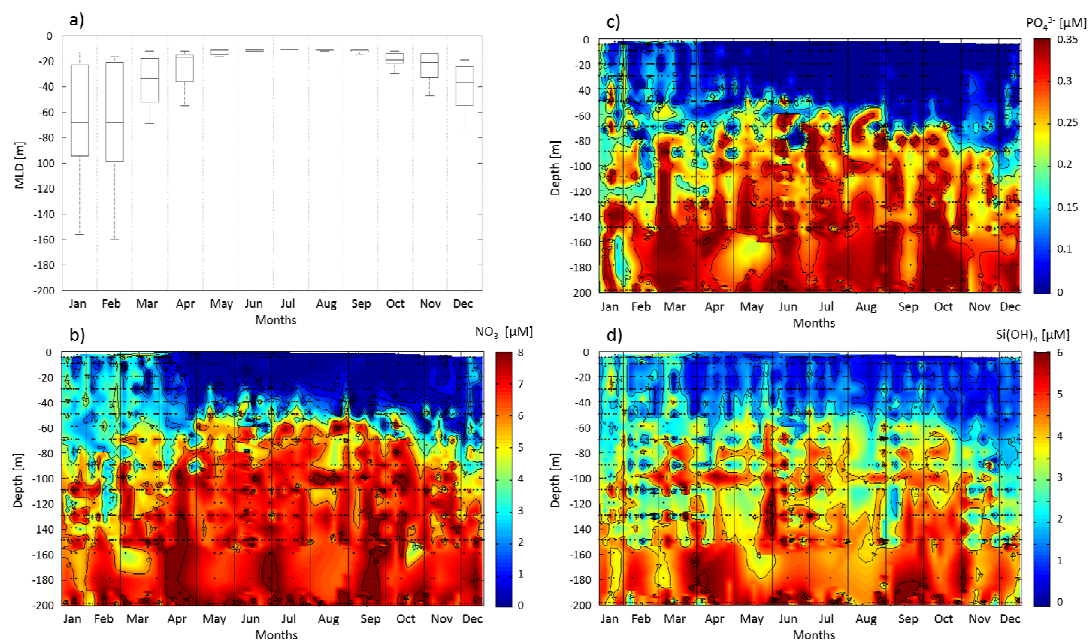
Fig.5

Fig. 6

Fig.7