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SUPPORT COHERENT AND COORDINATED ASSESSMENT OF BIODIVERSITY AND MEASURES ACROSS MEDITERRANEAN FOR THE NEXT 6-YEAR CYCLE OF MSFD IMPLEMENTATION

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"Report on the consolidated GES definition(s) for the pelagic habitat (D1C6) based on MSs assessments"

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SUPPORT COHERENT AND COORDINATED ASSESSMENT OF BIODIVERSITY AND MEASURES ACROSS MEDITERRANEAN FOR THE NEXT 6-YEAR CYCLE OF MSFD IMPLEMENTATION

Support Mediterranean Member States towards implementation of the MSFD new GES Decision and programmes of measures and contribute to regional/subregional cooperation

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Coordinator

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Scope

The criterion for pelagic habitats (D1C6, Descriptor 1, 2017/848/EU) considers the condition of the habitat type as a whole for its biotic and abiotic features and functions. Good environmental status (GES) under this criterion must take into account different pelagic broad habitat types with variable salinity, coastal, shelf and oceanic/beyond shelf as main types, as well as other habitat types where their need is identified through (sub)regional cooperation. The comparison of GES definitions for pelagic habitats has shown that the degree of coherence between the eight Mediterranean member states is currently low, with GES mostly being defined on a conceptual basis. In order to define tailored GES for pelagic habitats, and thus fulfil the first general objective of the call (Support for the (sub)regional assessment of the extent to which GES has been achieved), phytoplankton and zooplankton communities as relevant biotic components need to be included as indicators of pelagic habitat.

Deliverable D2.3, produced as part of Task 2.3, builds on the results of Tasks 2.1 and 2.2 and includes several case studies covering a range of prevailing natural conditions in different sub-regions of the Mediterranean, including the Adriatic, Aegean, Tyrrhenian and Ligurian Seas. The case studies are tailored to phytoplankton and zooplankton communities and include specific activities such as the definition of temporal and spatial scales for testing, the selection of appropriate templates for data analysis and comparison, and the definition of reference/baseline/threshold/trigger conditions against which the actual or potentially changing situation can be compared. The expected outcome is a step towards a harmonized GES definition for pelagic habitats based on the findings from these case studies.

One of the main objectives of this document is to provide recommendations resulting from the comprehensive synthesis of the work carried out under all ABIOMMED Activity 2 tasks. This guidance document could help direct future efforts to integrate pelagic habitat components into biodiversity descriptor assessment systems not only for Member States but also for non-European countries that are Parties to the Barcelona Convention. It also contributes to the development of the assessment system for MSFD Descriptor 4 (D4). The established think tank of plankton experts will continue to provide ongoing collaboration, advice and expertise after the completion of the project and establish links with other descriptors where plankton plays a crucial role, such as D4 and D5.

Executive Summary

The conceptual definition of Good Environmental Status (GES) for pelagic habitats is consistent with the holistic assessment of their biotic and abiotic characteristics and functions as set out in Criterion D1C6 of Descriptor 1 - Biodiversity (Commission Decision 2017/848/EU). Although pelagic habitats are interconnected, their plankton communities show considerable variability in the different (sub)regions of the Mediterranean Sea, making direct comparability difficult. The lack of consistent thresholds and baseline values for plankton biodiversity indicators has led to different methodological approaches in the Member States. The aim of this deliverable is to address these gaps by proposing tailored recommendations derived from the comprehensive synthesis of work across all ABIOMMED Activity 2 tasks, focusing on phytoplankton and zooplankton communities that play a critical role in marine food webs and are sensitive to environmental pressures.

Despite advances in molecular biology, satellite remote sensing and biogeochemical modelling, it remains difficult to distinguish human-induced changes from natural variability in plankton communities. As previous research has shown, plankton diversity indices prove to be reliable indicators of environmental change and anthropogenic impacts mainly when abrupt or significant disturbances occur, especially in areas with lower biodiversity. The effectiveness of these indices depends on a uniform level of taxonomic classification, which requires time-consuming efforts by highly qualified experts. While this is possible in regions with low species diversity, such as high latitudes, it is a major challenge in a diverse and highly dynamic ecosystem such as the Mediterranean Sea, as it requires deep taxonomic knowledge and has to deal with both the limits in observation techniques and the presence of cryptic species.

This deliverable presents the results of case studies on plankton diversity in different subregions of the Mediterranean Sea. For phytoplankton, these studies build on previous projects, while focusing on a more detailed investigation of temporal diversity patterns. In addition, the Italian case study included in this report examines spatial patterns at relatively high resolution. For zooplankton, where the development of indicators in the region has faced challenges including slow progress in the standardization of methods, considerable research effort and historical data collection methods, the present results report the first collaborative study of indicators in the Mediterranean using a common methodology.

Phytoplankton case studies covering the eastern Adriatic, the northern Adriatic, Italian regions and the Aegean Sea, aimed to capture different temporal and spatial aspects. Long-term analyses were carried out in the eastern and northern Adriatic, showing increasing richness and decreasing abundance, especially in the oligotrophic eastern Adriatic. Stations in the open sea showed more pronounced trends, suggesting that environmental changes generally affect phytoplankton communities independently of anthropogenic influences. In both the eastern Adriatic and the northern Gulf of Trieste, the observed changes were consistent with trends towards increased oligotrophy and reduced pollution, suggesting that the changes are due to broader factors such as climate change.

The Italian case study underlined the considerable temporal and spatial variability of phytoplankton communities in the Italian regions. Despite minimal differences between transect stations, alpha diversity indices showed limited discriminatory potential, while beta diversity indices such as LCBD proved to be potentially valuable tools to identify changes. On the contrary, studies of the LTER Senigallia-Susak dataset highlighted differences between coastal and offshore stations related to water circulation and water masses, overshadowing direct anthropogenic influences. The shift to erratic community dynamics at LTER stations underlined the influence of climatic and hydrological factors at the mesoscale and emphasized the importance of long-term ecological research for understanding large-scale trends like climate change.

The zooplankton case studies marked a crucial initial step towards understanding the diversity of zooplankton in the Mediterranean Sea on a larger scale. With datasets spanning more than a decade (Croatian and Greek) or providing broader spatial coverage (Italian), the studies included five Marine Reporting Units (MRUs): Tyrrhenian, Adriatic, Ionian Sea, Aegean, and Levantine Seas. However, the sub-regional differences in monitoring frequency and duration, and selection of zooplankton parameters posed a challenge for the comparison of results, highlighting the complexity of zooplankton community dynamics.

The extensive datasets revealed findings such as that microzooplankton in the central eastern Adriatic can serve as a potential indicator, suggesting an extension of spatial coverage in the eastern Adriatic. The Italian case study emphasized the need to test the indices at the local level and suggested a monthly homogeneous sampling frequency. Overall, the studies underlined the complexity of zooplankton dynamics and advocated for standardized methods, a spatial consideration, and further research on the mechanisms shaping (meso)zooplankton communities in the context of anthropogenic pressures and impacts. The potential benefits of functional diversity and the selection of appropriate indicators for (meso)zooplankton in the context of environmental pressures need to be further explored.

Overall, both the phytoplankton and the zooplankton showed considerable fluctuations in diversity, which were more related to the prevailing conditions than to direct anthropogenic influences. The importance of data from long-term ecological research (LTER) for understanding changes in the plankton community over time, particularly in relation to climate change, is emphasised throughout the report.

In order to improve the harmonization of GES definitions for pelagic habitats in the Mediterranean Sea, recommendations are made based on the comprehensive synthesis of ABIOMMED Activity 2 to guide future efforts to include pelagic habitat components in biodiversity descriptor assessment systems.

1. The importance of assessment scales: Comprehensive spatial coverage of monitoring stations in the subregions and Marine Reporting Units (MRUs) is advisable, with the inclusion of satellite data and modelling products to track phytoplankton trends on a broader scale. This needs to go hand in hand with a multi-temporal approach that allows the inclusion of climate regimes in assessments and helps to distinguish between anthropogenic influences and natural variability, recognizing the challenges involved.

2. Importance of investigating the planktonic community as a whole, including all phytoplankton and zooplankton groups/size classes. It is recommended to include in the assessment picophytoplankton and microzooplankton.

3. For a comprehensive understanding of environmental changes, it is advisable to integrate data from long-term ecological research (LTER) stations with data from regional monitoring stations. To this end, the maintenance and possible expansion of the LTER network is recognised, which would build on its adaptability to incorporate new methods.

4. Support a more uniform and consistent sampling frequency across Member States for meaningful cross-regional comparisons, with monthly sampling for phytoplankton and at least seasonal for zooplankton.

5. Evaluation of trends and trust in expert judgment: As an alternative to rigid thresholds, a different, expert-based approach to GES is recommended, assessing regional trends and changes, emphasizing that there are no specific thresholds in the Mediterranean.

6. Establish connections to Descriptor 4 and focus on changes in food webs considering observed trends in phytoplankton and zooplankton communities. Including multiple trophic guilds could allow easier detection of cascading effects of different natural and human influences.

7. Continuation of cooperation through a working group of multidisciplinary experts. The group could operate under the MSFD umbrella or the Barcelona Convention and aim for standardized monitoring protocols and a harmonized approach across the Mediterranean Sea.

Notwithstanding the currently insurmountable challenges in defining GES, reference conditions and thresholds, detailed information on the taxonomic analysis of plankton samples will always be necessary to correctly interpret the patterns of other variables such as biomass or abundance of plankton components (e.g. phytoplankton and mesozooplankton) or functional groups on which other indicators may be based.

1 Introduction

The good environmental status (GES) for pelagic habitats (criterion D1C6 of Descriptor 1 - Biodiversity) should be conceptually defined as the totality of their biotic and abiotic features and their functions (Commission Decision 2017/848/EU). The GES must be defined and assessed for pelagic broad habitat types (variable salinity, coastal, shelf and oceanic/beyond shelf) within the Marine Reporting Units, although other habitat types may also be considered if their need is identified through (sub)regional cooperation. Pelagic habitats are interconnected but not directly comparable in terms of plankton communities, and differences between parts of (sub)regions are often greater than differences between pristine and impacted areas that are geographically close to each other (Francé et al., 2021).

The biotic components of the pelagic habitat, i.e. the phytoplankton and zooplankton communities, are theoretically relevant indicators for the definition of GES, as they form the basis of the marine food web, are mostly commercially unexploited and vulnerable to environmental pressures. Currently, the study of plankton indicators in the Mediterranean focuses on indicators of the status of plankton assemblages, mostly related to coastal waters (as defined by the Water Framework Directive) and on specific case studies related to environmental pressures, in particular eutrophication. However, there is a consistent lack of thresholds and baseline values for plankton biodiversity indicators, and thus inconsistent methodological approaches to biodiversity assessment in the Member States. In recent years, various methods have been used to monitor and study pelagic communities, ranging from classical methods to approaches combining optical imaging and molecular data. However, these emerging methods have often been applied in the context of regional-scale research projects and have not yet been used to improve the spatial and temporal resolution of data collection for GES assessment. Collaboration with these scientific fields (e.g. molecular biology, satellite remote sensing, automated optical/imaging techniques, biogeochemical modelling) is recommended to increase the amount of data relevant for GES assessment.

Although the definition of GES for each indicator depends on both regional characteristics and the availability of data, it must be consistent with the overall need of the MSFD to monitor key pressures through the use of similar and coherent criteria at EU level. Member States are required to produce a report on GES, accepting a range of indicators, thresholds and integration methods for each habitat type. A common approach to assessing pelagic environmental status is to examine changes in the plankton community. In general, biological communities are assessed using three categories of indicators, depending on which taxa are targeted: phytoplankton only, zooplankton only, and a combination of phytoplankton and zooplankton indicators. There are advantages and disadvantages depending on the category and metrics of each indicator.

In the scientific literature, there are a variety of plankton indicators that have been developed and/or used for the Mediterranean region, all aimed at assessing the state of the marine environment (e.g. Varkitzi et al., 2018). One of the problems in improving assessment based on plankton diversity indices is to capture the response of plankton communities to human impacts, which is usually difficult and often non-linear (Francé et al., 2021). Zooplankton indicators for GES are particularly challenging and until recently were mainly produced on a regional basis and are still under development.

Linked to this is the difficulty of defining indicators that can be used to distinguish changes due to human influences from the natural variability (in time and space) of typical planktonic communities in coastal areas and open seas. An important aspect is the inclusion of areas with different pelagic habitat characteristics to distinguish between structural and/or functional differences of plankton communities (Garmendia et al., 2013; Varkitzi et al., 2018). Furthermore, the applicability of diversity indices to assess the state of the marine environment in a management context depends on the objective of the study, its ecological relevance, the mathematical properties of a given index and the ease of interpretation by stakeholders (OSPAR, 2017).

Given the variety of indices, it is often difficult to decide which is the best way to measure diversity. The method for selecting a diversity index is based on whether it meets certain functional criteria - ability to distinguish between sites, dependence on sample size, which component of diversity is being measured, and whether the index is commonly used and understood. The choice of indicators, i.e. evenness, species richness and biodiversity indices, was largely based on the scheme proposed by Magurran and Mc Gill (2011), with the main advantages of using diversity indices being their advanced development in the scientific literature and their ease of calculation (OSPAR, 2017). In the case of the phytoplankton community, diversity indices based on abundance and richness are usually calculated for the micro- and nanoplankton communities (i.e. excluding the pico-fraction), which (i) are identified to a minimal extent at the genus or species level, and (ii) includes also mixotrophic and heterotrophic species and could provide additional information for the assessment of pelagic habitats (Domingues et al., 2008), in contrast to the use of indicators based solely on Chlorophyll-a.

The MSFD assumes that monitoring zooplankton can be useful for detecting environmental changes and anthropogenic pressures in nature (Serranito et al., 2016). As zooplankton are very sensitive to changes, the response to disturbance is evident in a shorter time compared to higher trophic levels. This short response time and zooplankton ubiquity make them a key group among the biological components listed in Table I of Annex III of the MSFD (Cochrane et al., 2010). As part of ABIOMMED Activity 2 and Task 2.2, we conducted a systematic review of the existing literature to describe the current state of knowledge on the development and application of zooplankton-based indicators and indices in environmental assessment and monitoring (Deliverable D2.2a). The review of zooplankton indicators revealed that none of the available zooplankton-based status indicators are designed or have defined thresholds for the Mediterranean Sea and its subregions due to various difficulties. According to the catalogue of indicators by Varkitzi et al. (2018), the development of indicators is mainly based on the following zooplankton metrics: total abundance, total biomass, copepod abundance, % copepod abundance, copepod biomass, % copepod biomass (as copepods are the most abundant group in the mesozooplankton community), microphagous species biomass, % microphagous species biomass, cladocerans/copepods ratio, rotifers+cladocerans/copepods ratio, and zooplankton mean size. However, the development of useful zooplankton indicators in the Mediterranean Sea has lagged behind other European seas, generally hampered by slow progress in the standardisation of methods and metrics, as well as large research efforts and a long history of data collection that have favoured individual approaches and a low degree of synchronisation between zooplankton research groups in the Mediterranean.

As part of ABIOMMED Activity 2 and Task 2.1, a topical review of phytoplankton indicators for the regional seas was undertaken, and the potential use of phytoplankton indicators was critically evaluated, focusing on indicators of diversity (Deliverable D2.1a). The review of indicators in the OSPAR region (North-East Atlantic) revealed that the best-designed indicators for marine phytoplankton and zooplankton communities have been developed for the assessment of environmental status but are still under development. A common feature of the use of many indicators for pelagic habitats is their continuous development and/or improvement based on the acquisition of new knowledge, the constant review of their suitability and the search for better alternatives. In the OSPAR region, the pelagic habitat has been assessed using three common indicators that consider plankton communities at different levels of organisation (PH2, PH1, PH3; OSPAR, 2017). These indicators use the changes in abundance of life form pairs based on functional traits to indicate ecological changes in the habitat and also use taxonomic diversity indices to indicate changes in community structure. In the Baltic Sea, HELCOM assesses pelagic habitat using different indicators for the open and nearshore marine areas. Further progress has been made by McQuatters-Gollop et al. (2022) in developing a biodiversity status assessment system with categories that either correspond to indicator thresholds or simply take into account the change in the indicator over time in terms of impact based on expert judgement. However, the authors emphasise that the assignment of indicators to these categories currently has no formal link to policy regimes such as OSPAR or MSFD for GES assessments. Such categories could be a good starting point for a better definition of GES in relation to pelagic habitats, even if there are no thresholds. To date, no operational indices other than “Chlorophyll-a” are used in the Mediterranean Sea to assess the status of the pelagic habitat, although there have been several studies at sub-regional or local scales where different indicators have been tested. Some of the indicators have been proposed for further testing, e.g. size-based metrics, diversity and dominance metrics, and metrics based on bloom frequency. Under the EcAp and IMAP of the Barcelona Convention, two common indicators are proposed to assess the pelagic habitat in the Mediterranean (Habitat distributional range and status of species and communities typical of the habitat), for which a common reference list of pelagic habitat types must first be agreed.

Policy regulation and management measures will depend on the cooperation of all Member States, e.g. in the selection of representative indicators and methods to integrate indicators for the GES assessment at the habitat level. The current pelagic assessment foresees an exchange between this project ABIOMMED (Mediterranean Sea) and other ongoing EU-funded projects NEA PANACEA (North-East Atlantic) and HELCOM BLUES (Baltic Sea) and the support of this cooperation. Finally, when addressing GES for pelagic habitats, the links between diversity and other MSFD descriptors such as food web and eutrophication need to be considered to ensure consistency at MSFD level.

The work carried out as part of ABIOMMED Activity 2 was based on the assumptions described by Magliozzi et al. (2021). In particular, the composition of plankton communities and their patterns of variability are determined by a multitude of interconnected factors and processes acting at different temporal and spatial scales. This complexity generally limits the current understanding of the observed patterns, and the resulting uncertainty further limits the distinction between natural variability and anthropogenic influences, making it difficult to establish a direct, straightforward link between an anthropogenic pressure and an indicator. Consequently, D1 pelagic habitat indicators must generally be considered as state indicators capable of detecting relevant changes in plankton community dynamics.

Biodiversity indices (e.g. H'-Shannon Wiener's diversity index) have been proposed in several regions or programmes. However, our current knowledge of plankton composition and diversity is in most cases still at the level of characterising patterns using more or less sophisticated statistical models that are rarely combined with ecological theories and hypotheses (e.g. Buttay et al., 2017) or in the best cases with still relatively simple models (e.g. Buttay et al., 2022). Only recently new modelling approaches for plankton diversity were proposed, based on functional traits (e.g. Le Gland et al. 2021), but these are not yet available at higher taxonomic resolution. In the absence of a precise understanding of the causal processes driving patterns of plankton diversity, the general application of these indices themselves as reliable state indicators must therefore be considered with extreme caution.

As we have seen in previous research (e.g. Francé et al., 2021), only in cases of abrupt or strong environmental change, including high levels of anthropogenic pressure, and in areas of lower diversity, diversity indices show the potential as reliable indicators of state or pressure. In addition, diversity indices require a uniform taxonomic classification level of the analysed organisms, which is time consuming and requires highly qualified experts. This premise may be relatively easily achieved in areas of low diversity (high latitudes, e.g. Ibarbalz et al., 2019), where the total number of species to be classified is low. However, in areas of high diversity such as the Mediterranean Sea (Siokou-Frangou et al., 2010), this is particularly challenging as it depends on the specific taxonomic knowledge of the research expert, on the limits of the observation techniques (e.g. classification of phytoplankton species under the microscope) or on the occurrence of cryptic species.

This deliverable includes the results of the case studies on the plankton diversity across different Mediterranean sub-regions. For phytoplankton, they mostly represent the continuation of the work done in previous projects (e.g. MEDCIS) with a more detailed inspection into the temporal dimension of diversity patterns. However, with the inclusion of the Italian case study that dealt with a relatively high spatial resolution of data also the spatial patterns were studied in detail. For zooplankton component, we present valuable results on diversity patterns in different case study areas. This is the first comprehensive study of zooplankton indicators with an agreed methodology in the Mediterranean Sea, of which development has lagged behind other European Seas, generally hampered by slow progress in standardization of methods and metrics as well as large research efforts and long history of data collection that have favoured individual approaches and low levels of synchronization among Mediterranean zooplankton research groups.

2 Case studies

2.1 Phytoplankton case studies

2.1.1 Selection of case studies

The premise in the selection of case study areas and data for the pelagic component of phytoplankton was to consider the pelagic habitat as a whole of interconnected communities but exposed to different prevailing conditions. Although efforts were made to cover as many different sub-regions of the Mediterranean subregions, the decision was ultimately based on the availability of suitable phytoplankton community data (community structure and abundance).

The areas foreseen in the project proposal for the case studies were the Adriatic Sea, the Eastern Mediterranean – Aegean and Ionian Seas - and the Western Mediterranean. Although the final selection of case studies for phytoplankton depended mainly on data availability, the case studies presented below followed this scheme. The Adriatic Sea region was covered by three different case studies. The Croatian case studies were conducted with data from two localities in the eastern part of the basin, Mali Ston and Kaštela Bay, while the Slovenian case study covered the northernmost part of the Adriatic, the Gulf of Trieste. The western Adriatic Sea was covered by the Italian case study, which also included the Ionian, Tyrrhenian and Ligurian Seas. The Aegean Sea was also covered by the Greek case study, but with limited data. The Western Mediterranean was partially covered by the data sets from the Tyrrhenian and Ligurian Seas.

Two different types of case studies were considered in this selection, involving different spatial and temporal scales. On the one hand, the case studies in Croatia, Slovenia and (at least partially) Greece worked with a fairly long, multi-year data series with monthly data, thus enabling the analysis of trends. On the other hand, the Italian case study covered a geographically wide area (i.e. Adriatic, Ionian, Tyrrhenian and Ligurian Seas), but the data sets cover only a few years, allowing an analysis that focused more on the spatial aspects, i.e. on the differences between the sub-regions and between the stations along the onshore-offshore axis. The arrangement of the sampling sites along the Italian coasts, i.e. a gradient of three sampling sites with increasing distance from the coast (i.e. 3, 6 and 12 nM), also allowed the study of the possible effects of the prevailing natural conditions on the phytoplankton communities.

Once the case study areas and the temporal and spatial scales for the analyses had been determined, the most appropriate template for data analysis and comparison was created, using the methods from the earlier MEDCIS project as a basis (Francé et al., 2021). The datasets were prepared for the analyses following the same quality assurance protocol, according to the LifeWatch metadata and data templates already provided for the MEDCIS project by the University of Salento. It was agreed that the calculation of the indices would be based on data collected at genus level in order to solve, at least partially, the problem of data being analysed by different experts.

2.1.2 Methods

The data sets are of varying length: from one to twelve years in the period 2001–2020, and the data were collected with monthly, bimonthly or seasonal frequency. For the case studies of Croatia, Slovenia and Greece, the degree of anthropogenic impact was assessed for each sampling site by expert judgement. Levels of impact were categorized as low (marked with 1), moderate (marked with 2) and severe impact (marked with 3), while stations with no or minimal impact were classified as reference conditions (marked with 0). In order to ensure maximum coherence between the categorisation of pressures and impacts of sampling sites in different sub-regions, a common matrix of pressure categories (Francé et al., 2021, Table A1) was established as defined by Lugoli et al. (2012) and Simboura et al. (2016). As Francé et al. (2021) revealed that there were no significant differences between phytoplankton communities from stations with impact categories 0, 1 and 2, they were all treated as reference conditions (for the pressures).

Alpha diversity indices were selected to represent different aspects of the phytoplankton community composition, namely richness, diversity, evenness and dominance (Cozzoli et al., 2017, Francé et al., 2021).

The Richness was simply presented as the number of taxa (R') identified in a given sample (Fisher et al., 1943).

Shannon - Wiener's Diversity Index H' (Shannon, 1948) takes into account both the abundance and evenness of taxa in a given community. The H' increases with the number of taxa in the community and can theoretically reach very high values. In practice, the H' for biological communities does not appear to exceed 5.0. It was calculated with the following formula:

$$H' = -\sum_{i=1}^{R} p_i \times \ln p_i$$

where p_i is the proportion of individuals in taxon *i* and is estimated as (n_i/N), where n_i is the number of individuals in taxon *i* and N is the total number of individuals in the community.

Pielou's Evenness Index E' expresses the ratio between the realized Shannon-Wiener diversity of a sample (H') and its maximum possible value (as a logarithm of R'), i.e. the expected value of H' if all taxa had an identical number of individuals (Pielou, 1975). It was calculated as:

$$E' = \frac{H'}{\log R'}$$

Berger-Parker's Dominance Index BP' (Berger and Parker, 1970) is the simplest measure for the numerical significance of the first most abundant species. The formula is:

$$BP' = \frac{n_1}{N}$$

where n_1 is the abundance of the most abundant species and N is the total abundance of the sampled community.

We tested also some beta diversity indices.

Rao's quadratic entropy is a measure of diversity of ecological communities defined by Rao (1982) and is based on the proportion of the abundance of species present in a community and some measure of dissimilarity among them. For the species taxonomic dissimilarity matrix, we referred to Warwick and Clarke (1995), including taxonomic separation:

$$\Delta = \frac{\sum \sum_{i<j} w_{ij} x_i x_j \ + \ \sum_i 0.\, x_i \,(x_i - 1)\,/2}{\sum \sum_{i<j} x_i x_j \ + \ \sum_i x_i \,(x_i - 1)\,/2}$$

Where x_i is the abundance of the *i*th species and w_{ij} is the "distinctness weight" related to the hierarchical classification of species *i* and *j*.

In the case of the Italian case study, we also calculated the Local contribution to beta diversity (LCBD) and Importance value index (IVI), following Rombouts et al. (2019).

Local contribution to beta diversity (LCBD) was used to see how much each observation of different sites contributed to the total community variance in time, thus to assess the change in the community among stations and years. The average composition of a community yields a value near 0, while large values may indicate degradation or a disturbance event. LCBD was computed following Legendre and De Cáceres (2013) using Hellinger as dissimilarity coefficient.

Importance value index (IVI) was used to define the most important taxa in a given phytoplankton community and to define differences between sub-regions. It was calculated as follows:

$$IVI = RD_i + RF_i$$

where RD_i is the relative density and RF_I is the relative frequency, each calculated as follows:

$$RD_i = \left(\frac{n_i}{N}\right) * 100$$

where n_i is the number of individuals of the genus *i* and N is the total number of individuals of all the genera.

$$RF_i = \left(\frac{f_i}{F}\right) * 100$$

where f_i is the number of occurrences of the genus *i* and F is the total number of occurrences of all the genera

Beside these, abundance of phytoplankton is also presented along the indices.

In case of sufficiently long time series of phytoplankton community data (Croatian and Slovenian case studies), trend analyses were performed on the indices' values and abundance. First, annual means and coefficient of variance (COV) were calculated for all indices. A linear model was calculated with raw data. Besides, a model showing periodic components and one showing deseasonalized trend (with the LOESS method) were computed.

In the Italian case study, the seasonal distribution of the selected indices was presented with box plots and the statistical significance of the differences between coastal and offshore stations of selected transect were calculated with Wilcoxon test. The Non-Metric Multi-Dimensional Scaling (NMDS) was performed for each season on phytoplankton group abundances (dinoflagellates, diatoms, coccolithophores and phytoflagellates) to compare the sub-regions.

Statistical analysis was performed using statistical software R version 4.2.2 (R Core Team, 2022).

2.1.3 Croatia

The dataset from Croatia consisted of data collected at 6 stations in the period 2007-2021 (Figure 1). Selected stations were sampled under national MSFD and WFD monitoring programs, as well as various projects of Institute and Fisheries. Four stations are in the central Adriatic on the transect from Kaštela Bay across the island of Hvar to the island of Vis, while the other stations are in the southern part of the Adriatic in the Bay of Mali Ston. Two coastal stations from Kaštela Bay (ST101 and ST103) with a depth of 10-50 m, were under significant anthropogenic influence until 2004, when a wastewater collector was installed (Šolić et al., 2010, Skejić et al. 2014). Two other stations representing open waters (CJ 008 and CJ 009) are located on the central Adriatic transect towards the Italian coast and have a depth of 75 and 100 m, respectively. Stations PL105 and FP05 are in the Bay of Mali Ston, which is situated between the Pelješac peninsula and the mainland on the south-eastern Adriatic coast. The two stations were sampled in different time periods but are close to each other and can be considered as the same station (station PL105 on Figure 1). Both stations represent reference conditions (impact category 0) (Ninčević Gladan et al., 2015, Skejić et al., 2015). Station PL105 was sampled at least four times per year and up to seven times from 2001 to 2009 (except in 2002, when it was sampled only once). Station FPO5 was sampled three to four times a year in 2015, 2017 and 2019. The depth of stations PL105 and FPO5 is 14 and 16 m, respectively.

The data format followed the LifeWatch metadata and data templates provided by the University of Salento. After collection, all datasets were checked for taxonomic accuracy using AlgaeBase. A total of 2292 samples were collected in the vertical profile of all stations.

Station ST103 represents polluted conditions (impact category 3). It was sampled at least four times per year from 2007 to 2020 (2007 and 2010) and up to nine times per year. The results are presented in three layers (station depth 18 m): Surface (0 m, 5 m), middle layer (10 m, 15 m) and bottom layer (17 m, 18 m).

Station ST101 represents the conditions with impact category 2. It was sampled at least 8 times per year and up to 12 times from 2007 to 2020. The results are presented in three layers (station depth 37 m): Surface (0 m, 5 m), middle layer (10 m, 20 m) and bottom layer (30 m, 35 m). Both stations ST103 and ST101 are in Kaštela Bay.

Station CJ008 represents the reference conditions (impact category 0). It was sampled at least 4 times in 2007 and 9 to 11 times per year in the remaining period from 2008 to 2020. In the period 2008-2017, the surface layer was analysed in an integrated sample (1-30 m), while in the period 2017-2020) discrete water samples for each depth were analysed. The results are presented in three layers (station depth 80 m): Surface (0-30 m, 10 m, 20 m, 30 m), middle layer (50 m) and bottom layer (78 m).

Station CJ009 represents reference conditions as it is in open waters (impact category 0). In many studies this station was selected as reference due to it oligotrophic character (Marasović et al., 2005). It was sampled from 2007 to 2020 at least 4 times in 2007 and 9 to 11 times per year during the rest of the period. In the period 2007-2017, the surface layer was analysed in an integrated sample (1-30 m), while discrete water samples were analysed in the period 2017-2020. The results are presented in three layers (station depth 100 m): Surface (0-30 m, 10 m, 20 m, 30 m), middle layer (50 m, 75 m) and bottom layer (100 m).

Figure 1: Map of the Croatian case studies' area with the sampling stations (eastern Adriatic Sea)

2.1.3.1 E Adriatic, Mali Ston

The surface layer of station PL105 shows a clear increase in genus taxonomic richness during the study period. The variability is not remarkably high, except in 2000, when the deviations increase sharply compared to all other measurements (Figure 2). For the middle layer, the deviations are much larger, as they are significantly influenced by the hydro-morphological conditions of the bay itself (Suppl. Figure S1). There also lots of underwater springs in the bay. As far as abundance is concerned, the deviations are considerable throughout the study period, as they are strongly dependent on sampling fluctuations. The highest abundances were recorded in 2001 and 2007, followed by sharp decrease in abundances next year (2002 and 2008). Thus, the standard deviation also fluctuates during the study period (Figure 3). The Shannon-Wiener diversity index closely follows the pattern of taxonomic richness of the genera. The Shannon index shows a visible trend of increase from 2001, with a decline in 2007. There are also large fluctuations in this index, especially in 2007-2008 (Figure 4). The Pielou-Evenness index at station PL 105 is following almost same pattern as Shannon index. There is trend of increasing variability (Figure 5). Berger-Parker's dominance in the surface layer of stations PL105/FP05 in the period 2001-2019 is inconsistent (Figure 6) while Rao index almost identical as Shannon (Figure 7).

Annual means and linear trends in the middle layer of the station PL105 for all indices are shown in the Suppl. Figures S1-S6.

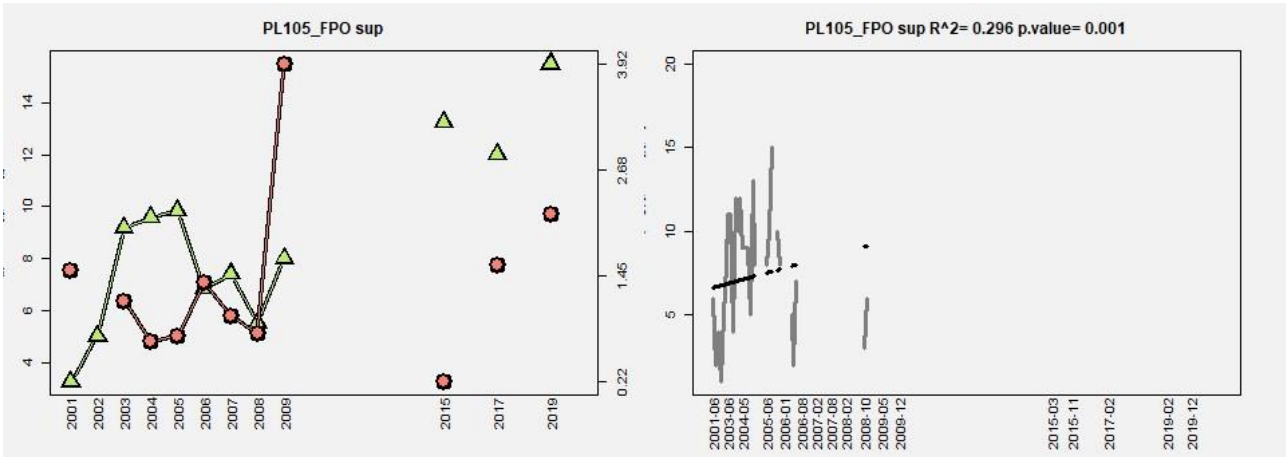

Figure 2: Genus richness in the surface layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); right panel: linear model.

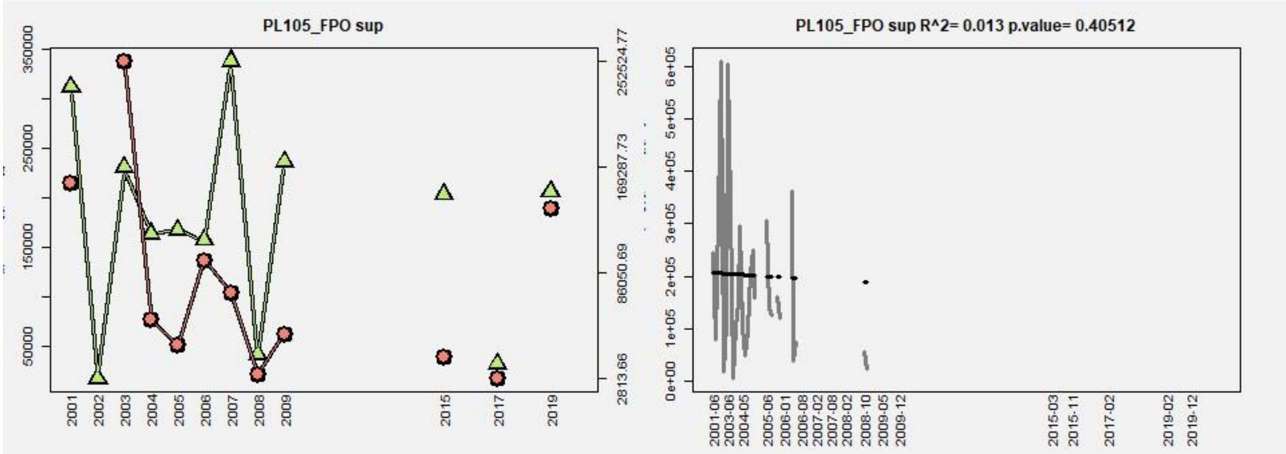

Figure 3: Phytoplankton abundance in the surface layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); right panel: linear model.

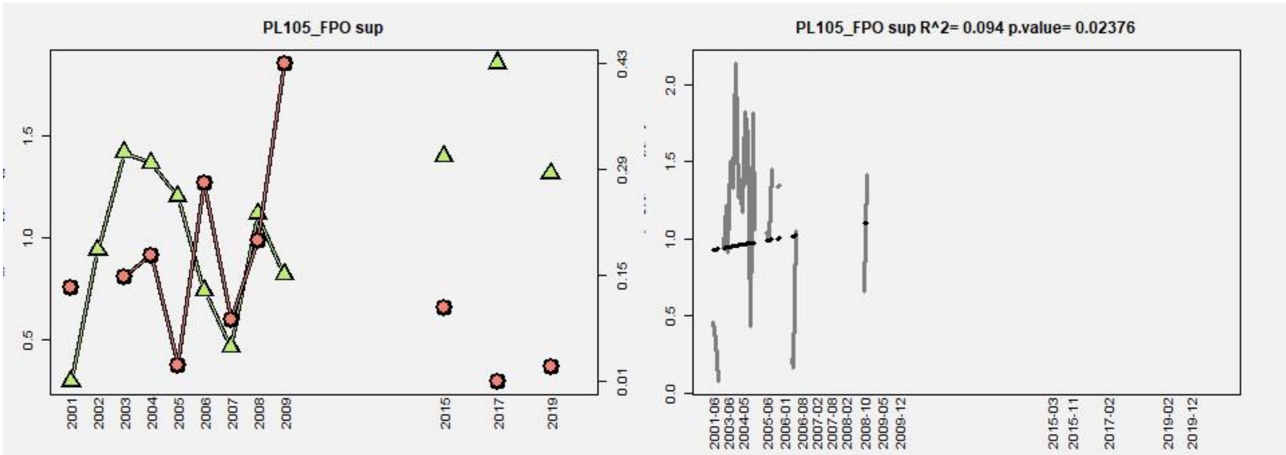

Figure 4: Shannon - Wiener’s diversity index in the surface layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); right panel: linear model.

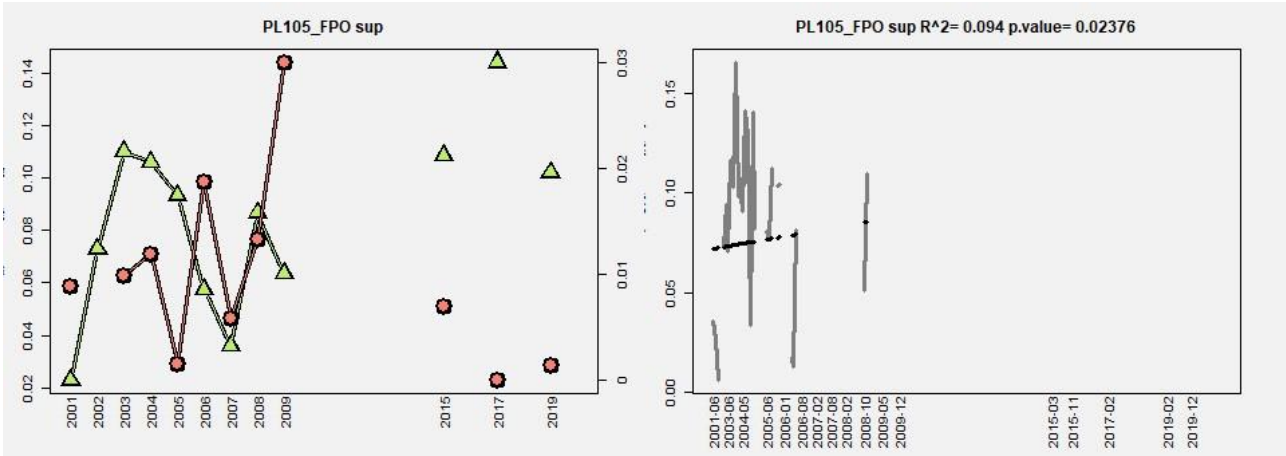

Figure 5: Pielou’s evenness in the surface layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); right panel: linear model.

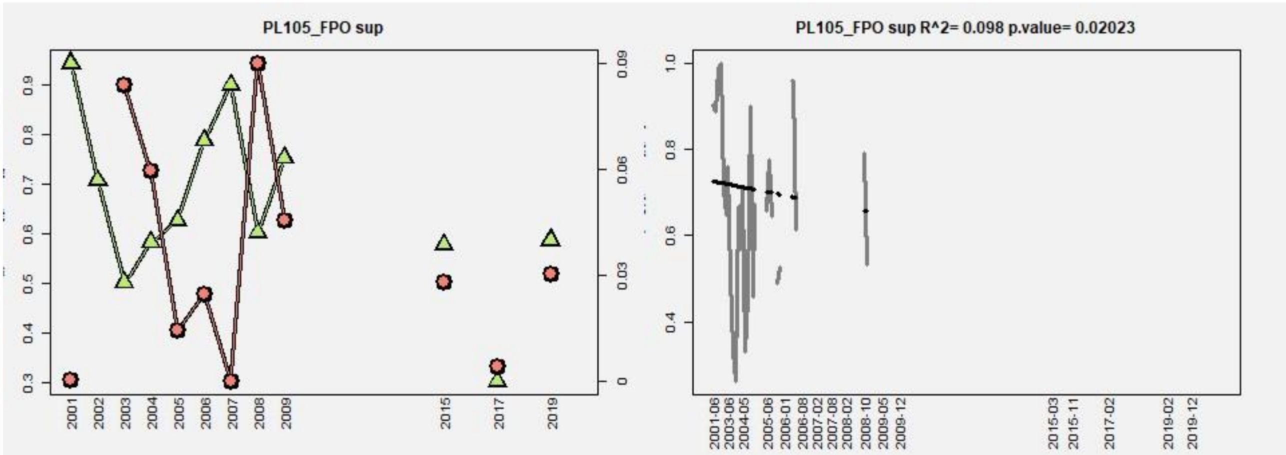

Figure 6: Berger-Parker’s dominance in the surface layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); right panel: linear model.

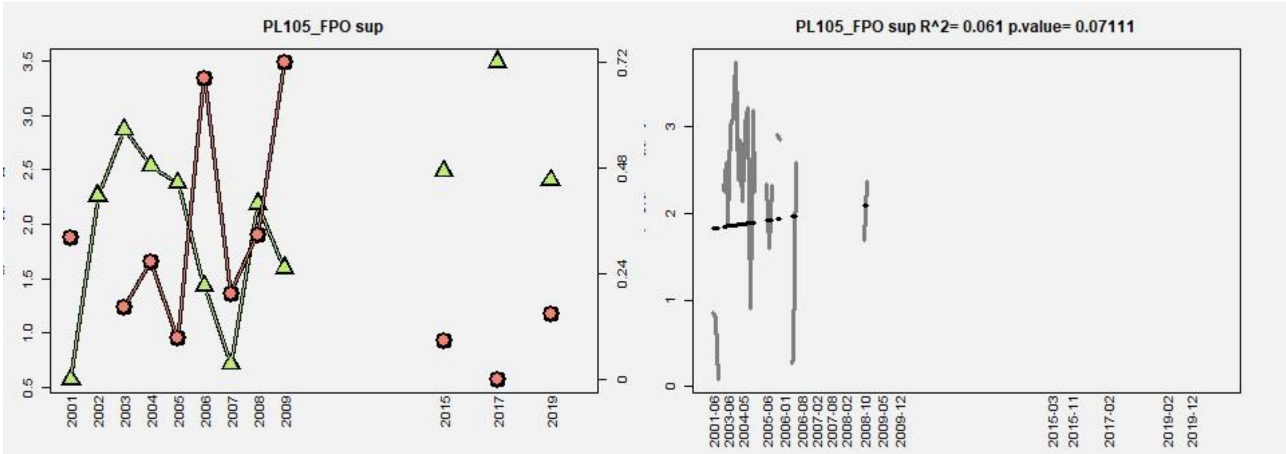

Figure 7: Rao's index in the surface layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); right panel: linear model.

2.1.3.2 E Adriatic, Kaštela – Hvar – Vis transect

The selected stations are located on a transect coast-offshore in Central Adriatic and they under different trophic pressure. Linear annual means and linear trends of tested indices at the surface layer of stations of the transect are presented in Figures 8-13, while trends other than linear (periodic components and deseasonalized model) for the surface layer and in all results for middle and bottom layers of the transect stations are presented in the Suppl. Figures S7-S30.

Genus taxonomic richness for ST103, as most impacted Adriatic site (impact category 3), shows unusual increasing linear trend at the surface layer (Figure 8). The COV was higher at the beginning of the studied period 2007-2013, while it stabilized from 2016 to 2020. In the middle and bottom layer of station ST103, the COV is extremely high until 2015, but the increasing trend is identical to that of the surface layer (Suppl. Figures S7). Like station ST103, station ST101 (which is in the same bay) shows the same increasing linear trend in taxonomic genus richness. COV values generally follow the same upward trend as genus richness, although COV decreases sharply from 2019 onwards. The smallest deviations were observed in 2011 and 2014 (Figure 8). Diversity also increases at stations CJ009 and CJ008 during the study period, which is reflected both in the number of genera recorded and in the measured diversity indices. At CJ008 and CJ009, there was a large jump in genus richness from 2015 to 2016 (Figure 8). In contrast to the coastal stations, the COV at these two open stations decreases from the beginning of the study period. This means that the indices fluctuate less strongly. Regarding abundance, it is visible that along the entire transect abundance is declining, except for station CJ008. For this station there was a decline in abundance until 2015 and 2016, after which numbers increased again to an extremely prominent level in 2019 (Figure 9).

The Shannon-Wiener diversity index in the surface layer for all stations of the whole transect shows a linear upward trend (Figure 10), a similar pattern to the genus richness. Such increasing trend was also observed for the evenness index, i.e. the Pielou evenness (Figure 11). For the two stations open stations CJ009 and CJ008 increasing trend in genus richness is even more pronounced compared to the coastal stations at the transect (ST101 and ST103).

Consequently, the dominance indices decrease as the two previously mentioned indices increase, so that, for example, the dominance of Berger-Parker decreases significantly throughout the area, suggesting that there is no genus that constantly dominates this area and affects biodiversity (Figure 12).

Similarly to richness, diversity and evenness also the Rao's entropy index shows a significantly positive trend at all stations of the transect except at station ST101 (Figure 13).

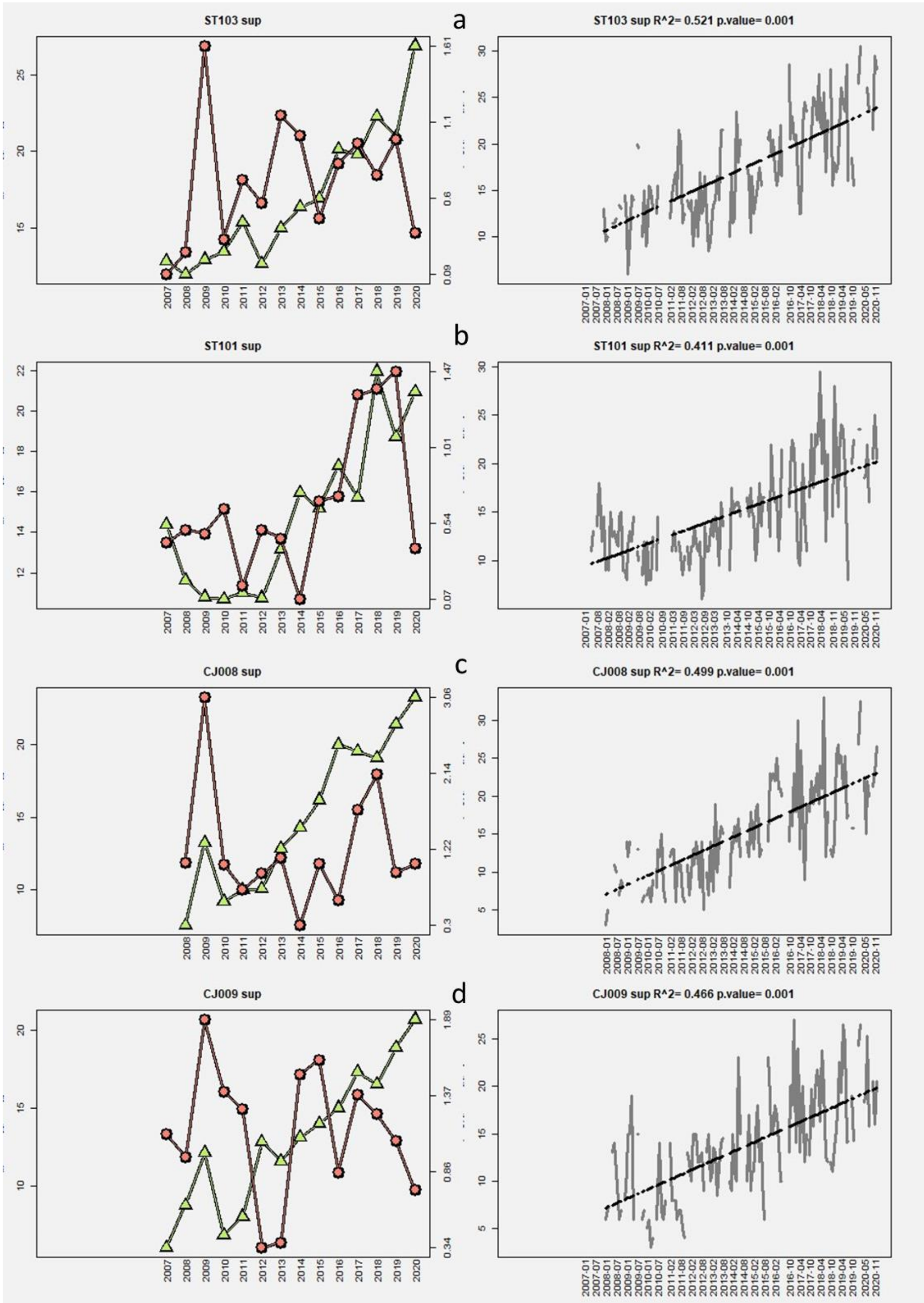

Figure 8: Genus richness in the surface layer of stations ST103 (a), ST101 (b), CJ008 (c) and CJ009 (d) in the period 2007-2020. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); right panels: linear model

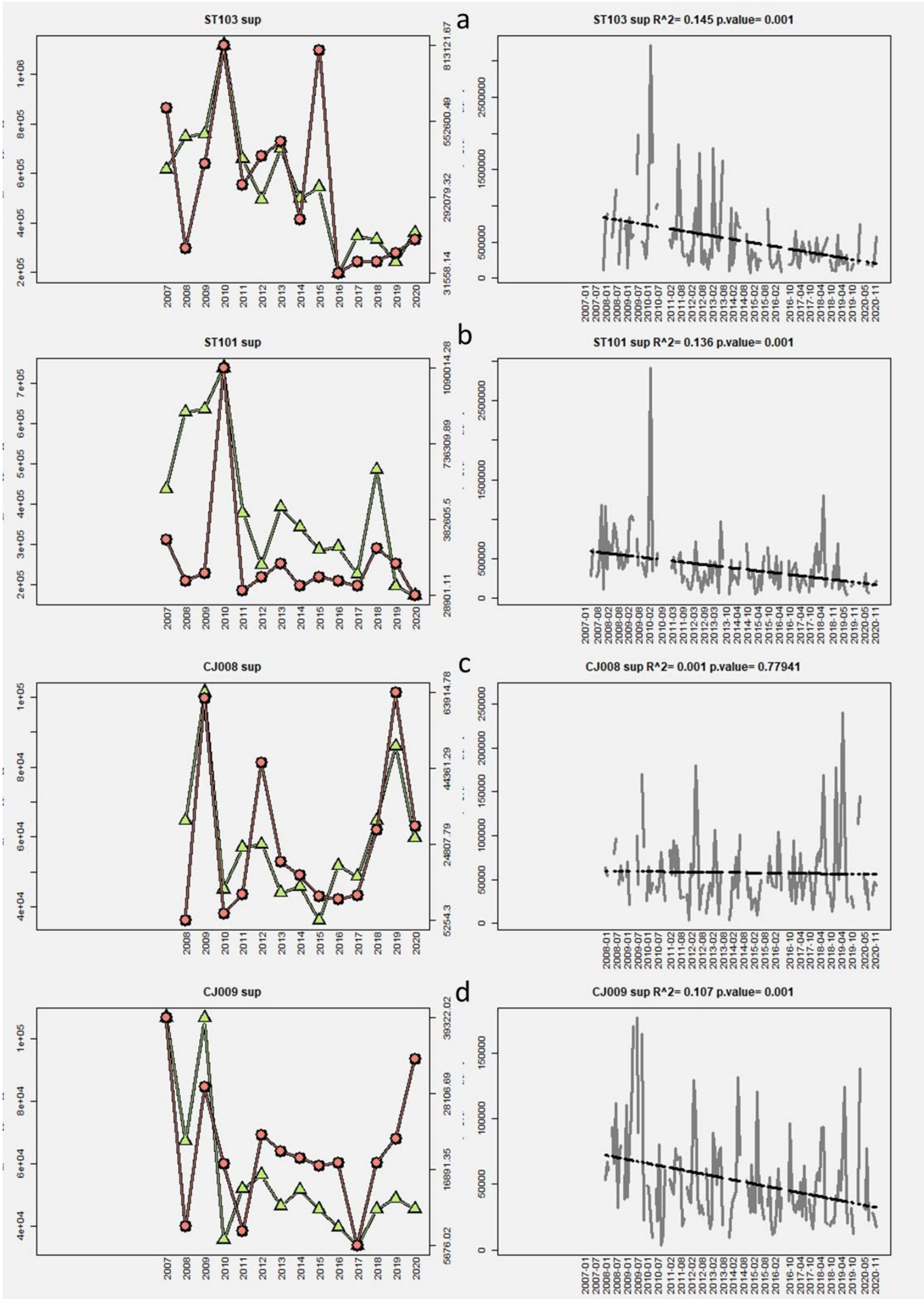

Figure 9: Phytoplankton abundance in the surface layer of stations ST103 (a), ST101 (b), CJ008 (c) and CJ009 (d) in the period 2007-2020. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); right panels: linear model

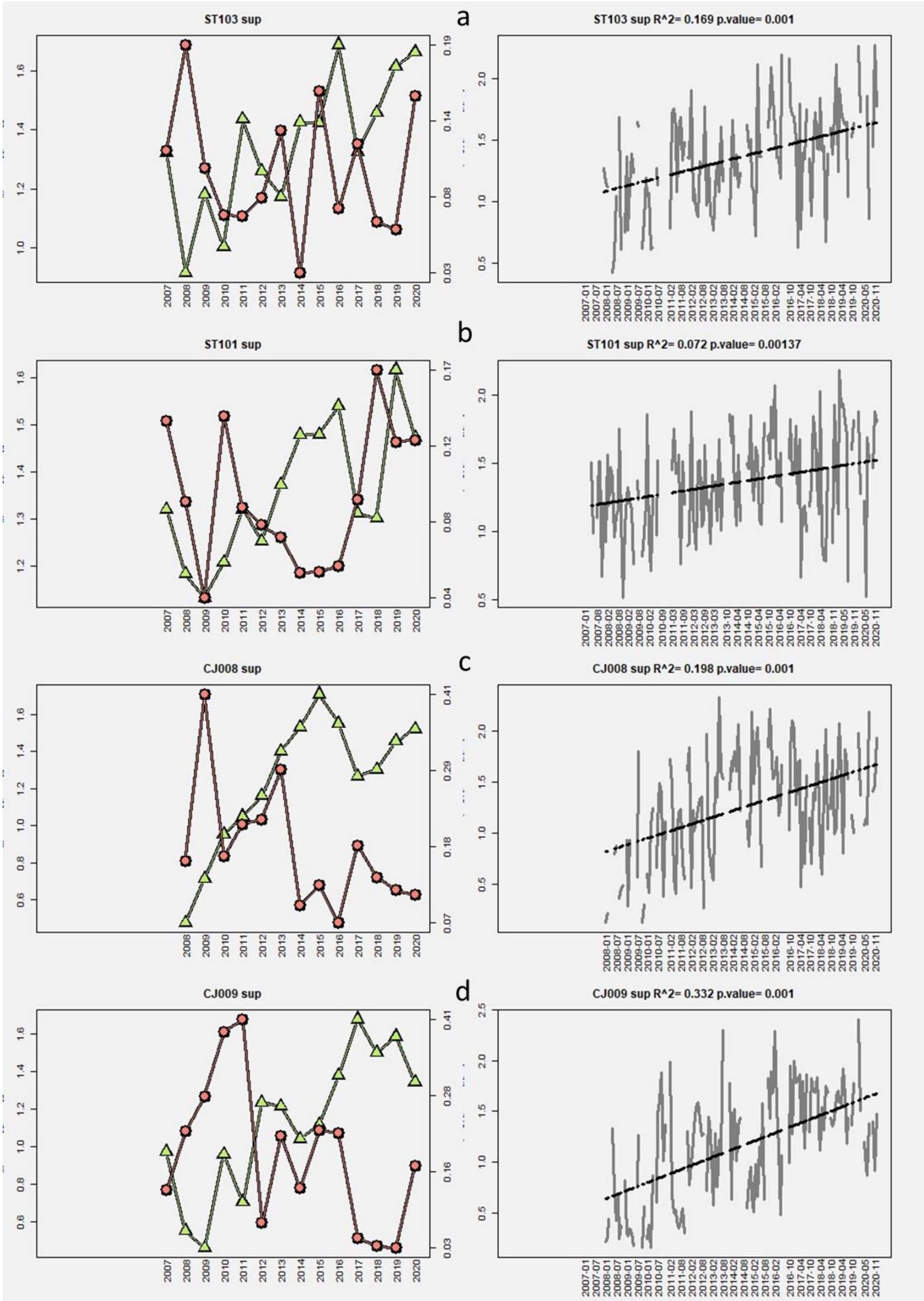

Figure 10: Shannon - Wiener's diversity index in the surface layer of stations ST103 (a), ST101 (b), CJ008 (c) and CJ009 (d) in the period 2007-2020. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); right panels: linear model

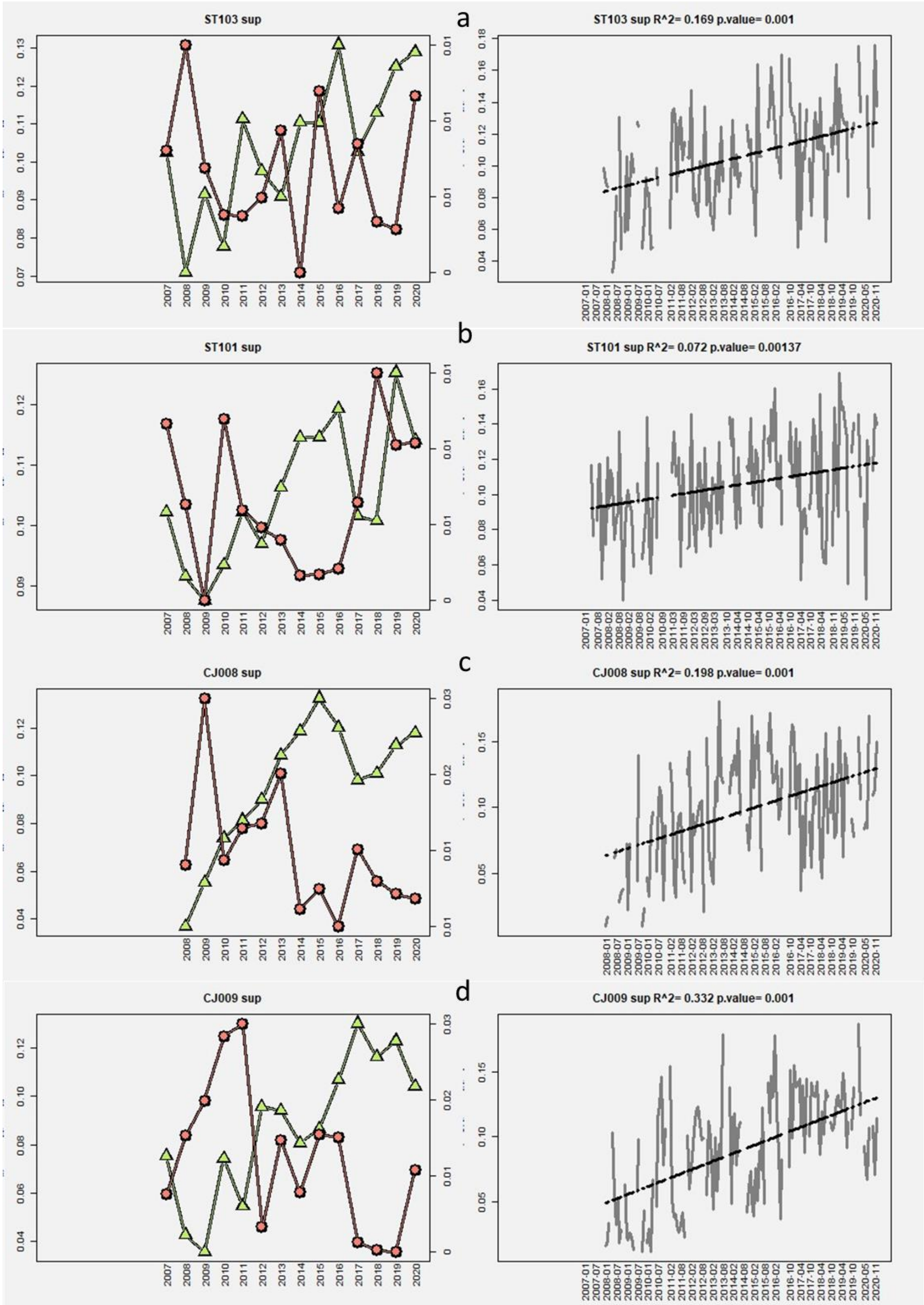

Figure 11: Pielou's evenness in the surface layer of stations ST103 (a), ST101 (b), CJ008 (c) and CJ009 (d) in the period 2007-2020. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); right panels: linear model

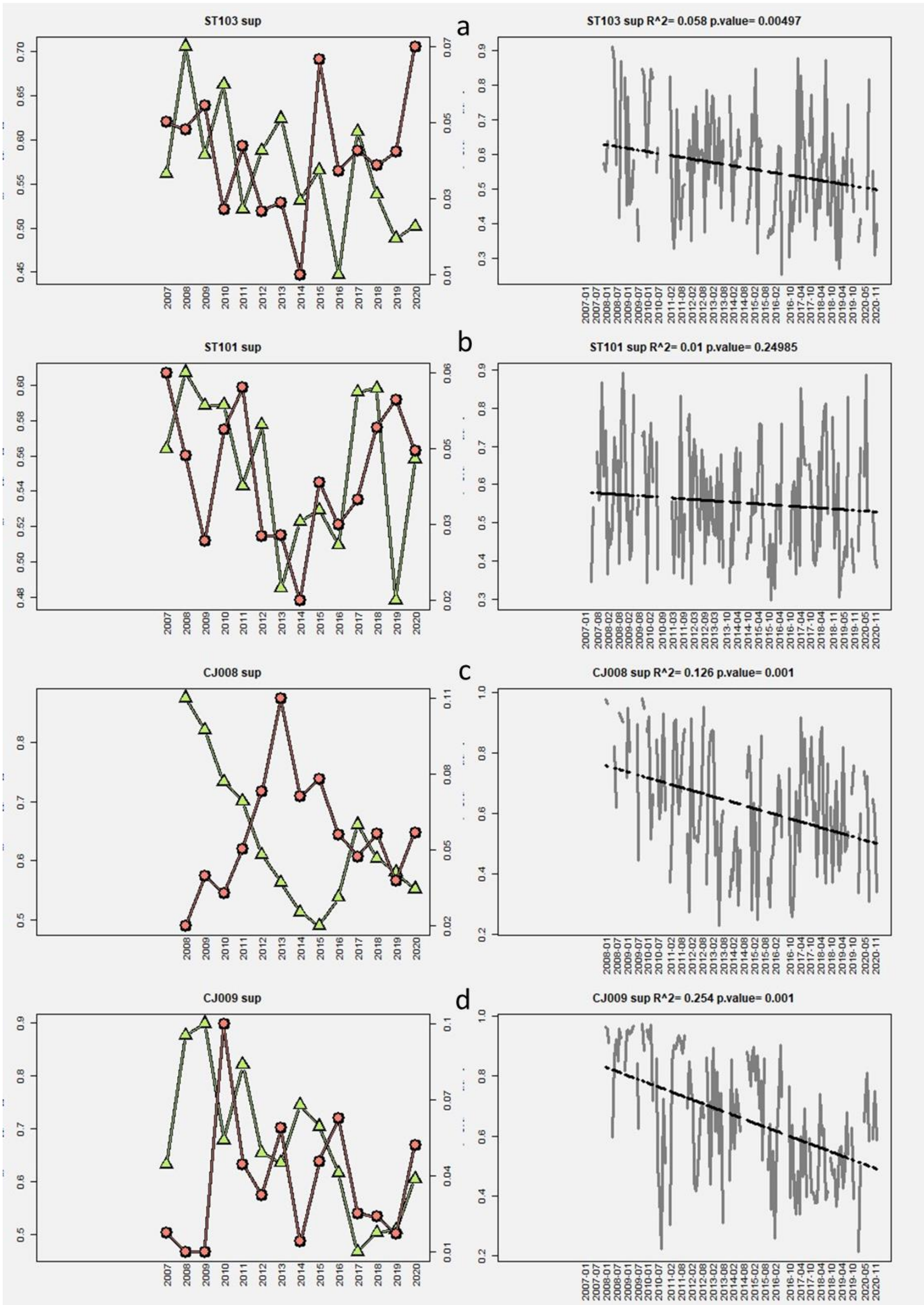

Figure 12: Berger-Parker's dominance in the surface layer of stations ST103 (a), ST101 (b), CJ008 (c) and CJ009 (d) in the period 2007-2020. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); right panels: linear model

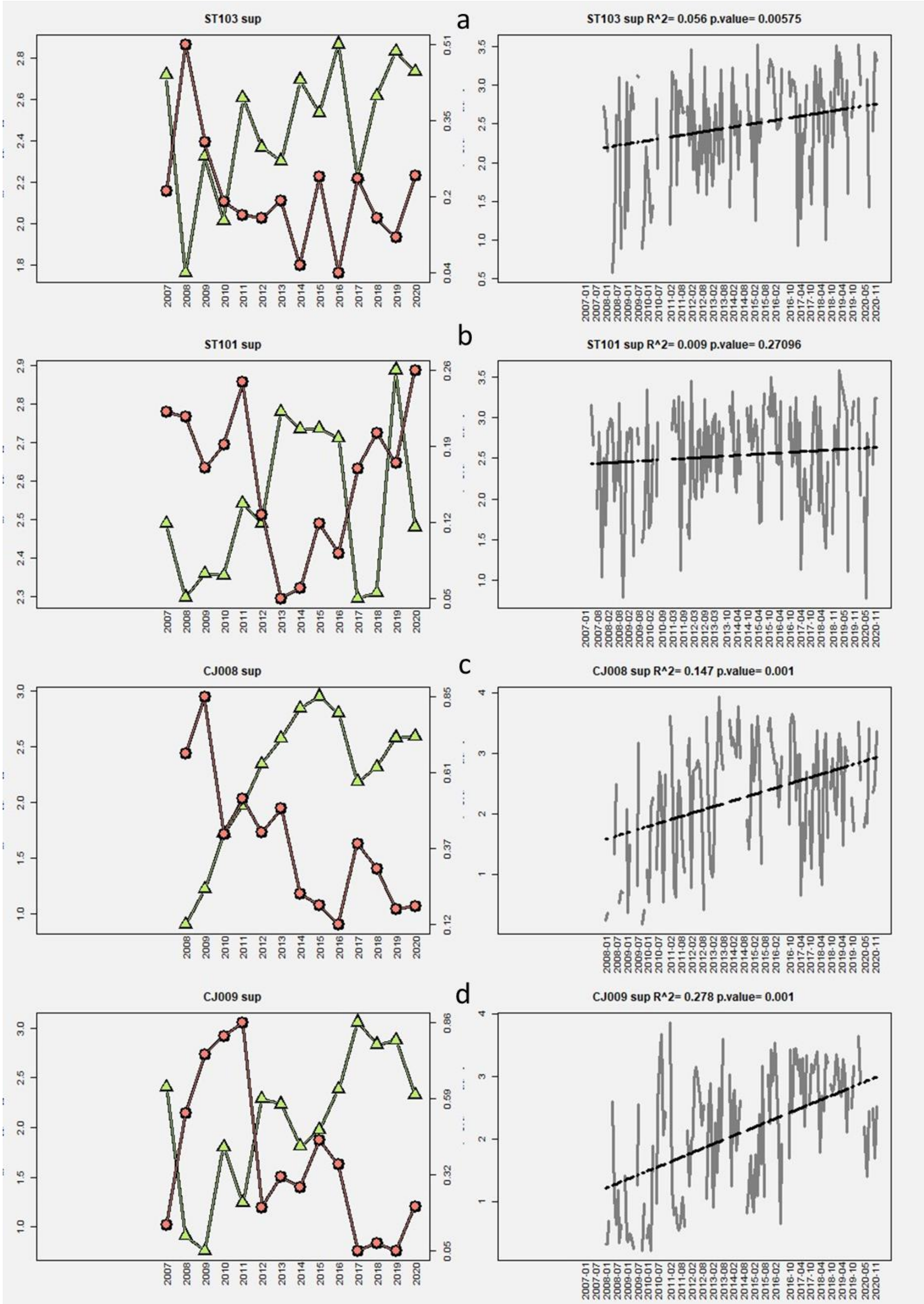

Figure 13: Rao's index in the surface layer of stations ST103 (a), ST101 (b), CJ008 (c) and CJ009 (d) in the period 2007-2020. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); right panels: linear mode

Conclusions

The results of this 14-year phytoplankton case study show an increasing trend in the number of genera (S) at all investigated stations. This trend can be observed with different fluctuations (COV) at both the coastal and open stations. In contrast, the total amount of phytoplankton did not increase significantly during the study period, but actually decreased. The abundances of phytoplankton correspond to the prevailing environmental conditions and confirms the coast-offshore gradient of phytoplankton found in previous studies (Marasović et al., 2005, Ninčević Gladan et al., 2010).

The analysed diversity indices (Shannon-Wiener's, Pielou's, and Rao's indices) also showed an upward trend during the study period, although it was less pronounced than the richness of genera. This trend is only slightly more pronounced at the stations in the open sea and at all depths investigated. This shows that, despite the different hydrodynamic conditions in these areas, there are no considerable differences in the temporal dynamics of the studied indices between impacted or non-impacted sites. It is also worth mentioning that in the last decades, none of the investigated sites could be characterized as heavily polluted, as was the case of the site ST103 back in the 1980s. There were no obvious seasonal fluctuations in diversity indices and genus richness. In terms of community composition, diatoms and phytoflagellates predominated throughout the study period. Only dinoflagellates showed a more pronounced seasonal signal, but their abundances were moderate and not comparable to the values of the period 1970-1990, when they were known to be equal or even superior to those of diatoms (Ninčević Gladan et al., 2010). Changes in the ratio of these two groups have been used as an indicator of short- and long-term environmental change in a variety of ecosystems (McQuatters-Gollop et al. 2019), but their importance has weakened in the eastern Adriatic in recent decades as diatoms predominate and the only apparent change in dinoflagellates is seasonally influenced.

All this suggests that the increasing diversity observed in the studied area of the eastern Adriatic cannot be attributed solely to the different degree of anthropogenic pressure, but that other large-scale trends such as climate change (e.g. Henson et al., 2021) should also be taken into account.

2.1.4 Slovenia

Part of the dataset from the Slovenian part of the Gulf of Trieste was already used for the large-scale analysis of phytoplankton diversity indices during the MEDCIS project - Support Mediterranean MSs towards Coherent and coordinated Implementation of the second phase of the MSFD (Francé et al., 2021). For the ABIOMMED project, the dataset was amended with recent data, so that data comprised the period 2005–2020.

Five sampling stations were selected, all sampled under the national MSFD and WFD monitoring programs: 000F, 00CZ, 000K, 00MA and 0DB2 (Figure 14). Samples for phytoplankton community analyses were taken at 4 standard depths: at the surface, at 5 m, at 10 or 15 m and near the bottom, with monthly frequency. All sampling stations are located in the Slovenian Sea, which is part of the Gulf of Trieste, the northernmost semi-enclosed bay of both the Adriatic (sub-region) and Mediterranean Sea (region). The southern boundary of the Gulf of Trieste is formed by an imaginary line between the towns of Savudrija (Croatia) and Grado (Italy). The bay has an area of 548 km^2 and an average depth of 16.4 m. The central and partly south-eastern part of the bay is mostly deeper than 20 m, while the north-western part is shallower. The maximum depth in the Gulf of Trieste is 38 m.

Slovenian coastal waters are affected by a range of natural and anthropogenic influences. There is an intense water mass exchange with the Adriatic Sea at the open boundary, which influences the oceanographic characteristics of the gulf. Besides, the gulf is affected by the freshwater inputs that are largely dominated by the Soča (Isonzo) River outflow in the northern part of the gulf. Minor rivers and streams outflowing along the Slovenian coast contribute only marginally and locally to overall freshwater inputs. The water column remains mixed throughout the winter, while in the spring, increased freshwater inflows and surface layer warming contribute to stratification, which intensifies during summer (Malačič and Petelin, 2001). The Gulf of Trieste is a crossroads of human influences, from intense maritime traffic to fisheries and aquaculture (primarily mussel farming), all of which place significant pressure on the coastal sea. In the past, phytoplankton blooms, which contribute to the development of hypoxia or even anoxia in the bottom layer, were the main consequence of eutrophication in this area (Kralj et al., 2019). Long-term studies have revealed significant spatial and, more importantly, temporal variability in phytoplankton biomass and community structure, reflecting rapidly changing hydrological and nutritional conditions in the Gulf of Trieste (Mozetič et al., 2012; Vascotto et al., 2021). In the recent past, however, a significant decline in chlorophyll-a concentrations and changes in the structure of the phytoplankton community have been observed throughout the northern Adriatic (Cabrini et al., 2012; Cerino et al., 2019; Mozetič et al., 2012, 2010; Totti et al.,2019a; Vascotto et al., 2021), mainly due to the decrease in nutrient concentrations, especially phosphates.

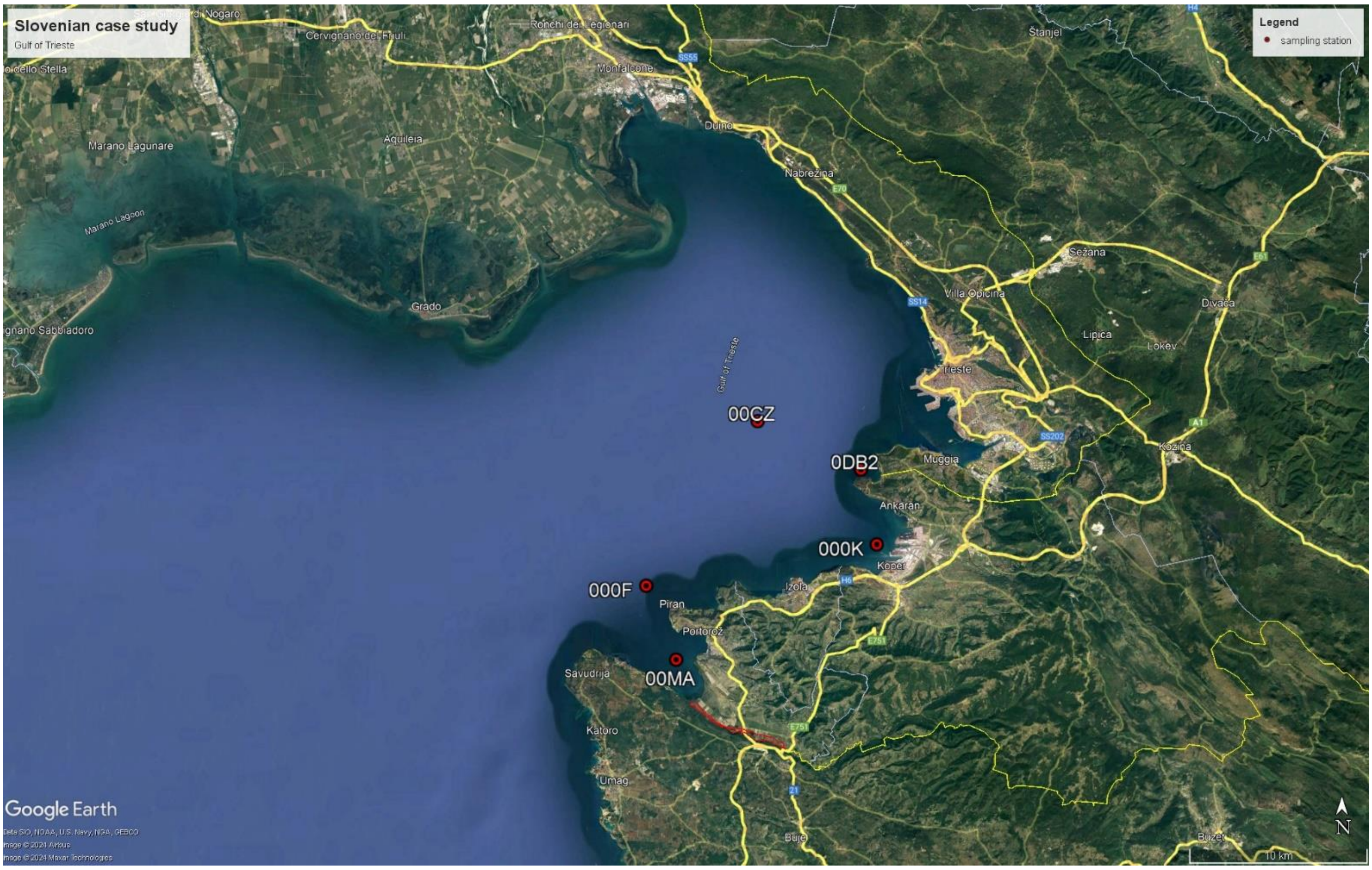

Figure 14: Map of the Slovenian case study area with the sampling stations (northern Adriatic Sea, Gulf of Trieste)

2.1.4.1 N Adriatic, Gulf of Trieste

Sampling station 000F represents reference conditions (impact category 0). It is located at the southern entrance to the gulf, where the direct effects of freshwater inputs and other land-based influences are minimal. Therefore, also the phytoplankton biomass is comparably low. Sampling station 000F was sampled in the period 2005-2020 with monthly frequency at three to five depths. Results are presented in three layers (station depth 21 m): surface (0 m, 5 m), middle (10 m, 15 m) and bottom layer (21 m). The middle layer was sampled only till 2013.

Sampling station 00CZ represents reference conditions (impact category 2). It is located in the central part of the Gulf of Trieste, where the influence of the Soča River outflow is particularly pronounced. This also affects the phytoplankton chlorophyll biomass, which is usually the highest among the Slovenian stations. Sampling station 00CZ was sampled in the period 2009-2013 with monthly frequency at three to four depths. Results are presented in three layers (station depth 24 m): surface (0 m, 5 m), middle (10 m) and bottom (24 m). The bottom layer was sampled only in 2012 and 2013.

Sampling station 000K represents impacted conditions (impact category 3). It is located in the middle of the Bay of Koper, which is mainly influenced by the outflows of the smaller Slovenian rivers Rižana and Badaševica and the densely populated coast. The chlorophyll-a biomass here is generally one of the highest in the Slovenian sea. Sampling station 000K was sampled in the period 2007-2011 with monthly frequency at four depths. Results are presented in three layers (station depth 16 m): surface (0 m, 5 m), middle (10 m) and bottom layer (16 m).

Sampling station 00MA represents reference conditions (impact category 0). It is located in Piran Bay, which is fed by the smaller Drnica and Dragonja rivers, but the surrounding area is not densely populated, and the cumulative impact is lower. The chlorophyll biomass here is among the lowest in the Slovenian sea. Sampling station 00MA was sampled in the years 2007, 2008 and 2010, 2011 with monthly frequency at four depths. Results are presented in three layers (station depth 16 m): surface (0 m, 5 m), middle (10 m) and bottom (16 m).

Sampling station 0DB2 represents impacted conditions (impact category 3). It is located between two densely populated areas, Koper and Trieste, and at the same time on the edge of mussel farming areas. Also here, the phytoplankton biomass is also among the highest in the Slovenian sea. Sampling station 0DB2 was sampled in the years 2007, 2008 and 2010, 2011 with monthly frequency at four depths. Results are presented in three layers (station depth 18 m): surface (0 m, 5 m), middle (10 m) and bottom layer (18 m).

The annual means of selected diversity indicators and trend analysis showed the most reliable results for the station 000F, which host the longest time series among Slovenian sampling stations (surface layer in Figures 15-20, middle and bottom layer in Suppl. Figures S31-S36). For the genus taxonomic richness an almost steady rise was observed in the surface layer in the period 2005-2020, with only a transient decrease in 2018-2019 preceded by a peak in 2016-2017 as evidenced by deseasonalised trend (Figure 15). The COV was high but almost always steady, showing the intraannual variability of the richness. In the middle layer the trend was also positive, although the data time series was shorter here (Suppl. Figure S31a), while in the bottom layer the positive trend was very low, though still significant (Suppl. Figure S31b). COV exhibited more variation in the middle and bottom layers in comparison to the surface one.

Annual means of phytoplankton abundance at station 000F did not follow the trend of richness but showed substantial interannual variability, almost mirrored in COV (Figure 16, surface layer and Suppl. Figure S32a and b, middle and bottom layers, respectively). Both linear and deseasonalized trends show a slight decrease of abundance over the study period only in the bottom layer (Suppl. Figure S32). On the contrary, similar positive trend as for richness was found in the surface layer for Shannon - Wiener's diversity index (Figure 17) and Pielou's evenness (Figure 18), while that of Berger-Parker's dominance was negative (Figure 19). Annual means and COV for Shannon - Wiener's diversity index and Pielou's evenness had an inverse relationship, in the first period with low values of diversity and evenness, COV values were higher in comparison to the second period when higher values of diversity and evenness were accompanied by generally low COV. This imply that in years with higher diversity and evenness also the indices varied less on a monthly basis. The COV for Berger-Parker's dominance annual values was also lower and less variable in the second part of the study period, indicating that the values of dominance were also more stable intraannually. Similar trends of all these indices were found also in the middle and bottom layer of the station 000F (Suppl. Figures S33-S35).

Rao's index in the surface layer of the station 000F showed very similar behaviour to Shannon - Wiener's diversity, either in terms of annual means ad COV or trends (Figure 20). Also in the middle and bottom layer the Rao's index had a positive trend, though it was not so accentuated in the bottom layer (Suppl. Figure S36).

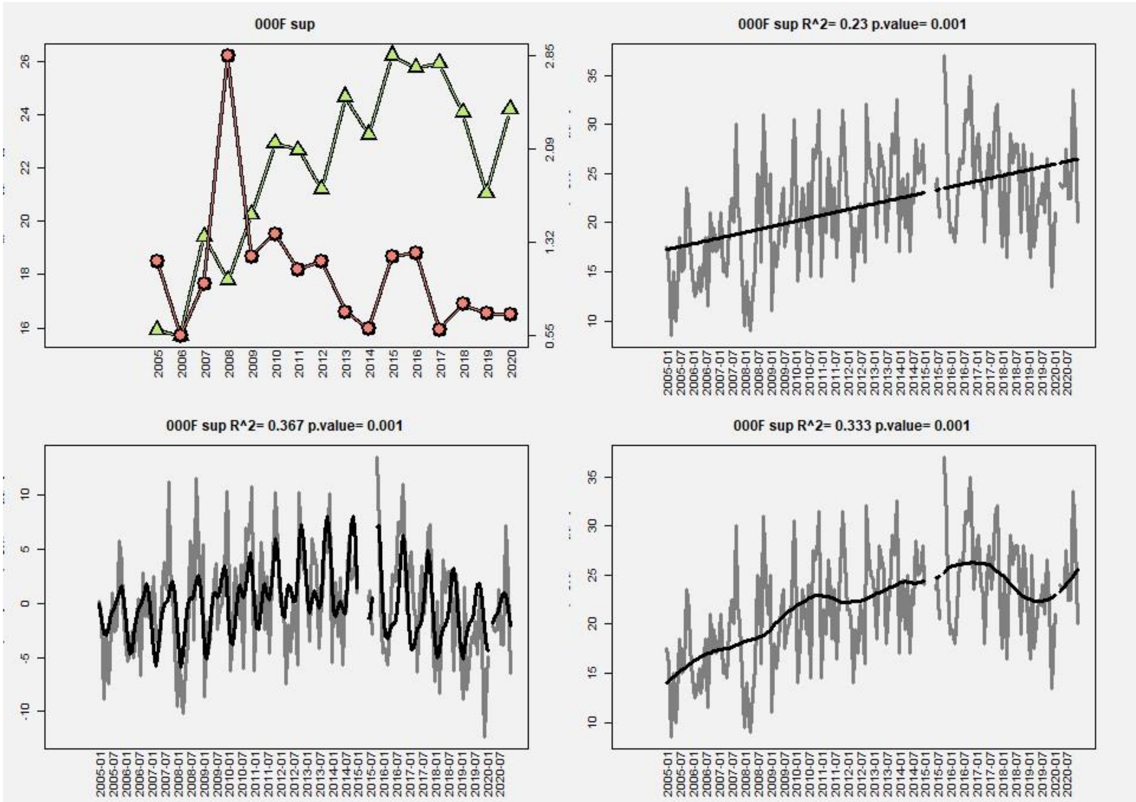

Figure 15: Genus richness in the surface layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

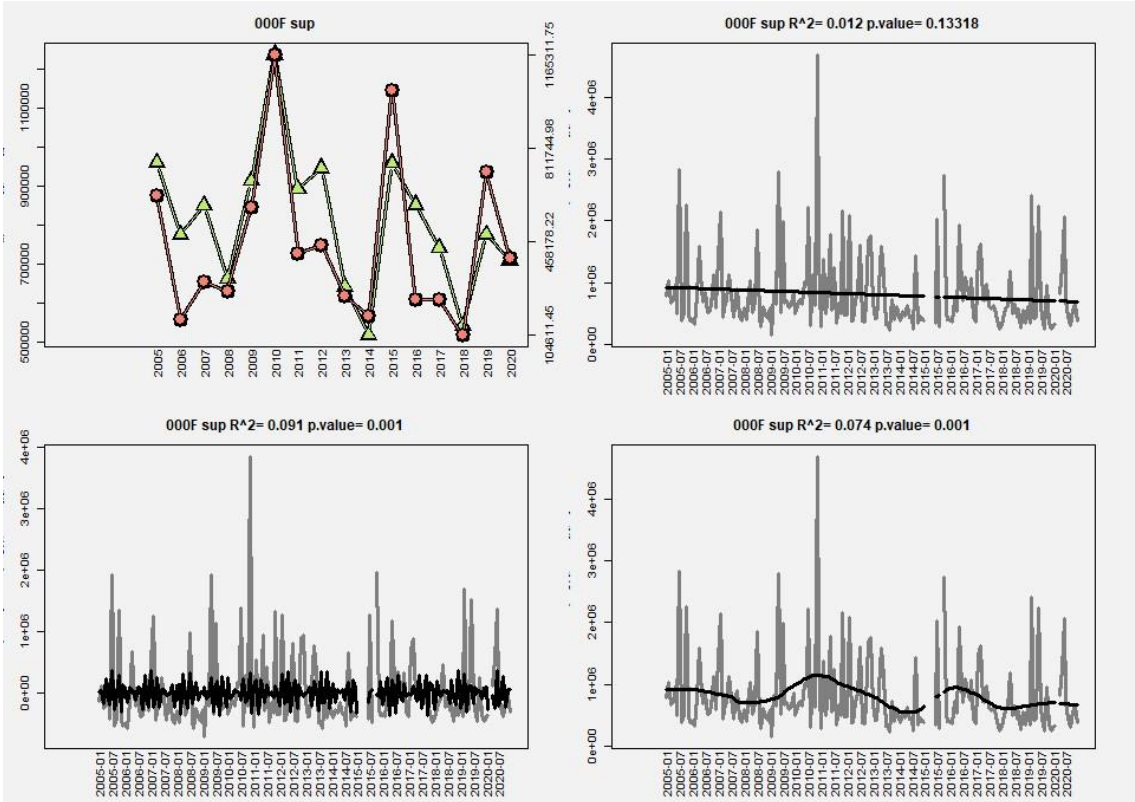

Figure 16: Phytoplankton abundance in the surface layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

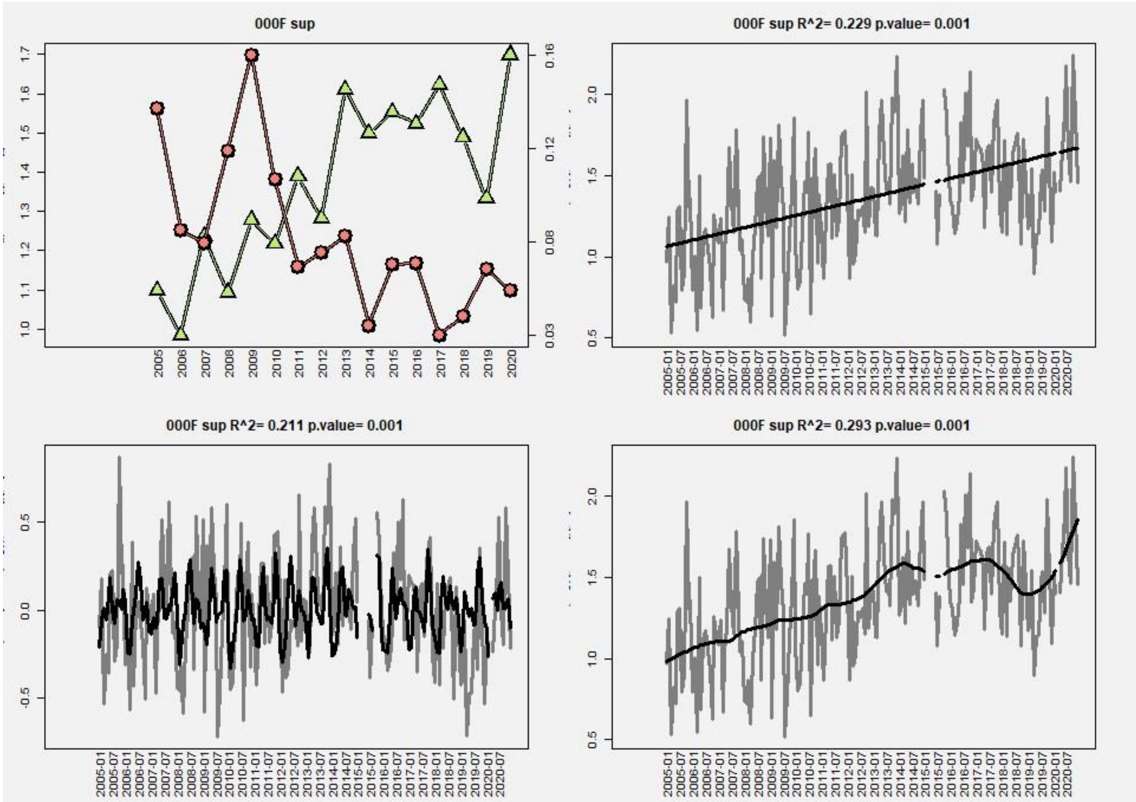

Figure 17: Shannon - Wiener’s diversity index in the surface layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

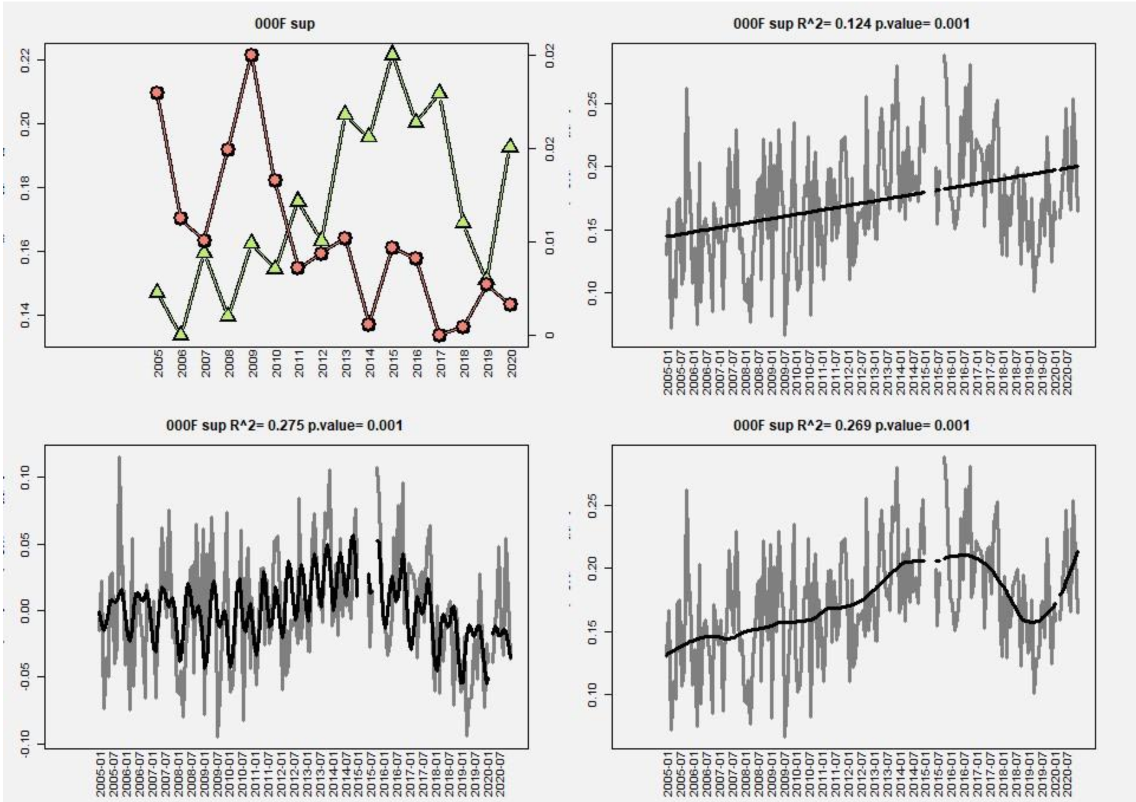

Figure 18: Pielou’s evenness in the surface layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

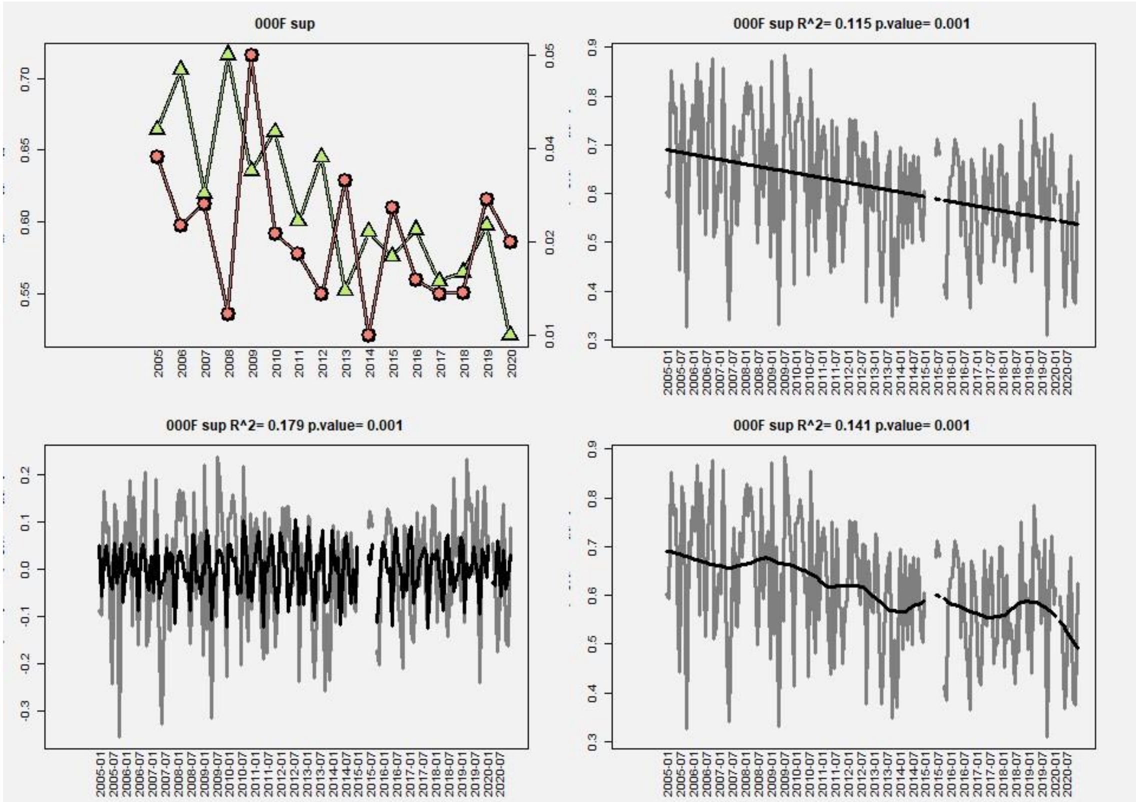

Figure 19: Berger-Parker's dominance in the surface layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

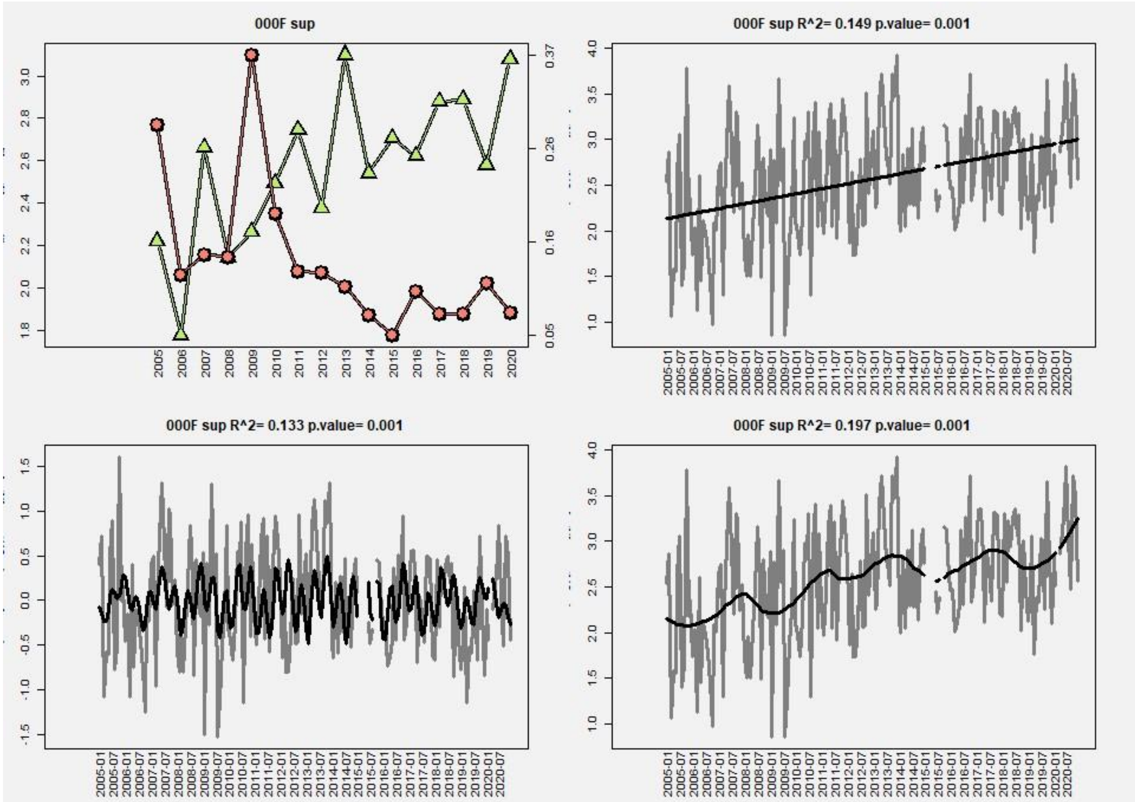

Figure 20: Rao's index in the surface layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

The available data series was substantially shorter at other sampling stations (00CZ, 000K, 00MA and 0DB2) covering the period of 5 years and wit a one-year gap at stations 00MA and 0DB2. However, the analyses showed quite similar trends to the one at station 000F: mostly statistically significant positive at all four stations for genus richness (Figure 21, Suppl. Figures S37, S43), statistically significant positive at the station 00CZ for Shannon - Wiener's diversity (Figure 23, Suppl. Figures S39, S46), and Pielou's evenness (Figure 24, Suppl. Figures S40, S47). However, for phytoplankton abundance (Figure 22, Suppl. Figures S38, S45) and Berger-Parker's dominance (Figure 25, Suppl. Figures S41, S48) no significant trends were observed, except for the latter at 00CZ sampling station (negative trend as in station 000F). Differently, Rao's index showed a statistically significant positive trend only at stations 00MA and 0DB2 (Figure 26, Suppl. Figures S42, S49).

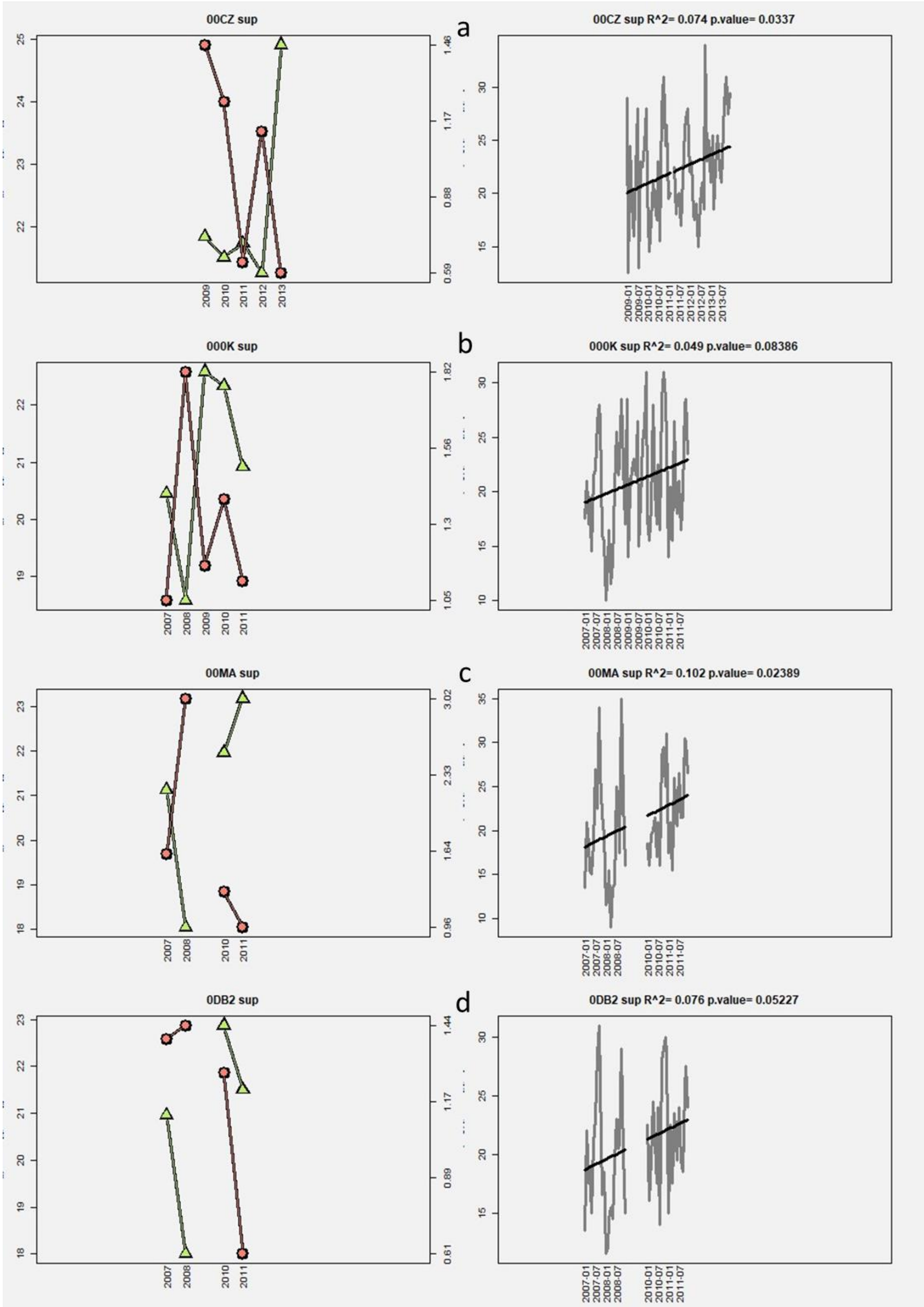

Figure 21: Genus richness in the surface layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

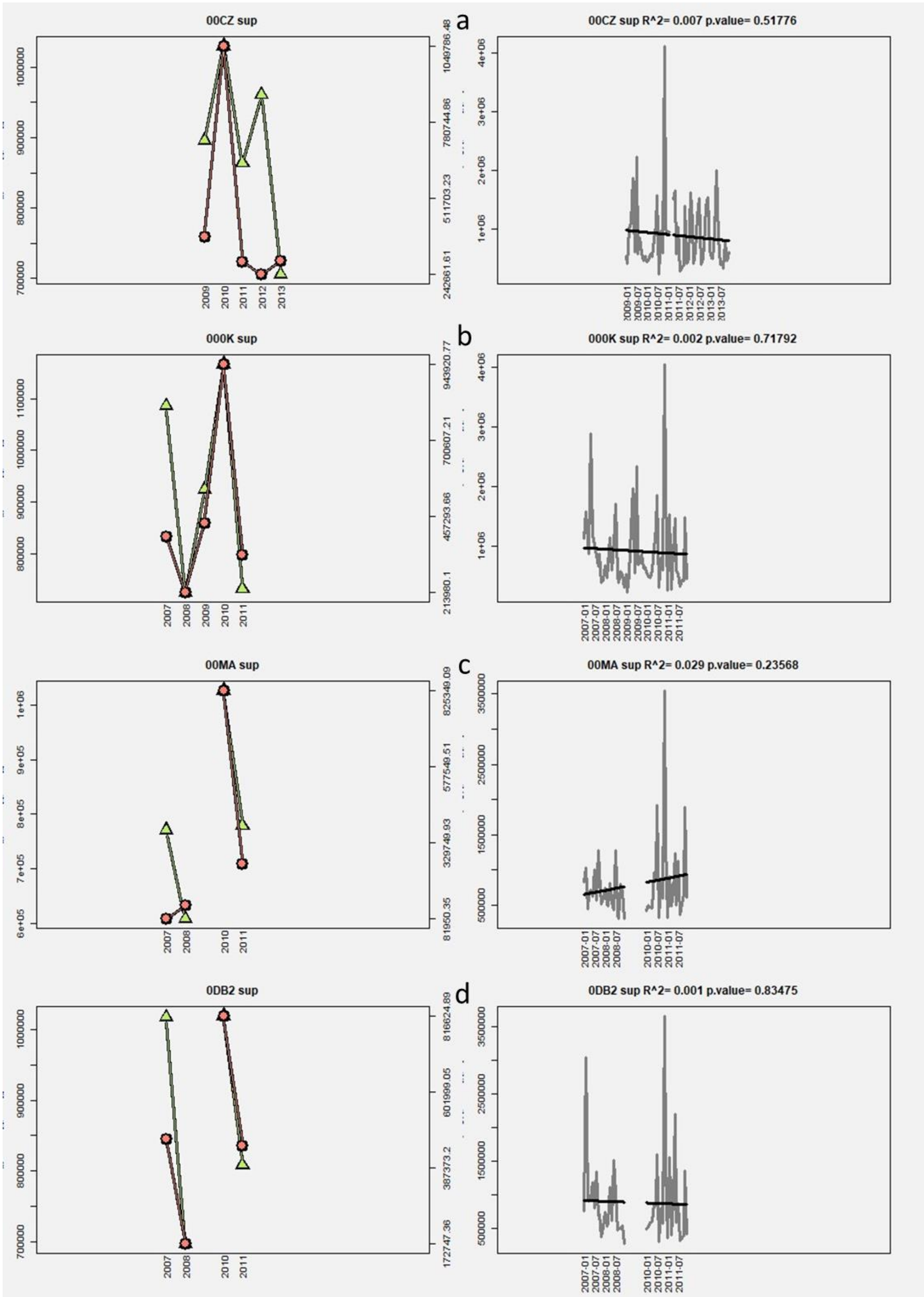

Figure 22: Phytoplankton abundance in the surface layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011 Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

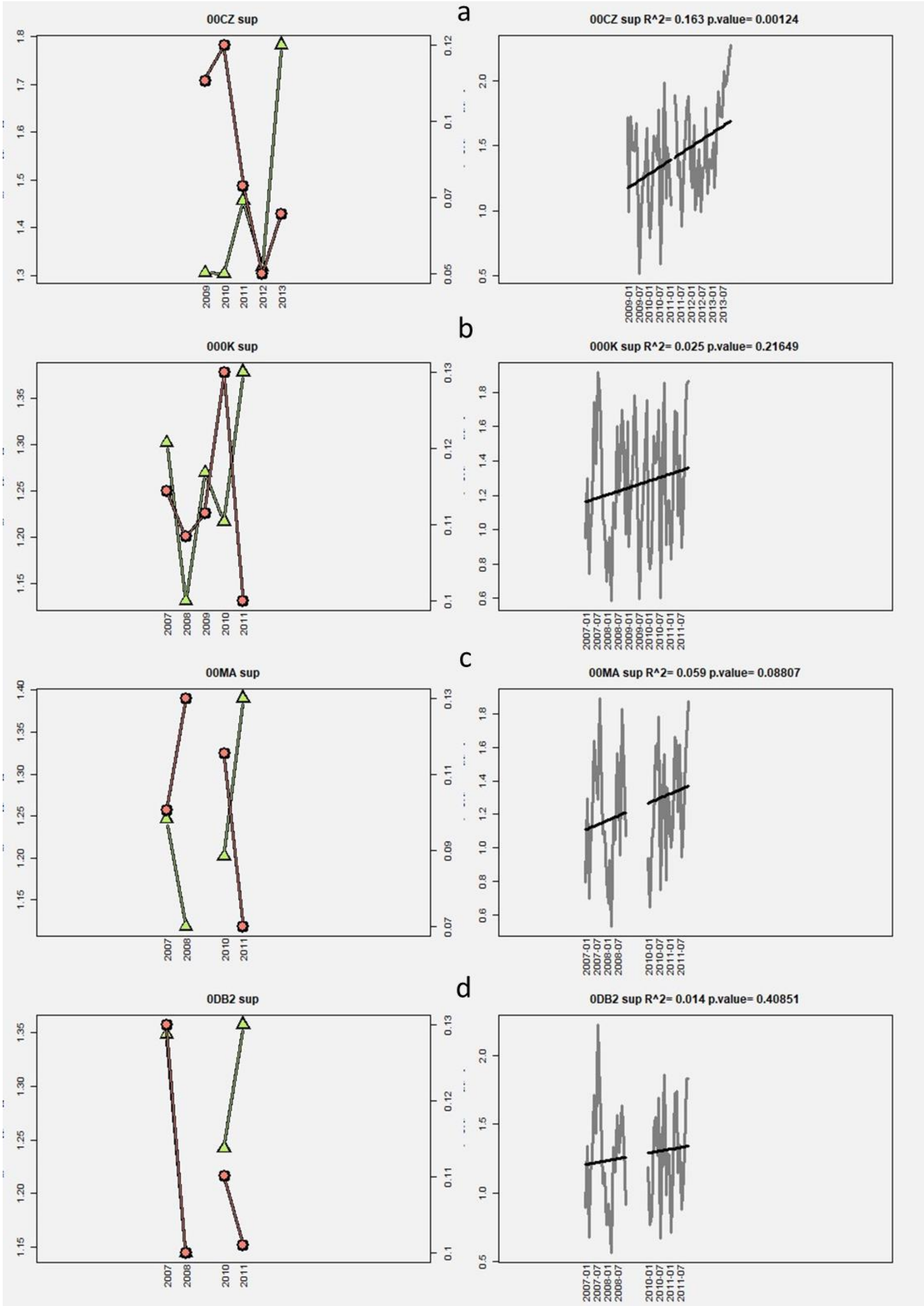

Figure 23: Shannon - Wiener's diversity index in the surface layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

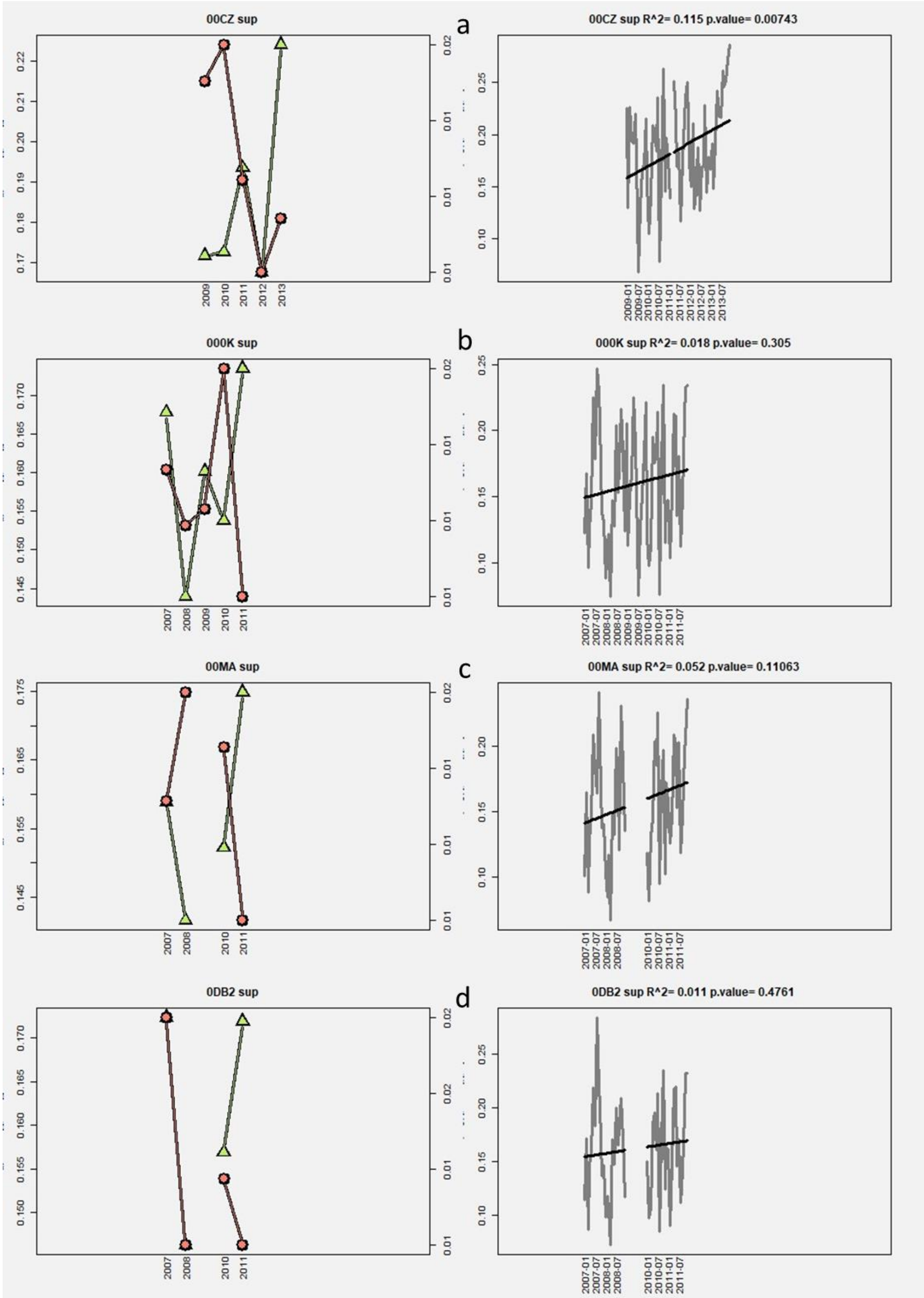

Figure 24: Pielou's evenness in the surface layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

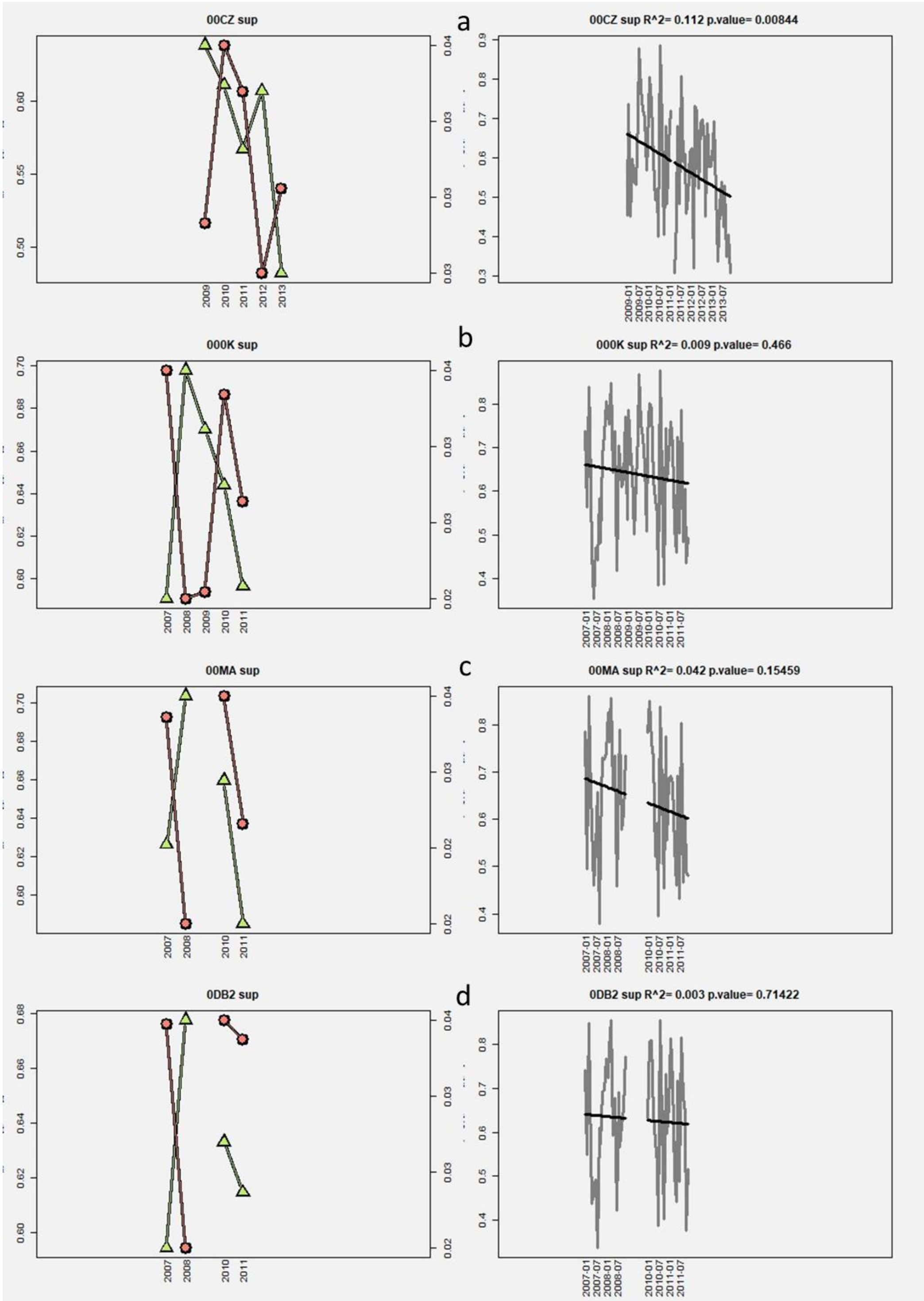

Figure 25: Berger-Parker's dominance in the surface layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

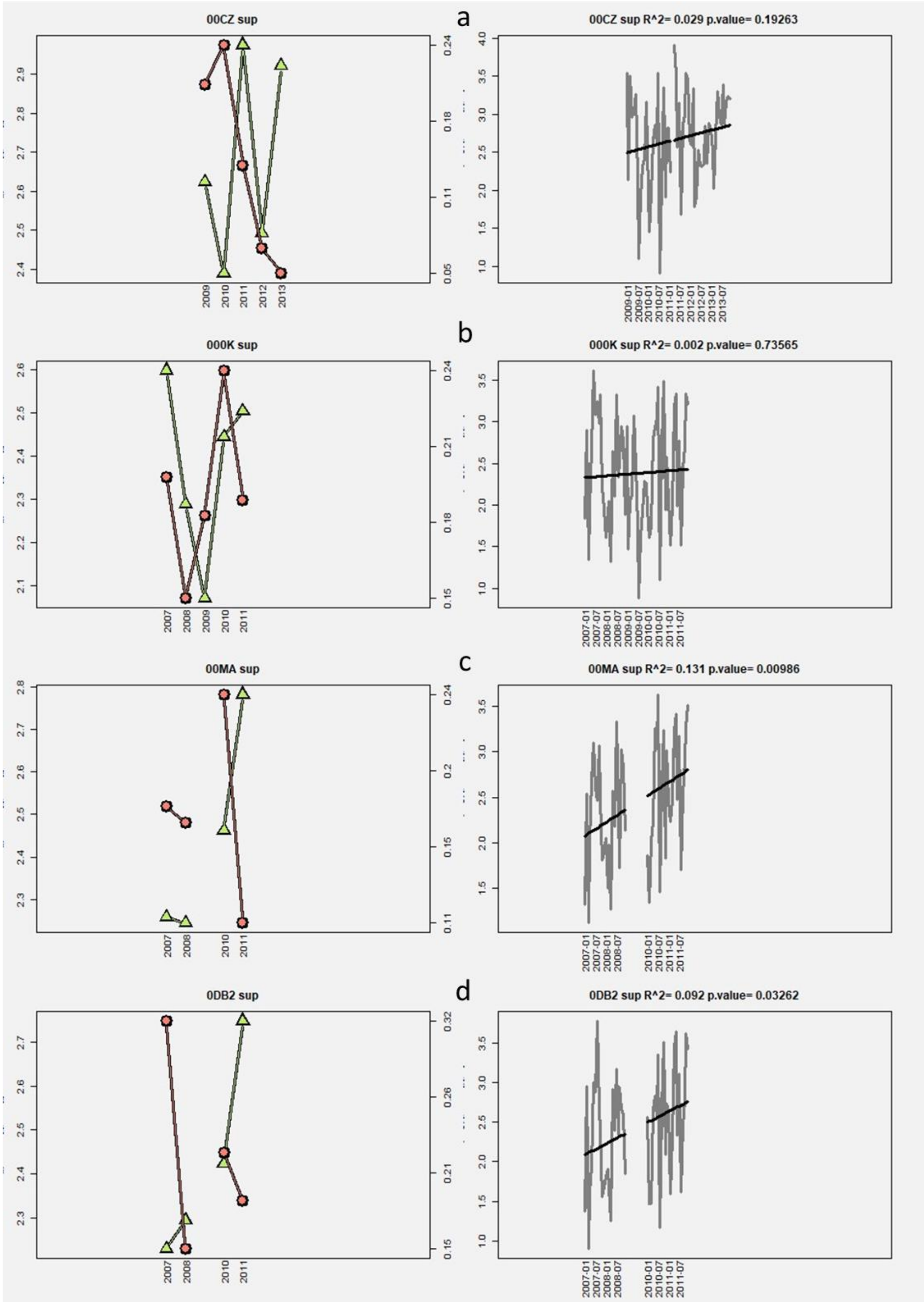

Figure 26: Rao's index in the surface layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

Conclusions

In summary, the analysis revealed consistent positive trends in genus richness in all Slovenian stations, but with different trends in phytoplankton abundance and diversity indices. Genus richness was on average slightly higher at stations in open waters (000F and 00CZ) than at other, more coastal stations, but the differences were very small, even if we take into account the different lengths of the time series. The same small differences in mean values were also observed for diversity and evenness, which were also slightly higher at the open-water stations, while the differences in dominance were reversed. Differences between stations were also observed in the results of the ecological status assessment using Chlorophyll-a, but always showed good to very good status (Francé et al. 2023). For Chlorophyll-a, the situation primarily reflected the reduced nutrient inputs via river discharges and precipitation in recent years and fluctuations in the same direction at all stations. The years 2018 and 2022 in particular were characterised by a pronounced hydrological drought, which led to a very low phytoplankton biomass at all stations. The dry year 2018 was also reflected in the abundance at station 000F, which was particularly low, but other indices were not particularly different. However, it must be emphasised that the phytoplankton community in the Gulf of Trieste underwent a profound change at the beginning of the 21st century (Mozetič et al., 2010, 2012) and that the reduced trophic status in the Slovenian Sea persists to this day.

The longer time series at station 000F certainly provides more reliable results, while the shorter data series at the other stations still show similar patterns, suggesting that the effects of environmental changes on phytoplankton communities are the same at all sampling stations, regardless of the different anthropogenic influences. For example, Vascotto et al. 2021 showed that the phytoplankton community at station 000F experienced a switch from a more predictable to a more erratic community dynamics, probably triggered by climatic and hydrological factors at the mesoscale. We believe that phytoplankton at other Slovenian sampling stations have also experienced similar changes that exceed those caused by anthropogenic influences. These facts make it extremely difficult to assess which conditions might represent reference conditions, and even to judge whether the observed trend in diversity indices indicates a better or worse state of the phytoplankton community. Rather, we suspect that they represent a continuum of different situations that are possible in a given environment and are caused by fluctuating/changing environmental conditions beyond the reach of human intervention.

2.1.5 Greece

Data from the Saronikos Gulf in the Aegean Sea was already used for the large-scale analysis of phytoplankton diversity indices during the MEDCIS project - Support Mediterranean MSs towards Coherent and coordinated Implementation of the second phase of the MSFD, together with data from other 5 sampling stations in Saronikos Gulf and 5 sampling station in Maliakos Gulf in central Greece (Francé et al., 2021). For the ABIOMMED project, we decided to use only the data from three sampling stations in Saronikos Gulf (S1, S7 and S11, Figure 27), because these datasets cover the longest period (although not very long compared to Slovenian and Croatia case studies).

The data from Saronikos Gulf cover the geographical area of the sub-region Mediterranean Aegean Levantine-Central Aegean. Saronikos Gulf is a coastal area near the Athens metropolitan area and the port of Piraeus, which communicates with the Aegean Sea to the south. The Bay of Elefsis (northern Saronikos) is on average 90 m deep, with limited water exchange, low freshwater inflows and therefore with strong seasonal stratification and low oxygen distribution. These characteristics, together with industrial and shipping activities, lead to the trapping and accumulation of nutrients and organic matter (Pavlidou et al., 2014; Pavlidou et al., 2019). The inner part of the Gulf is located near the port of Piraeus and receives the treated sewage of ~5 million people in the north. The southern inner part communicates with the outer part of Saronikos Gulf and receives the influence of the open Aegean waters. Fishing and aquaculture are common practices in Saronikos Gulf.

2.1.5.1 Aegean Sea, Saronikos gulf

Station S11 is located 5.5 nM off the coasts in the NW part of the Saronikos Gulf and represents reference conditions (impact category 0). It was sampled in the period 2008-2018, but the sampling frequency was usually very low, from once a year to 5 times per year. Results are presented in three layers (station depth 77 m): surface (2 m, 20 m), middle (50 m) and bottom layer (75 m). Only annual means of selected indices are presented due to low number of available data.

Station S7 is located near the Port of Piraeus, but still represents reference conditions though with impact category 2. It has the longest data set in the case study and was sampled in the period 2007-2018, but the sampling frequency was usually very low, from once a year to 5 times per year. Results are presented in three layers (station depth 77 m): surface (2 m, 20 m), middle (50 m) and bottom layer (75 m). Only annual means of selected indices are presented due to low number of available data.

Station S1 is located in the central part of the Bay of Elefsis and therefore represents impacted conditions with impact category 3. It was sampled only in the period 2013-2018, but the sampling frequency was usually very low, from once a year to 5 times per year. Results are presented in three layers (station depth 25 m): surface (2 m), middle (10 m) and bottom layer (20 m). Only annual means of selected indices are presented due to low number of available data.

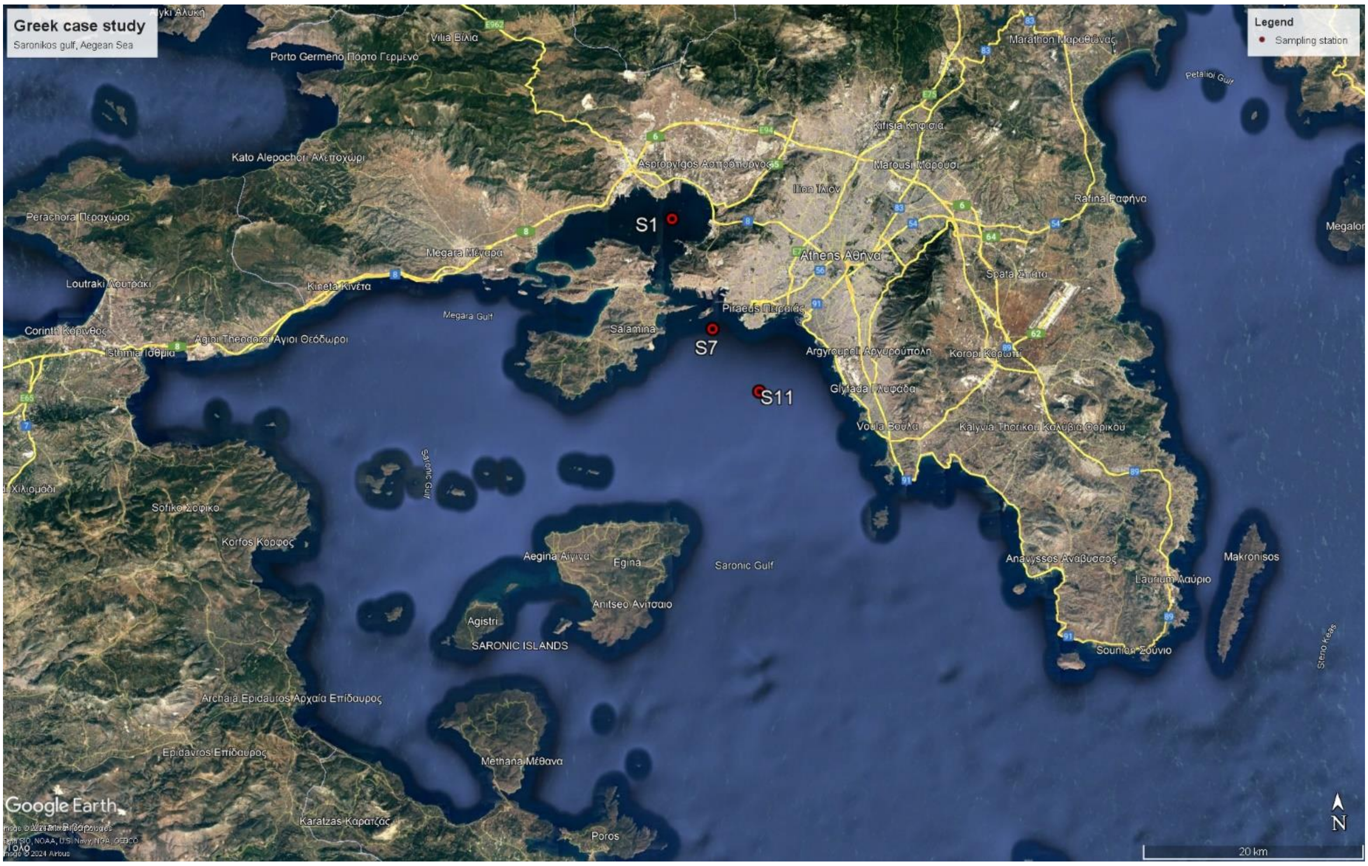

Figure 27: Map of the Greek case study area with the sampling stations (Aegean Sea, Saronikos Gulf)

The annual means of the set of selected diversity indices and phytoplankton abundance show above all a great interannual variability and no obvious pattern (Figures 28-30). For the station S11 the variability was greater in the period 2008-2010, while in the period 2013-2018 the annual means varied less, except for the genus richness (Figure 28). On average, the highest abundance was attained at station S1 (Figure 30), but this is true just for the last two years of the time series. The highest annual means of diversity and evenness were calculated for station S11, where also the dominance values were on average lower in comparison to other two stations. Nevertheless, apart from these very vague differences there is no obvious temporal pattern in none of the stations. If we take into account the very limited sampling frequency, we cannot draw any conclusion about the reflection of the impact category of the stations in the Saronikos Gulf.

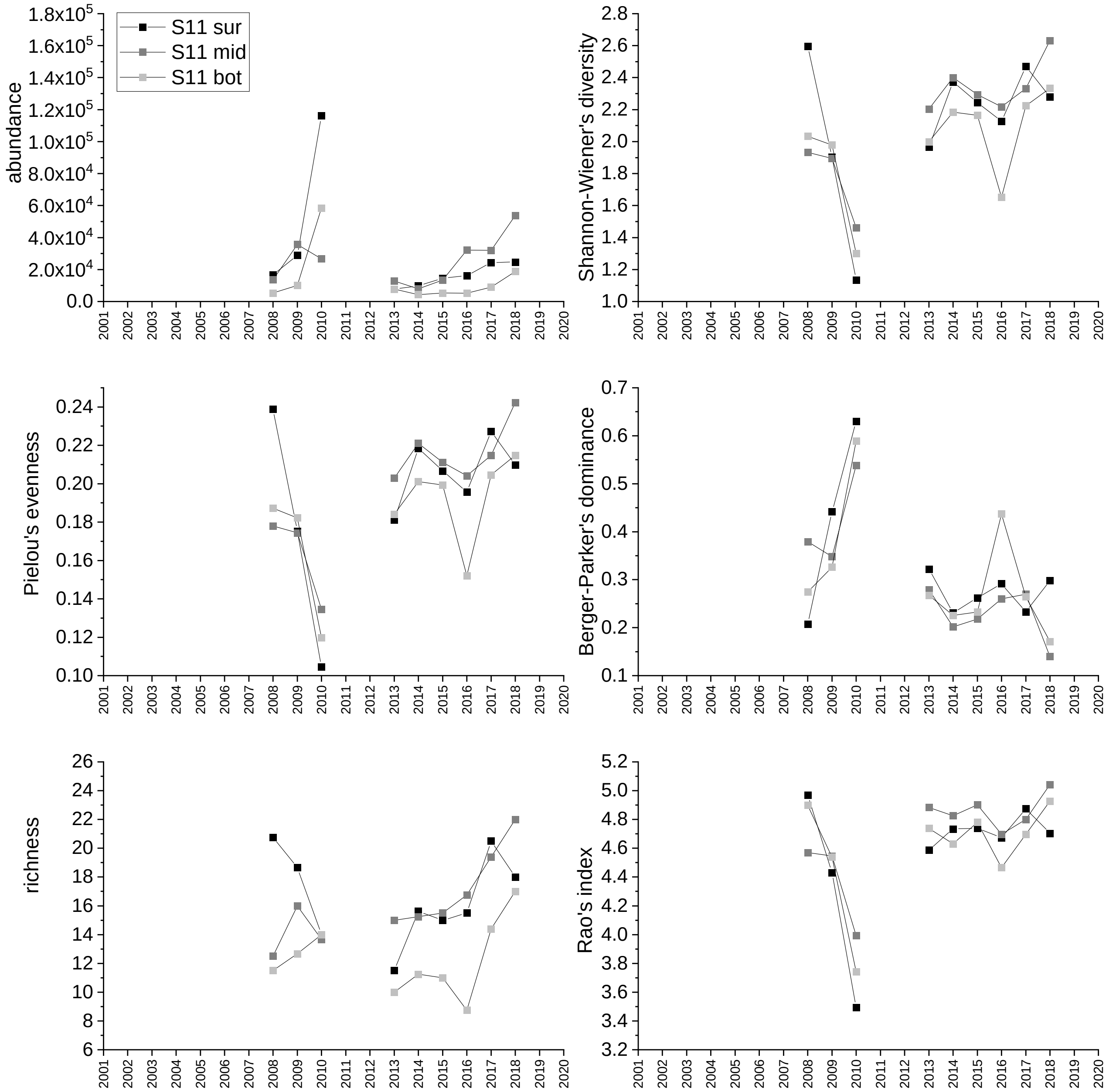

Figure 28: Annual means of selected diversity indices for station stations S11 in the period 2008-2018 in three water layers: sur (surface – 2 m, 20 m), mid (middle layer – 50 m) and bot (bottom layer – 75 m).

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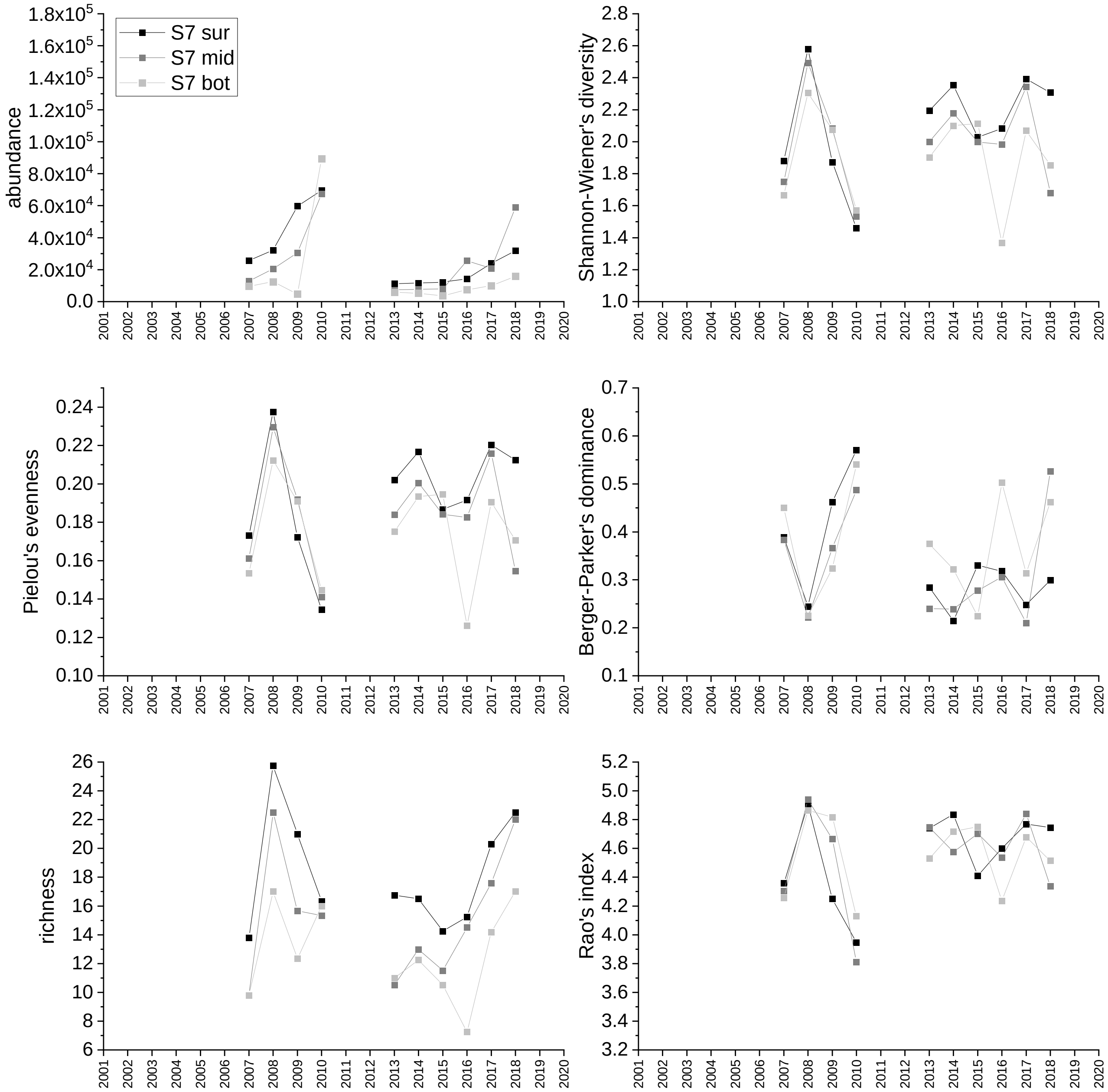

Figure 29: Annual means of selected diversity indices for station stations S7 in the period 2007-2018 in three water layers: sur (surface – 2 m, 20 m), mid (middle layer – 50 m) and bot (bottom layer – 75 m).

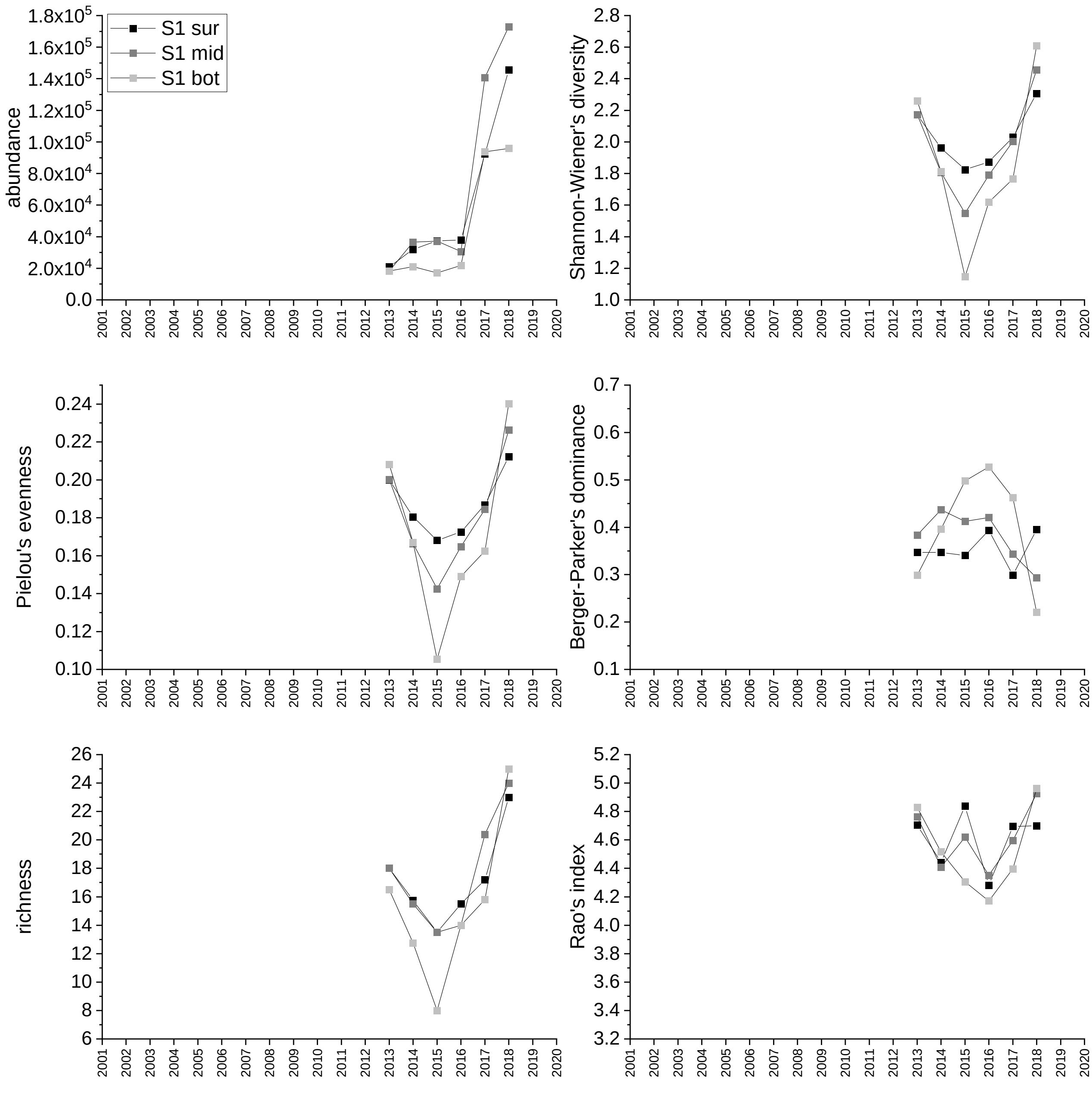

Figure 30: Annual means of selected diversity indices for station stations S1 in the period 2007-2018 in three water layers: sur (surface - 2 m), mid (middle layer – 10 m) and bot (bottom layer – 20 m).

2.1.6 Italy

2.1.6.1 Selection of Italian case study sampling stations and data information

Data of phytoplankton abundances were obtained from ISPRA for a total of 54 transects along the Italian coasts (Figure 31), from 2015 to 2020, although not homogenous in time and space. Each transect was characterized by coastal (3 nM), intermediate (6 nM) and offshore stations (12 nM).

Data were organized in a database and several quality checks were performed. For example:

- the names of taxa were checked on the official website (AlgaeBase) and updated.
- taxa and abundances values were checked, and errors were fixed.

A higher quality dataset was obtained, with 162 sampling sites (along the approximately 8,000 km of Italian coasts), 753 sampling dates, and 1,179 taxa included in 352 phytoplankton genera. Unfortunately, many data are not available at a species level.

For each MSFD subareas (Northern Adriatic, Middle Adriatic, Southern Adriatic, Ionian and Tyrrhenian Seas), the region with the highest homogeneity in terms of seasonal sampling dates among years were selected as representative of the area. For each region, one transect was chosen for the data analyses, as preliminary tests showed no differences among stations of the same region (e.g. among coastal stations of different transects of the same region). For regions facing different Seas (Apulia, Calabria and Sicily), one transect per each Sea was chosen.

It was not possible to calculate the relationship with anthropogenic pressures and activities as data on that pressures were not available. To override this, the coastal and offshore stations were compared, assuming that the coastal site was more impacted than the offshore one.

On the selected transects, the following analyses were performed on the coastal and offshore stations: diversity indexes (Shannon, Simpson and Rao) at the species level and Local Contribution to Beta Diversity (LCBD) at the genus level. Importance Value (at the genus level) was used to detect the spatial distribution of phytoplankton genera mainly affecting the local variability along the entire Italian coasts.

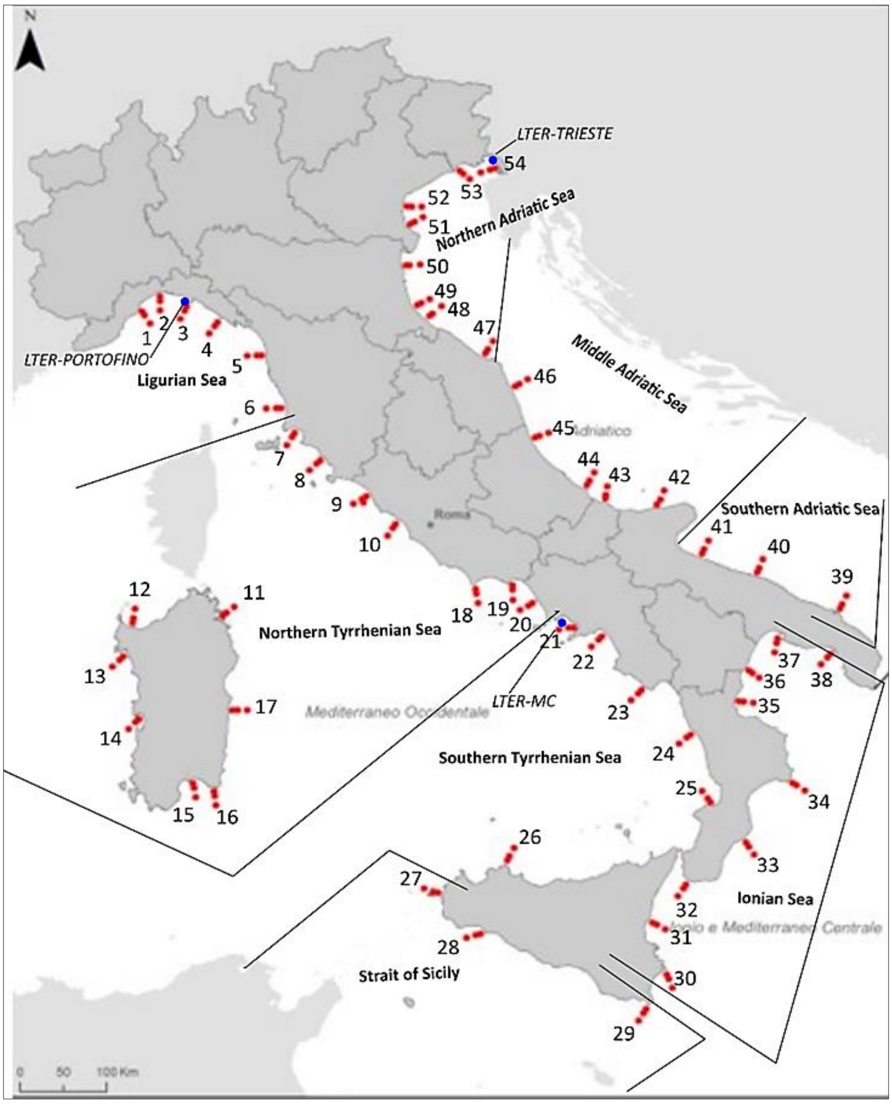

Figure 31: Map of the Italian coast showing sampling stations for the MSFD monitoring, where phytoplankton is monitored (red symbols). Marine regions and sub-regions are also shown.

2.1.6.2 Results of the Italian case study

The results for the selected indices for each transect (one transect per Italian region) are shown in Figures 32-35. In each transect, Shannon, Simpson and Rao showed no significant differences between coastal and offshore stations of the selected transect (Wilcoxon test, $p > 0.05$). However, this was mainly due to the scarcity of data (several missing samplings in the dataset).

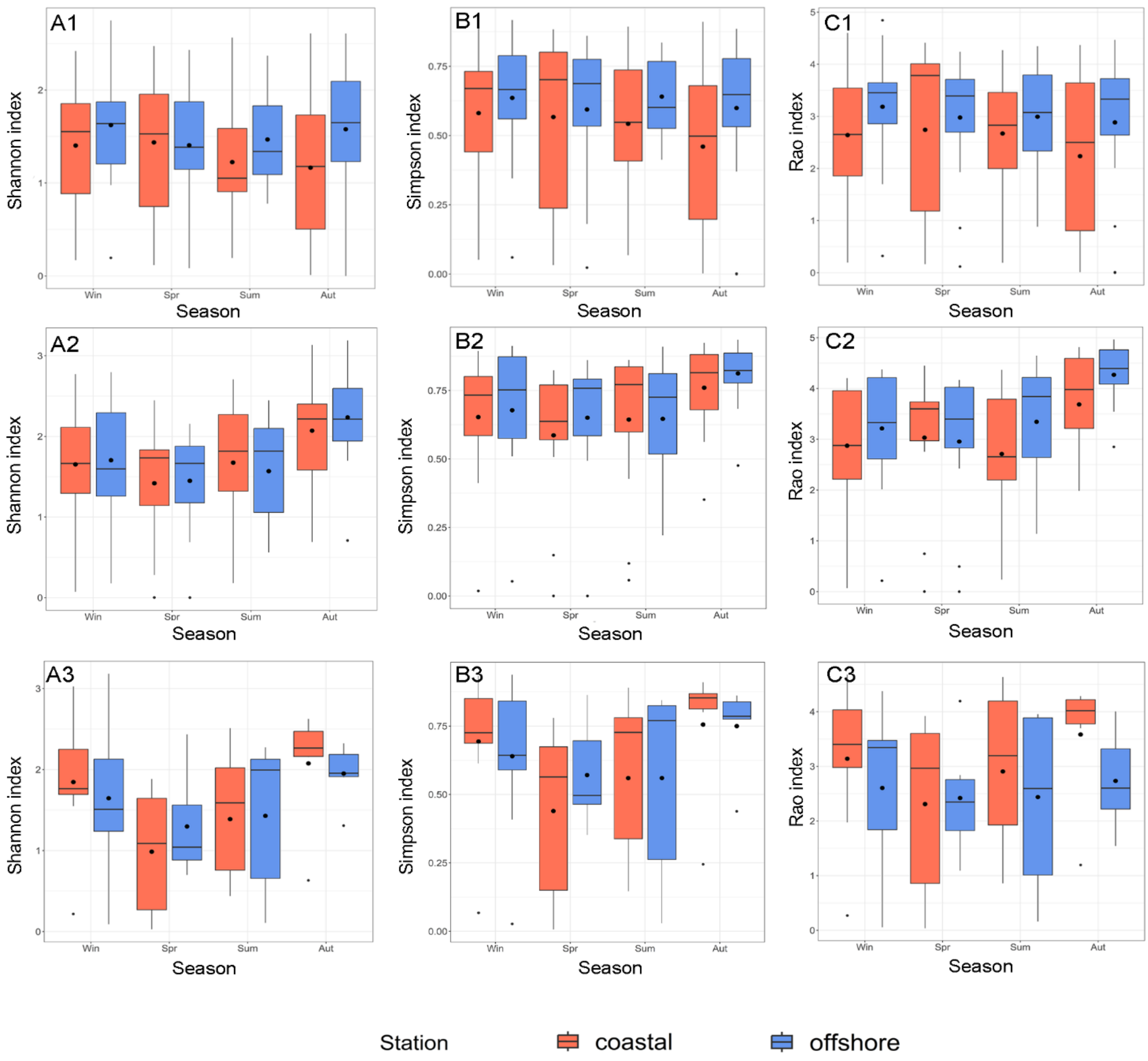

Figure 32: Shannon (A), Simpson (B) and Rao indices (C) of the coastal (orange) and offshore (blue) stations of the Adriatic transects Emilia- Romagna (A1, B1, C1), Marche (A2, B2, C2), Puglia (A3, B3, C3).

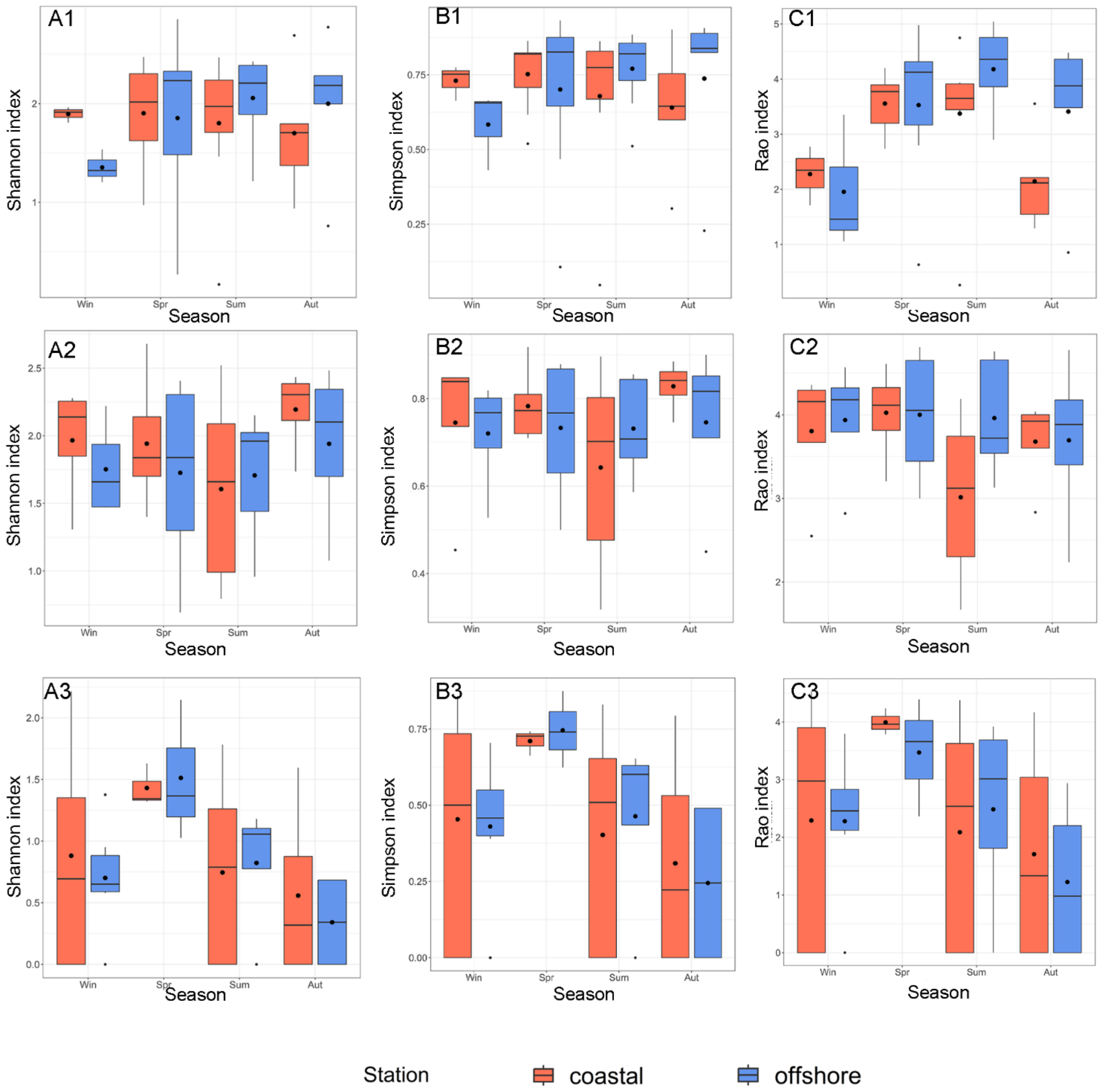

Figure 33: Shannon (A), Simpson (B) and Rao indices (C) of the coastal (orange) and offshore (blue) stations of the Tyrrhenian Sea transects: Toscana (1), Lazio (2) Campania (3).

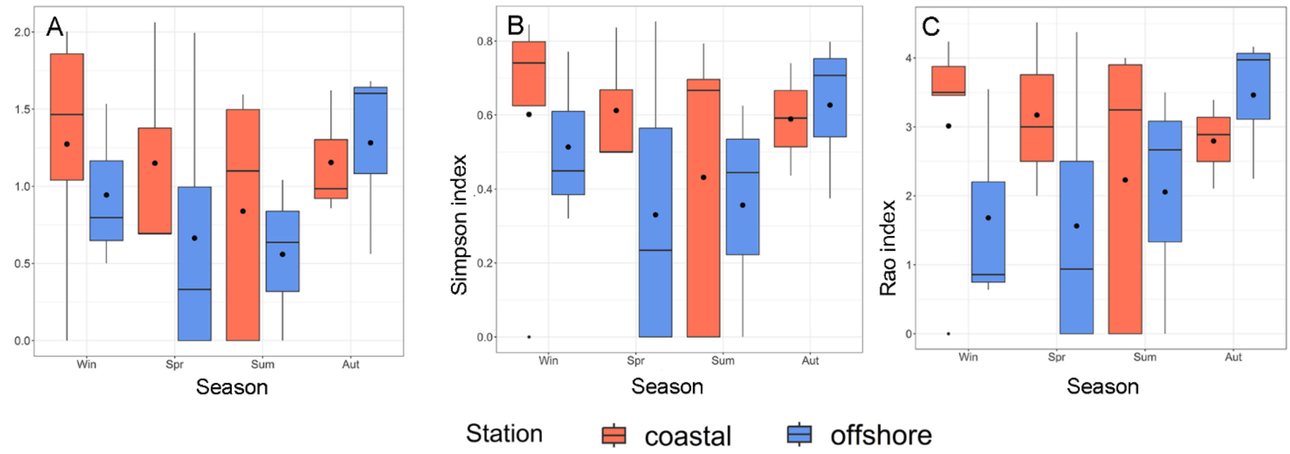

Figure 34: Shannon (A), Simpson (B) and Rao indices (C) of the coastal (orange) and offshore (blue) stations of the northern Tyrrhenian Sea - Ligurian Sea transects.

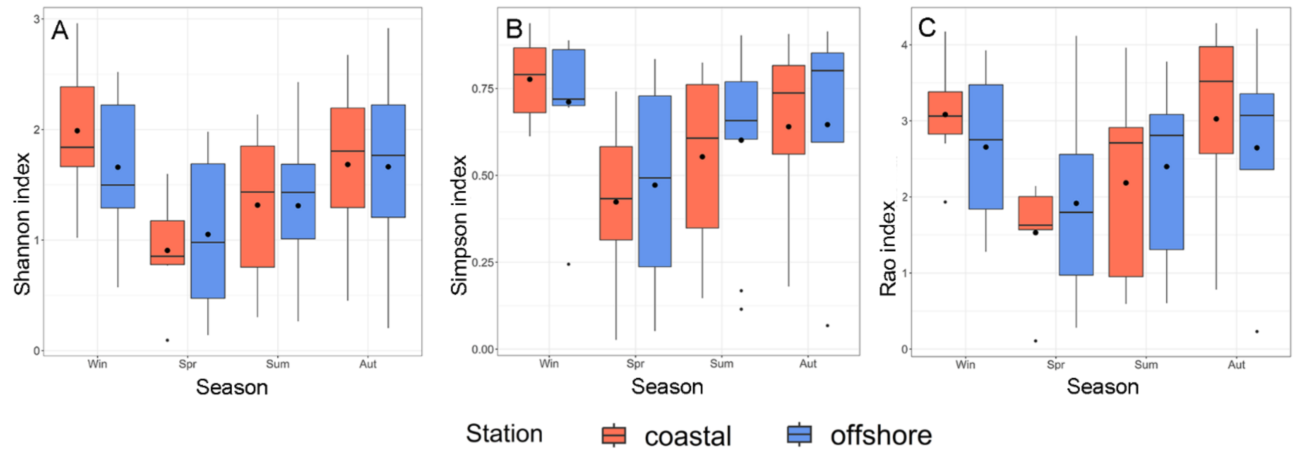

Figure 35: Shannon (A), Simpson (B) and Rao indices (C) of the coastal (orange) and offshore (blue) stations of the Ionian Sea transects.

Local Contribution to Beta Diversity (LCBD) calculated at the genus level highlighted only a few inter- and intra-annual differences in the phytoplankton communities in both the coastal and offshore stations of each transect, in each region, and no differences between the two types of station (Figure 36). However, it should be considered that the interannual variability should be calculated in a longer period.

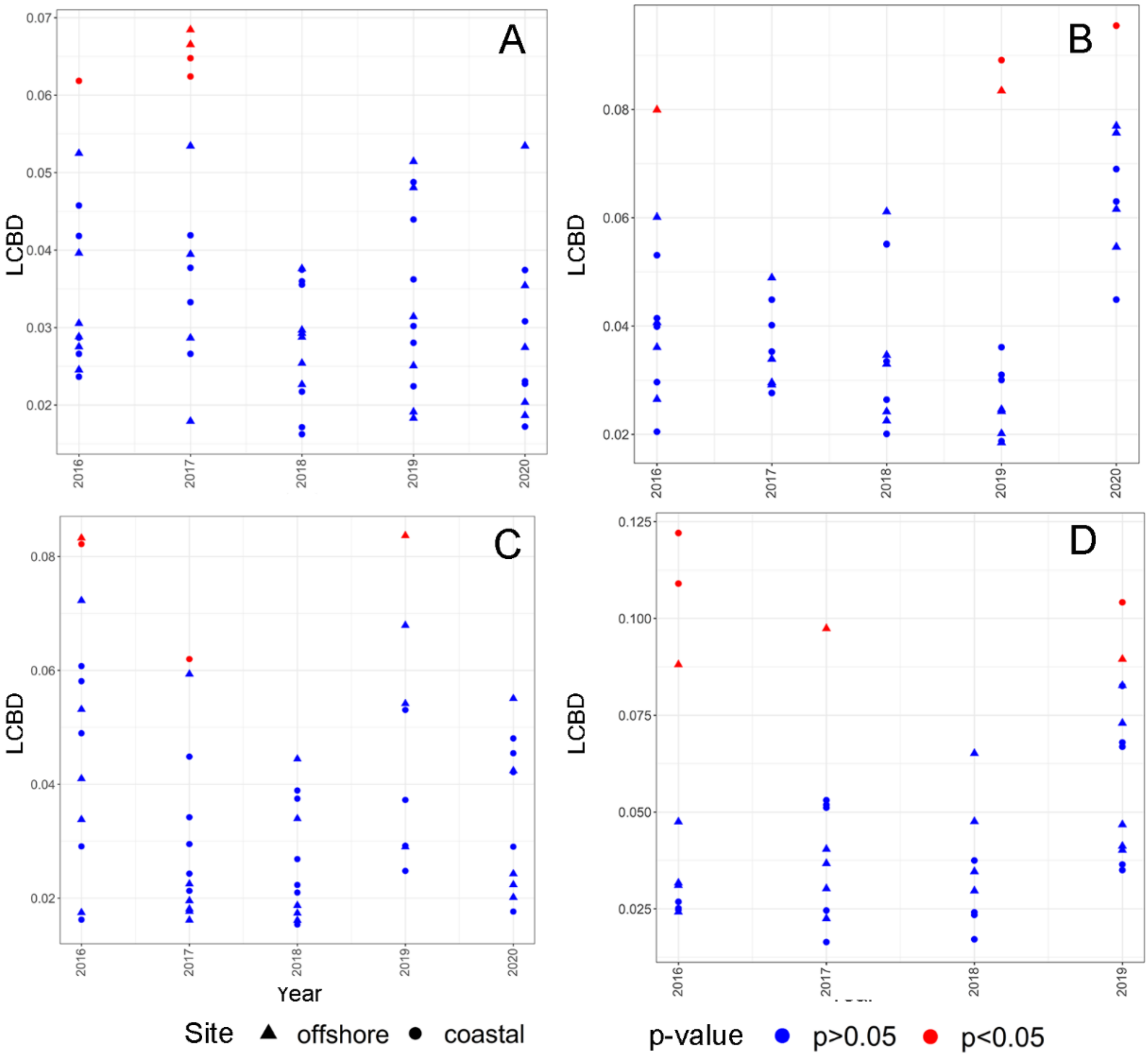

Figure 36: Local Contribution to Beta Diversity (LCBD), calculated at the genus level, for the Campania (A, southern Tyrrhenian Sea), Toscana (B, central Tyrrhenian Sea), Apulia (C, Ionian Sea) and Lazio regions (D, central Tyrrhenian Sea).

The Importance Value, calculated at the genus level showed which phytoplankton genera mainly affected the whole variability. This index showed that the community composition in terms of dominant genera differed among the Adriatic, Ionian and Tyrrhenian Seas (Table 1). The high similarities in main phytoplankton genera between the region along western Adriatic coast (Emilia-Romagna, Marche, Abruzzo and Molise) highlights the role of the Western Adriatic Current.

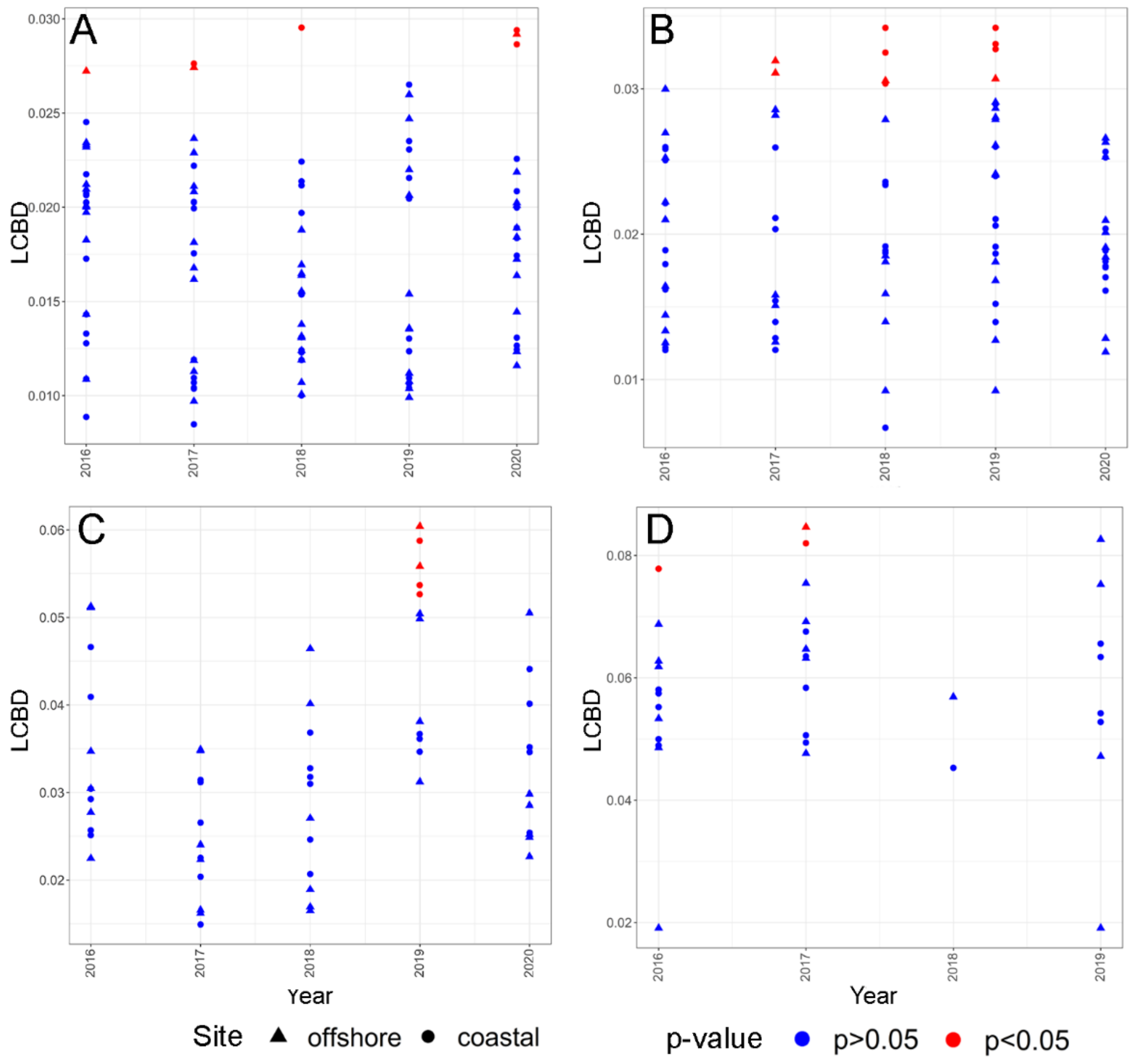

Figure 37: Local Contribution to Beta Diversity (LCBD), calculated at the genus level, for the Emilia - Romagna (A, northern Adriatic Sea), Marche (B, central Adriatic Sea), Apulia (C, Tyrrhenian Sea) and Liguria regions (D, central Tyrrhenian Sea).

LCBD and Importance Value could represent a useful tool for the identification of changes, although such identification should require longer datasets.

Table 1: The three most important phytoplankton genera for each Italian region as indicated by Importance Value Index

Mediterranean Sea Area	Region	Genus	Importance value
Adriatic Sea	Friuli Venezia Giulia	Chaetoceros	32.16018482
		Teleaulax	29.27047095
		Chrysochromulina	9.160749471
	Veneto	Chaetoceros	42.91843894
		Pseudo-nitzschia	24.4444617
		Tenuicylindrus	7.466389013
	Emilia-Romagna	Chaetoceros	52.47562262
		Skeletonema	35.39917177
		Pseudo-nitzschia	6.1681991
	Marche	Skeletonema	47.39912189
		Chaetoceros	45.63338763
		Pseudo-nitzschia	3.623970853
	Abruzzo	Skeletonema	77.14076635
		Pseudo-nitzschia	10.49741659
		Chaetoceros	4.562546143
	Molise	Skeletonema	64.03196356
		Chaetoceros	12.90493178
		Pseudo-nitzschia	7.039177176
	Puglia (Adriatic Sea transect)	Chaetoceros	35.62632136
		Pseudo-nitzschia	13.42117742
		Leptocylindrus	12.23596989
Tyrrhenian Sea	Liguria	Pseudo-nitzschia	34.69036206
		Chaetoceros	18.75485556
		Asterionellopsis	15.16279117
	Toscana	Tenuicylindrus	27.46217726
		Chaetoceros	27.27649072
		Leptocylindrus	23.79667421
	Lazio	Pseudo-nitzschia	32.75460522
		Leptocylindrus	17.47434783
		Chaetoceros	12.46768477
	Campania	Chaetoceros	24.41559612
		Navicula	15.71602434
		Gymnodinium	13.86792878
	Sardegna	Tripos	19.39591073
		Skeletonema	19.3832293
		Amphidinium	12.42972581
	Calabria (Tyrrenhian Sea transect)	Pseudo-nitzschia	23.09955135
		Gymnodinium	19.51040209
		Heterocapsa	9.865733675
	Sicilia (Tyrrenhian Sea transect)	Pseudo-nitzschia	36.12920514
		Leptocylindrus	21.71075182
		Leucocryptos	9.865785636
Ionian Sea	Puglia (Ionian Sea transect)	Chaetoceros	22.9506351
		Telelaux	21.58033044
		Gymnodinium	18.46847948
	Calabria (Ionian Sea transet)	Chaetoceros	20.57323919
		Gymnodinium	14.92429935
		Leptocylindrus	13.3553893
	Sicilia (Ionian Sea transect)	Pseudo-nitzschia	35.38894422
		Chaetoceros	24.47354661
		Leptocylindrus	8.923670252
	Basilicata	Heterocapsa	16.73780924
		Tetraselmis	10.94790778
		Gymnodinium	10.5166531

2.1.6.3 Comparison between regions

The Non-Metric Multi Dimensional Scaling (NMDS) performed for each season on phytoplankton group abundances among the Italian regions having the more accurate dataset (Emilia-Romagna and Tuscany), highlighted that marked differences occurred among regions in terms of phytoplankton abundances (Figure 38). Regions that have less accurate dataset (lower number of sampling dates) were not considered for the analysis.

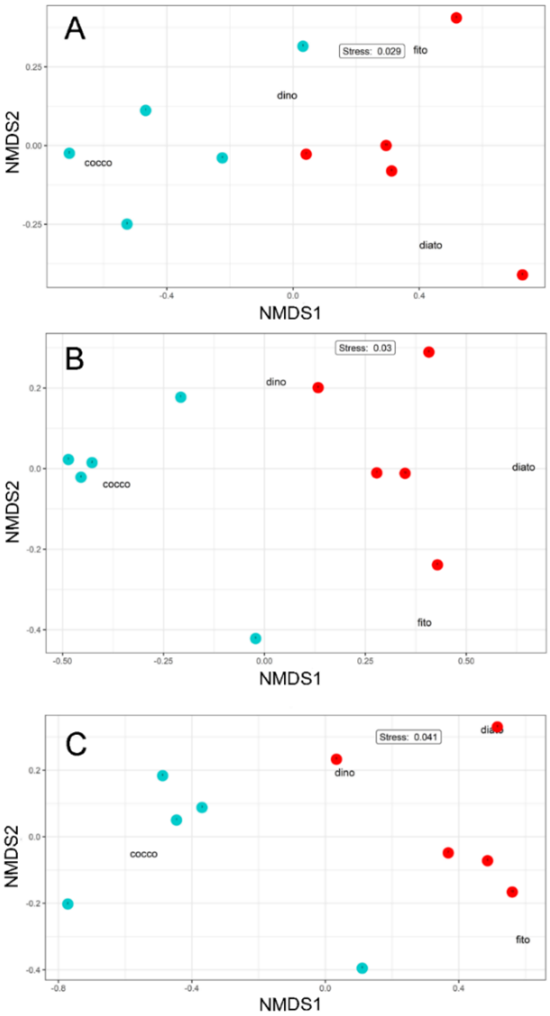

Figure 38: Non-Metric Multidimensional Scaling performed on the phytoplankton group abundances of the Emilia-Romagna (red circles) and Tuscany (blue circles) in spring (A), summer (B) and autumn (C). Winter was not considered due to insufficient data.

Conclusions

Marked seasonal and interannual variations in the selected diversity indices were observed at all sampling stations along the Italian coasts. There were no statistical differences between the sites of the same region, while communities from different regions resulted dissimilar in terms of both phytoplankton group abundance and indicator taxa. However, it was not possible to assess the coastal to offshore gradient as in many transects the sampling frequency was not adequate as the dataset obtained from the regional agencies only covered a very short period (2016-2020) and had a number of missing samples.

To overcome this problem, we used the stronger 40-year dataset from the LTER Senigallia-Susak transect (Totti et al., 2019a; Neri et al., 2022, 2023) for comparison within appropriate temporal and spatial scales. This LTER area is located at the southern edge of the northern Adriatic Sea, in an area where the Western Adriatic Current is narrow and clearly separates the nearshore from the offshore waters. The results (Totti et al., 2019a; Neri et al., 2022, 2023) showed that there was a clear difference between the coastal and offshore stations, both in terms of abundance, biomass, community composition and diversity of phytoplankton community. The physical structure of the water column and DIN concentrations were identified as the main drivers of these differences (Totti et al., 2019a). In terms of long-term changes, Totti et al. (2019a) showed that phytoplankton abundance and biomass have increased over the last decade, while the annual cycle became irregular and sudden diatom blooms occurred, reflecting the irregularity of meteorological events.

Although it was not possible to investigate the relationship between changes in phytoplankton community composition and anthropogenic pressures from human activities due to a lack of data, the observed differences between coastal and offshore stations can be interpreted as a response to environmental changes of both natural and anthropogenic origin, while still following a seasonal rhythm. In summary, the temporal variability (seasonal and between years) of phytoplankton diversity is much greater than the variability between the different sampling stations, which complicates the understanding of diversity patterns in response to environmental changes.

2.2 Zooplankton case studies

2.2.1 Selection of case studies

In the Mediterranean Sea, few studies have attempted to develop a biodiversity-based indicators, using time series of zooplankton data (Serranito et al., 2016; Villarino et al., 2020). None of the available zooplankton-based state indicators (see Tables 2, 3) is designed, or has defined thresholds, for the Mediterranean Sea and its subregions (Magliozzi et al., 2021). In the Mediterranean Sea, there are sub-regional differences in the frequency, duration, and spatial coverage of zooplankton monitoring and data availability. Furthermore, the high diversity of species within the different regions lead to the conclusion that a large amount of information needs to be summarized. Due to the sporadic occurrence, and sometimes uncertain taxonomic affinity of all but especially the most common planktonic organisms (i.e., copepods), it is difficult to distinguish the links to environmental change or human pressures on a species-by-species basis.

In the frame of the Task 2.2, based on the outcome of reviewing and assessing the existing approaches (indicators) for determining the zooplankton status, we selected the most suitable. These indicators will be used for testing in case studies and for further development. As this is the first attempt to combine zooplankton data from different sub-regions of the Mediterranean Sea for MSFD purposes, evaluation of data availability for each area has been performed, in order to increase geographical coverage. ABIOMMED partners from Croatia, Italy and Greece agreed to provide official data from their programs in the frame of MSFD, WFD and other national or European monitoring projects spanning the last 12 years. An extensive metadata file of all available data was compiled, including area coverage, station, sampling methodology, availability of environmental parameters, pressures and zooplankton parameters (zooplankton biomass, abundance, species/taxa abundance). After an extensive comparison and evaluation of the metadata catalogue, the involved partners (Croatia, Italy and Greece) have provided the zooplankton data including taxonomic classification (verified by WORMS) and abundance values (ind. m^{-3}) from the selected areas. According to the data availability, in total 5 MRUs examined (Tyrrhenian, Adriatic, Ionian, Aegean and Levantine Seas). A number of selected indicators are applied including their strengths and weaknesses in each selected area in determining GES for criterion D1C6.

2.2.2 Croatia

2.2.2.1 Central part of the eastern Adriatic Sea – Croatia: Microzooplankton

Microzooplankton data were collected in the central part of the Adriatic Sea at two coastal stations in Kaštela Bay and one station in the Split Channel (Figure 39, Table 2). The selected stations are located on a transect coast-offshore and they under different trophic pressure. Following the commissioning of the new sewage system at the end of 2004, water quality in the eastern part of the bay has improved significantly, but is still polluted by developed industry, shipping and trade. The Jadro River, which flows into the eastern part of the Bay near the JA10 sampling station, is the main source of freshwater with an average inflow of 10 m^3s^{-1} (Zore-Armanda, 1980). The geographical characteristics of the bay, its proximity to the mainland and anthropogenic influences have a major impact on the hydrographic parameters of the bay. During the warm season (July to September), the water renewal period is relatively long. Station JA13, on the other hand, is located in the Split Channel area and is characterised by relatively rapid aeration, including the intrusion of water masses from the open ocean and a lower trophic status. Previous studies indicated that there was an increase in phosphorus and nitrogen in Kaštela Bay and at the stations in the Split Channel due to anthropogenic influence, which led to the formation of a trophic gradient from the coastal area to the open sea (Kušpilić et al., 2010, Bojanić et al., 2012).

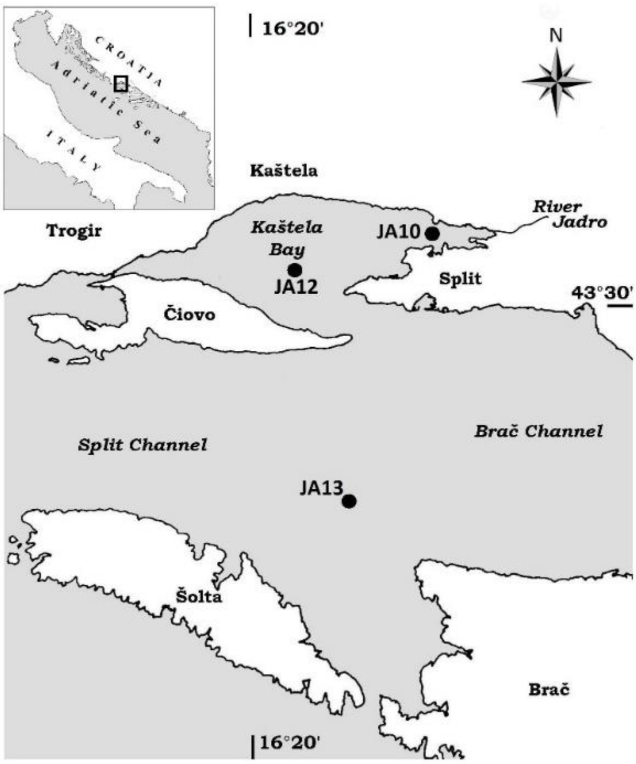

Figure 39: Study area with the sampling stations in the central Adriatic Sea

The microzooplankton samples were collected monthly or seasonally from January 2010 to December 2021. However, the period and frequency of sampling vary greatly from station to station (Figure 40, Table 2). At station JA12 the data set is the most complete, at station JA10 the matrix is slightly smaller and starts in 2012, and at station JA13 the data set is the shortest and sampling was done in cycles of two years. Plankton samples were collected at intervals of 5 m or 10 m depth from the water surface to the sea bottom (Kaštela Bay) or at 0, 5, 10, 20, 30 and 52 m depth (Split Channel) using 5-litre Niskin bottles. The samples were preserved in 2.5% formaldehyde seawater solution previously buffered with $CaCO_3$, as Lugol's solution stains the detritus and thus reduces visibility. Although the number of ciliates (except tintinnids) is underestimated due to this preservation technique, the ratio of the abundance of these organisms at the different stations is preserved, which can be useful for the assessment of the ecological status. Sedimentation and decantation methods were used to prepare the samples for microscopic analysis. Species were counted and identified using inverted microscopes ("Olympus" IMT-2) at 100x and 400x magnification, and abundance was expressed as the number of cells or individuals per litre (cells L^{-1} or ind. L^{-1}).

The microzooplankton data set, 998 samples in total, was collected within various programs of the Institute of Oceanography and Fisheries (IOR), e.g. Croatian National Monitoring Program, Systematic Assessment of the Quality of Transitional and Coastal Waters, Monitoring and Observation System for the Ongoing Assessment of the Adriatic Sea within the Adriatic Sea Monitoring Program, Phase II, JADMON project.

The microzooplankton data set includes the following functional groups: non-loricate ciliates, tintinnids, other protozoa (unicellular zooplankton organisms belonging to the phylum Foraminifera, Radiozoa and Myzozoa), copepod nauplii, post-naupliar copepods (juvenile copepods and small adult copepods), other micrometazoans (Rotatoria, Cladocera, juvenile organisms of Pteropoda, Chaetognatha, Tunicata and larvae of benthic organisms).

Operational indicators for microzooplankton:

- Monitoring the abundance of microzooplankton target groups (ciliates, copepod nauplii, postnaupliar copepods, other micrometazoans).
- Monitoring the population structure and the relationship between the selected microzooplankton groups (ciliates, copepod nauplii, postnaupliar copepods, other micrometazoans).
- Assessment of the state of the tintinnid community using different ecological indices: number of species (S), Shannon-Wiener (H'), Pielou (J') and Simpson (1-D).

Table 2: The three most important phytoplankton genera as indicated by Importance Value Index for Italian regions

Station	Latitude	Longitude	Station depth [m]	Sampling depths [m]	Total number of samples
JA10	43.530000 N	16.453333 E	18	0, 5, 10 and 17 m	164
JA12	43.518333 N	16.381667 E	37	0, 5, 10, (15, 20), 25, (30) and 35 m	710
JA13	43.426719 N	16.393519 E	52	0, 5, 10, 20, 30 and 52 m	124

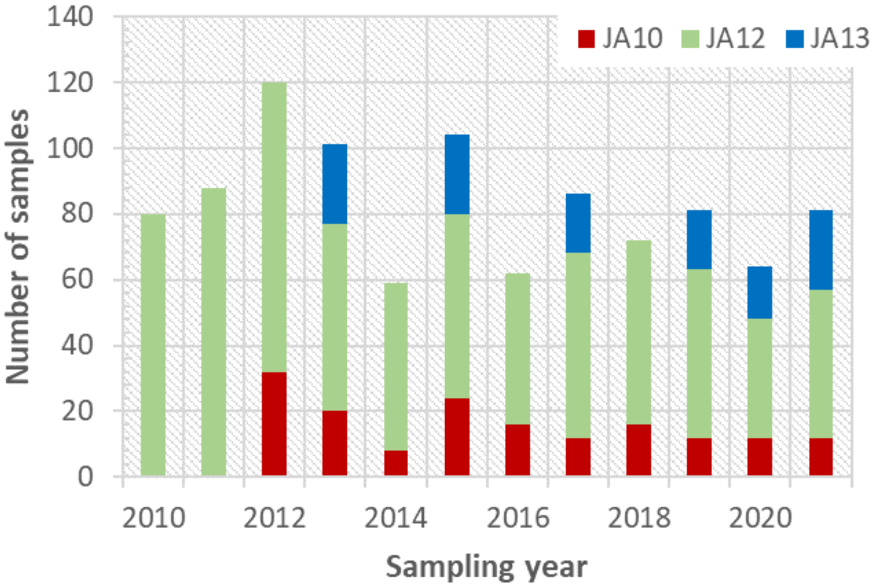

Figure 40: Number of microzooplankton samples collected per year at three stations along the coast-offshore transect in the central Adriatic Sea (Croatia).

Table 3: Descriptive statistics of abundances of microzooplankton target groups at three stations along the coast-offshore transect in the central Adriatic Sea.

Station	JA10			JA12			JA13		
Sampling period	2012-2021			2010-2021			2013-2021		
Abundance [cells or ind. L^{-1}]	Mean±SD	Max.	Geom.	Mean±SD	Max.	Geom.	Mean±SD	Max.	Geom.
Non-loricates	329.94±373.48	3662	237.48	172.64±120.19	865	137.98	167.94±147.27	918	127.79
Tintinnids	125.84±198.91	1714	69.08	54.59±62.49	686	-	47.82±66.66	461	-
Nauplii	78.68±66.94	314	56.98	42.76±38.22	384	33.68	32.94±24.86	158	26.64
Post-naupliar copepods	32.48±29.74	172	22.25	16.17±11.14	106	-	10.79±8.80	45	-
Other micrometazoans	39.91±36.90	238	27.93	16.31±13.64	114	-	6.91±4.42	23	-

A principal component analysis (PCA) was performed on a set of abundance data for microzooplankton target groups from 2010 to 2021 in the central part of the Adriatic Sea at three stations (Figure 41). The descriptive statistical data for the microzooplankton groups analysed are presented in Table 3 and refer to the mean and standard deviation as well as the geometric mean of the abundance of the organisms. The analysis extracted two factors linking functional groups with similar distribution patterns, e.g. ciliates and small metazoans (Figure 42). The spatial and seasonal distribution of abundance of these two groups is shown in Figures 43 and 44. Two factors explained 67.47 % of the variability. The first factor relates to micrometazoans (nauplii and post-naupliar copepods and other small metazoans) and explains 38.28 % of the variability. The second factor is significantly positively related to the ciliate component of the microzooplankton (non-loricates and tintinnids) and explains 29.19 % of the total variability. Both axes are strongly positively correlated with the station in the eutrophic part of Kaštela Bay (JA10) and negatively correlated with the channel station (JA13), indicating a clear relationship between the abundance of selected microzooplankton groups and the ecological status of the ecosystem.

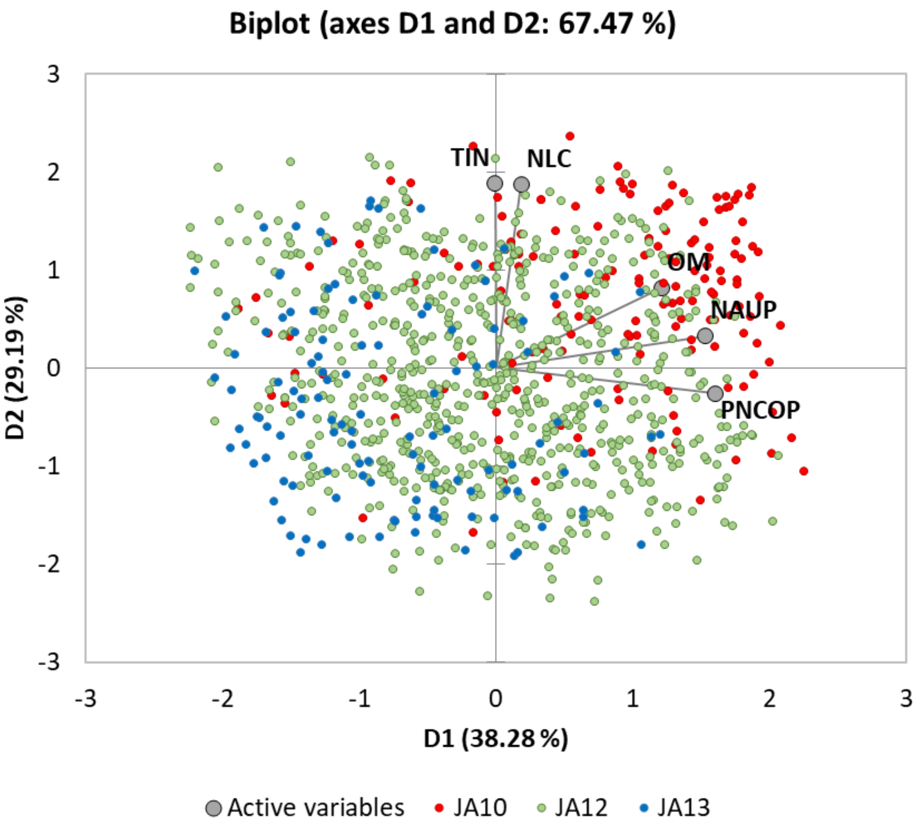

Figure 41: Ordering of microzooplankton target groups of non-loricate ciliates (NLC), tintinnids (TIN), copepod nauplii (NAUP), post-naupliar copepods (PNCOP) and other metazoans (OM) as active variables determined by principal component analysis (PCA) during the sampling period from 2010 to 2021 at three stations in the central Adriatic Sea. The sampling stations are overlaid as active observations and shown in red (coastal station JA10), green (station JA12 in the central part of the bay) and blue (channel station JA13) depending on their position on the coastal-offshore transect.

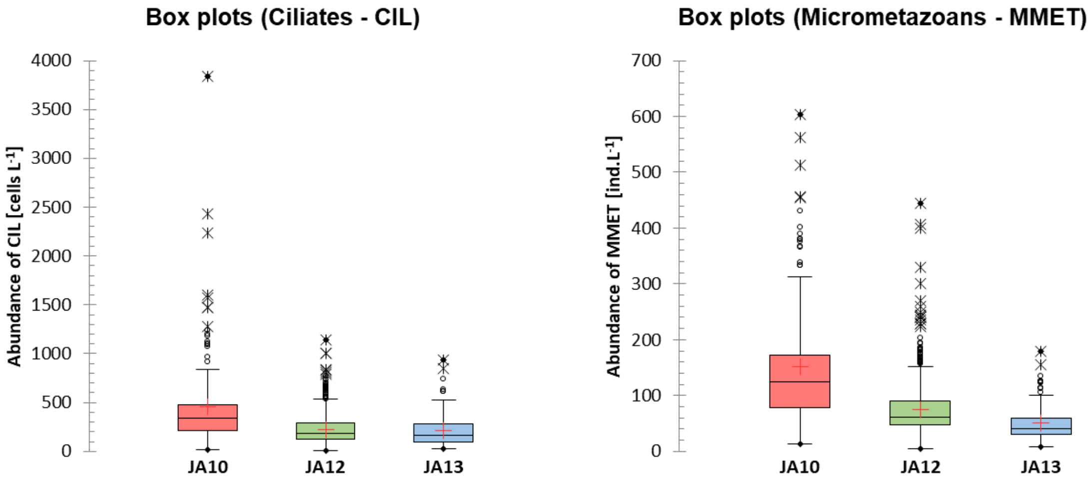

Figure 42: Spatial distribution of abundance of ciliates and micrometazoans in the study period 2010-2021 at three stations along the coast-offshore transect in the central Adriatic Sea.

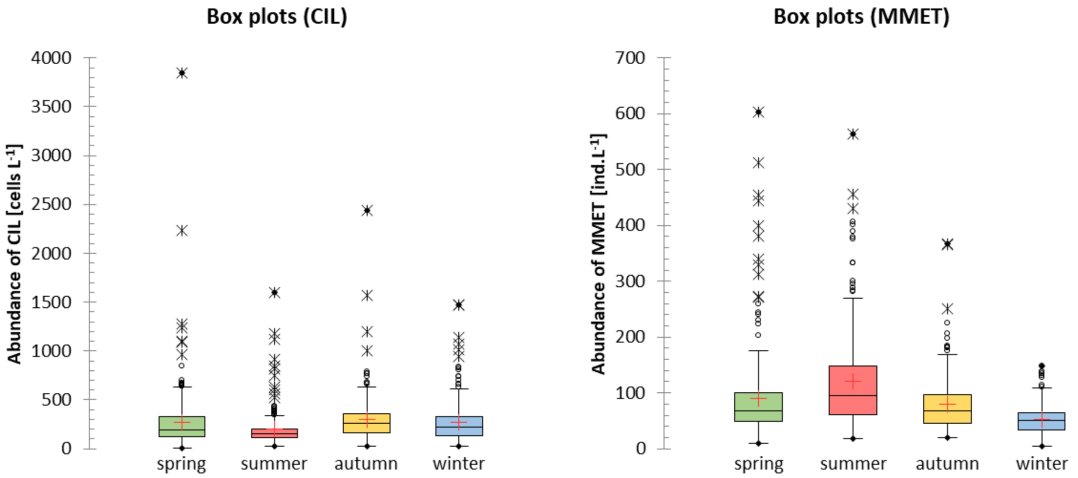

Figure 43: Seasonal distribution of abundance of ciliates and micrometazoans in the study period 2010-2021 at three stations along the coast-offshore transect in the central Adriatic Sea. (CIL, ciliates ad MMET, micrometazoans).

As the results of the PCA analysis showed a clear separation of the two components of the microzooplankton community e.g. ciliates and micrometazoans, the abundance ratio of these groups was examined in more detail on a spatial and seasonal scale (Figure 44).

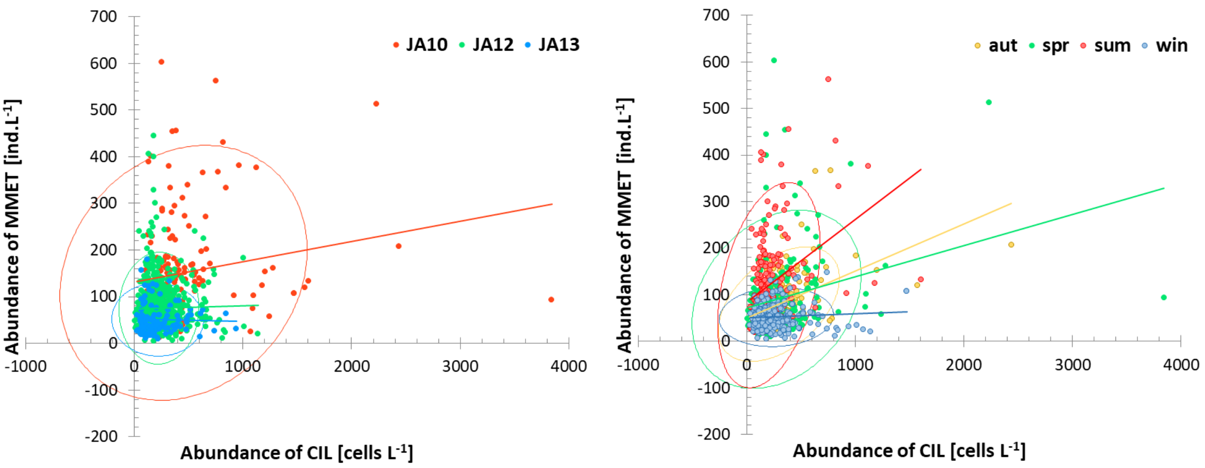

Figure 44: Scatterplot of ciliate and micrometazoan abundance during the research period 2010-2021 on a spatial and seasonal scale in the central Adriatic Sea with 95% confidence intervals and regression lines.

Similar to previous analyses of microzooplankton abundance, ecological indices of diversity (Shannon-Wiener index, H'), evenness (Pielou index, J') and dominance (Simpson index, 1-D) were also calculated at three stations in the central Adriatic (JA10, JA12 and JA13) over a slightly shorter period 2013-2021. It should be noted that the length of the data set is not the same for all stations (Figure 40). Alpha diversity indices were calculated for the tintinnid community only, and used to show changes in their spatial and temporal variability (year and season). Tintinnids were identified based on the morphology of lorica and the species description given by Kršinić (2010). The data set excluded indeterminate tintinnid species. The *Coxliella* forms were counted as undetermined tintinnids and were not included in the calculation of species diversity. The integrated values were used to estimate the abundance of tintinnids in the entire water column (at 4-6 sampling depths, depending on the station) and to calculate biodiversity indices.

The significance of differences in environmental indices between sampling stations, seasons and years was tested using the Kruskal-Wallis test (two-tailed test) followed by the post hoc Dunn's Multiple Comparison test. The spatial and temporal variability of these indices is shown in Figures 45-48. The Kruskal-Wallis test revealed significant differences (p=0.001) only in the seasonal distribution of all ecological indices (S, H', J' and 1-D), with winter being distinguished from all other seasons (Figure 47).

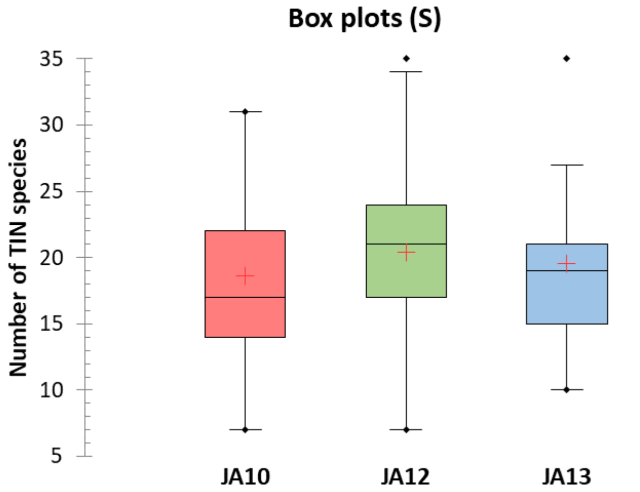

Figure 45: Number of tintinnid species at three stations in the central Adriatic Sea during the research period 2013-2021.

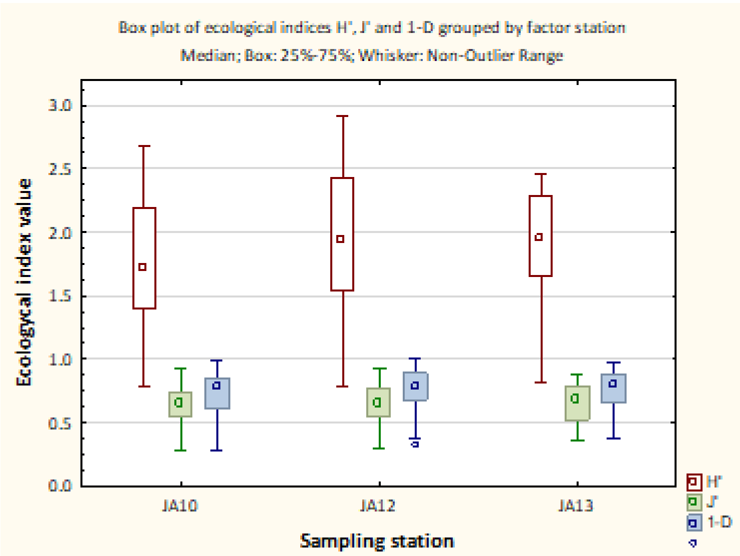

Figure 46: Box plots of ecological indices of diversity (Shannon-Wiener, H'), evenness (Pielou, J') and dominance (Simpson, 1-D) based on the abundance of tintinnid species at three stations in the central Adriatic Sea during the research period 2013-2021.

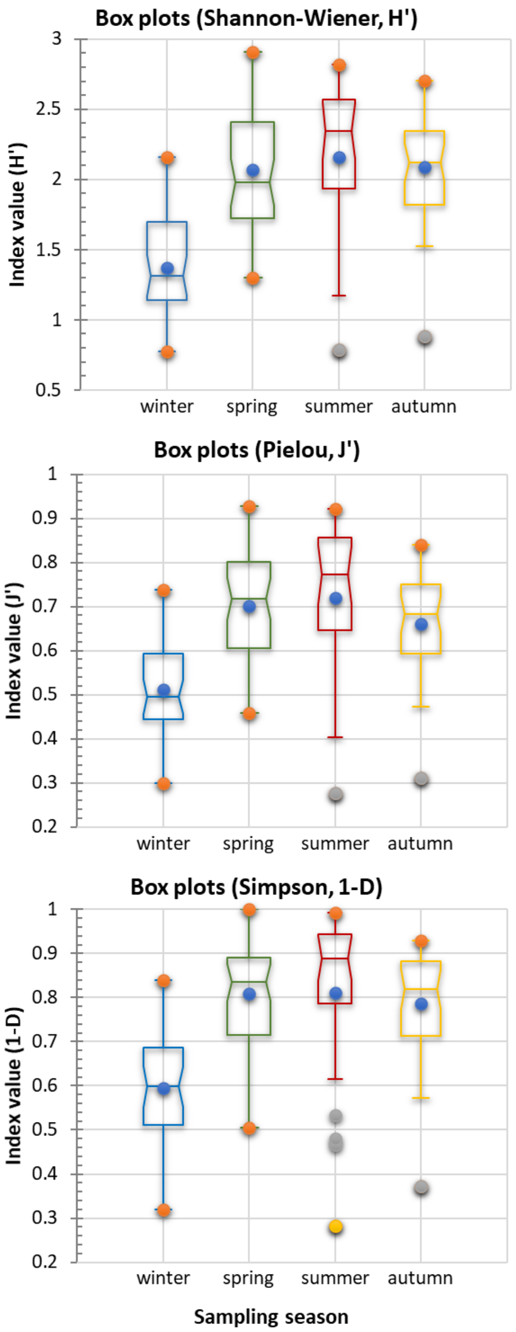

Figure 47: Box plots of seasonal variability of ecological indices of diversity (Shannon-Wiener, H'), evenness (Pielou, J') and dominance (Simpson, 1-D) based on the abundance of tinntinid species at three stations in the central Adriatic Sea during the research period 2013-2021.

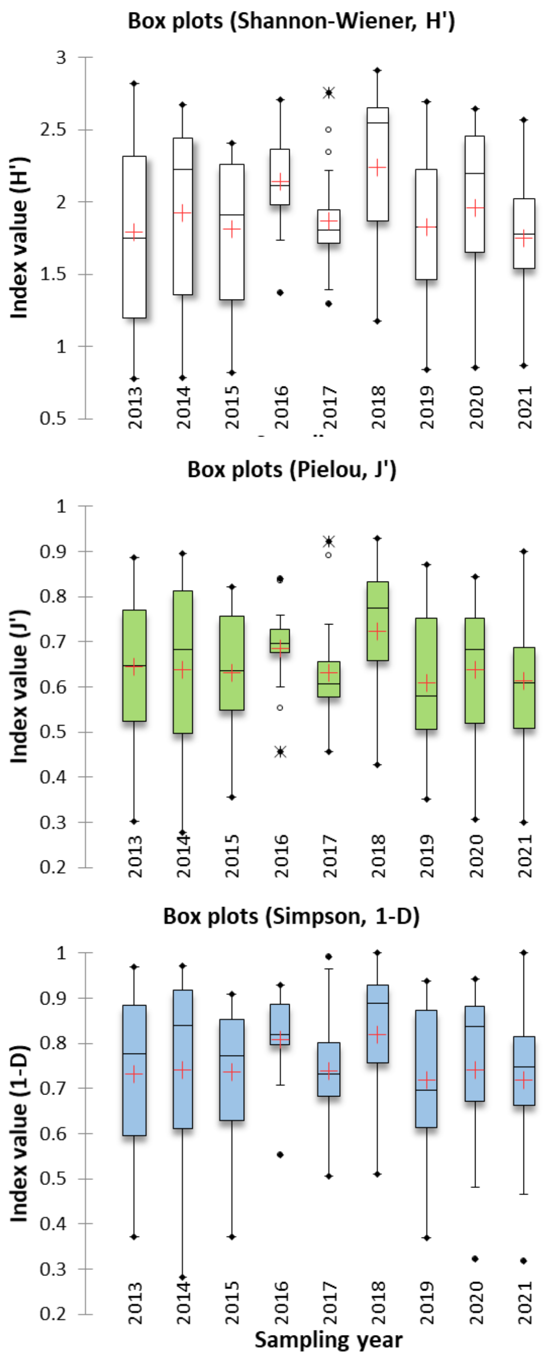

Figure 48: Box plots of multiannual variability of ecological indices of diversity (Shannon-Wiener, H’), evenness (Pielou, J’) and dominance (Simpson, 1-D) based on the abundance of tinntinid species at three stations in the central Adriatic Sea during the research period 2013-2021.

<u>Conclusions</u>

The catalogue of zooplankton indicators by life form pairs presented in this document (Table 3) for the micro-size category lists only the ratio of ciliates to microflagellates, which is potentially useful for assessing the transition from a primarily autotrophic to a more heterotrophic system. According to the presented data from our research in the central Adriatic Sea for the parameter microzooplankton, potential indicators can be based on the overall abundance of ciliates/protozoa and micrometazoans and their mutual relationship, as well as ecological indices of diversity, evenness and dominance for the tintinnid community. By analysing a larger data set and comparing it with data from other partner institutions, we will be able to apply and select the most appropriate indicators. The specific and inconsistent methodology of (micro)zooplankton sampling, the small number of experts and the extremely long data processing are the main obstacles we are facing today.

2.2.2.2 Central part of the eastern Adriatic Sea – Croatia: Microzooplankton

<u>Study area and sampling methodology</u>

The Croatian mesozooplankton dataset includes three stations monitored under the national monitoring for MSFD, supplemented with data from WFD monitoring (2000/60/EC) and various projects of the Institute of Oceanography and Fisheries in 2011-2021 period (Figure 49).

Figure 49: Microzooplankton case study area in central Adriatic Sea (Croatia)

Figure 50 summarizes the sampling frequency over the years in this case study. Similar to phytoplankton case studies, the data format followed the LifeWatch metadata and data templates, provided by the University of Salento. After the collection, all records were checked for taxonomic accuracy using WoRMS. Data cleaning and validation were performed manually, removing incorrect or incomplete data. The final data matrix consisted of 6401 entries. All three stations are also part of the Croatian phytoplankton dataset presented in this report.

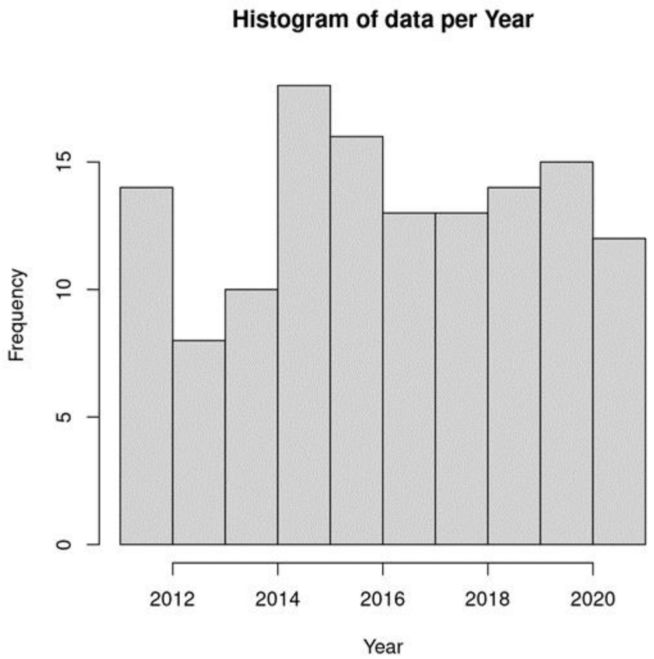

Figure 50: Frequency of zooplankton data collected per year in the Croatian case study from 2011 until 2021.

As reported for phytoplankton (Chapter 2.1.3.2), the selected stations are located along a coast-offshore transect in the central Adriatic Sea, characterized with different depths and varying degrees of anthropogenic influence. Station JA10 (Vranjic basin), with maximum depth of 18 m, is located in the eastern part of the shallow, semi-enclosed Kaštela Bay and receives organic matter and nutrients from the Jadro River, local agriculture and sewage, as well as pollutants from few small industrial plants and a shipyard along the coast (assessed as impact category 3). For decades, the Vranjic Basin was considered a hotspot of eutrophication in the coastal eastern Adriatic (Vidjak et al., 2006), but environmental conditions have recently improved. However, it is still characterized by higher trophic status and occasional nutrient extremes compared to outer parts of Kaštela Bay. The mesozooplankton data set at this station extends over 2014-2021 period, with the seasonal sampling frequency (4 times per year).

The station JA 12 (maximum depth 38 m) is located in the central part of Kaštela Bay. Despite the high degree of urbanization along the coast, this station has been exposed to only moderate anthropogenic pressure (impact category 2) since the commissioning of the modern sewage system in 2004, which led to disappearance of nutrient and oxygen extremes, decrease in bacterial abundance and production, reduction in phytoplankton biomass and restoration of the regular seasonal cycle (Šolić et al., 2010, Skejić et al., 2014). The mesozooplankton data set extends over 2011-2021 period. Sampling frequency at this station was near- monthly, but the number of analysed samples is lower, ranging from 4 to 10 samples per year.

The station JA 16 (maximum depth 103 m) is located just out of the coastal waters of the central Adriatic. The deep layers are influenced by open Adriatic water masses, mainly Levantine Intermediate Water (LIW) and North Adriatic Dense Water (NAdDW), which are characterized by low variations in chemical and physical parameters. This area is considered a reference area for the oligotrophic open waters in the central Adriatic (impact category 0) and has been regularly monitored since 1950s (Marasović et al., 2005). The mesozooplankton data set extends over 2015-2021 period, with the seasonal sampling frequency (4 times per year). Seasons were determined as follows: winter (January-March), spring (April-June), summer (July-September) and autumn (October-December).

Sampling methodology was consistent throughout the dataset, involving a 125 µm mesh Nansen net (total length 2.5 m, mouth area 0.25 m^2) towed vertically from near bottom to the surface. Abundances were expressed as number of individuals per cubic meter (ind. m^{-3}). Taxonomic identification was made at the species or genus level for main zooplankton groups (Copepoda, Cladocera, Appendicularia, Chaetognatha, Thaliaceaa, Siphonophora). Copepods were classified by stage (adults, copepodites). Larger planktonic crustaceans (Amphipoda, Euphausiacea, Mysidacea) and various larvae of benthic invertebrates and fishes were determined at higher taxonomic levels (class, order, phylum). All individuals found in a sample were used to calculate total abundance and number of taxa.

The data set is characterised by a relatively low fluctuation of taxonomic expertise, as only two analysts from the same laboratory were responsible for the analysis of the samples. The following analyses represent the initial results, with further work planned for the future linking the dataset primarily to the underlying abiotic and biotic environmental conditions in each broad habitat type.

RESULTS

Bulk properties and α diversity indices

The stations in the central Adriatic showed clear differences both in total abundance and in the number of mesozooplankton taxa (Table 4, Figures 51 and 52). The gradient in total abundance was consistent with the change in depth (15 m, 35 m, 100 m) and proximity to the coast, as is common in the central Adriatic, which is generally characterized by a very oligotrophic open sea (Šantić et al., 2023). Mesozooplankton community size reflects the trophic state of an area and can also be associated with anthropogenic nutrient enrichment (Vidjak et al., 2012), but this relationship is not unequivocal in the marine environment. The high seasonal and interannual variability in the size of the mesozooplankton community under natural conditions requires long-term series to establish reliable reference conditions for this indicator.

Table 4: Mean Total abundance (ind m^{-3}) including s.d., max. and min. values at three stations.

Station	Mean (ind m^{-3})	St dev (ind m^{-3})	Max (ind m^{-3})	Min (ind m^{-3})
JA10	16093.8	12836.56	61470.5	696.3
JA12	6921.0	3486.07	15983	1660
JA16	1182.7	730.37	4251.0	227.5

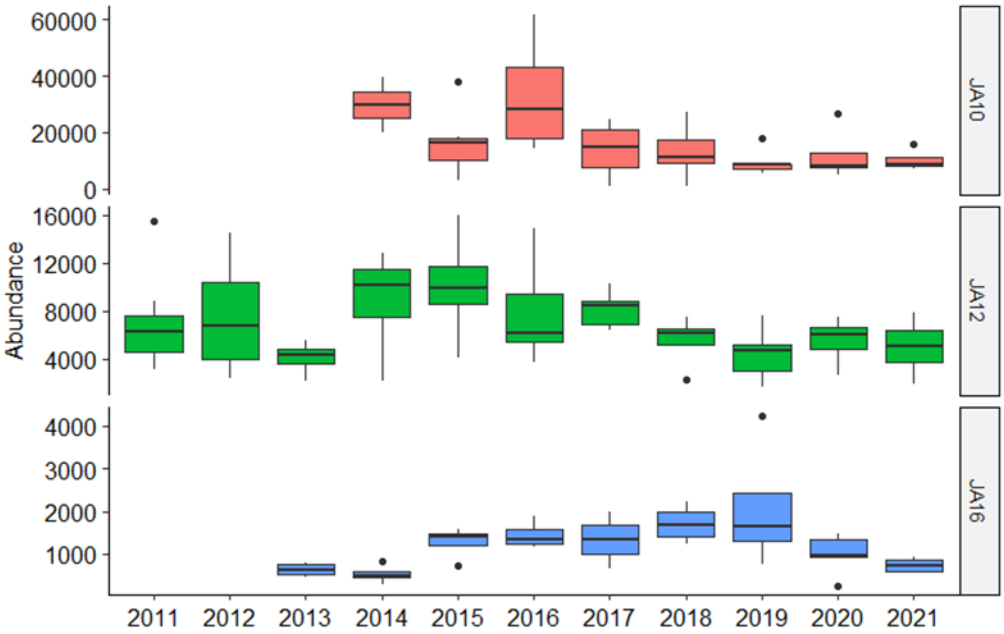

Figure 51: Distribution of total mesozooplankton abundances at central Adriatic stations

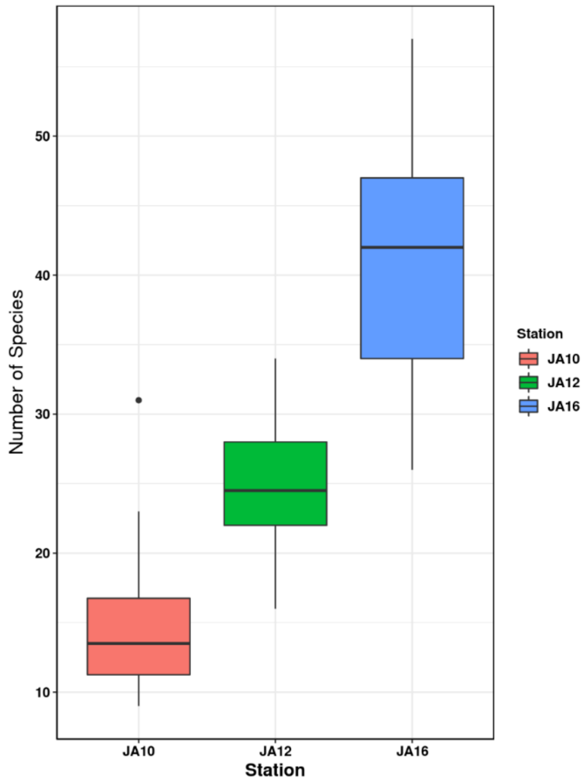

Figure 52: Number of mesozooplankton species at central Adriatic stations

Similar to the Greek mesozooplankton case study, 9 ecological indices of evenness, dominance, and diversity were calculated for three central Adriatic stations. The data matrix included all species-level records (either determined or sp.), while indeterminate juveniles, species complexes, and order-, class-, or higher-level data were not included. Figure 53 shows the spatial change of three selected diversity indices in the central Adriatic. In general, the indices were able to distinguish well the communities at each station, according to the prevailing natural conditions at each site. For example, the deep offshore station JA16 and the shallow coastal station JA10 represent the contrasts in terms of richness-abundance (Shannon index) and dominance (Berger-Parker index) of the mesozooplankton community, while station JA12 has intermediate values.

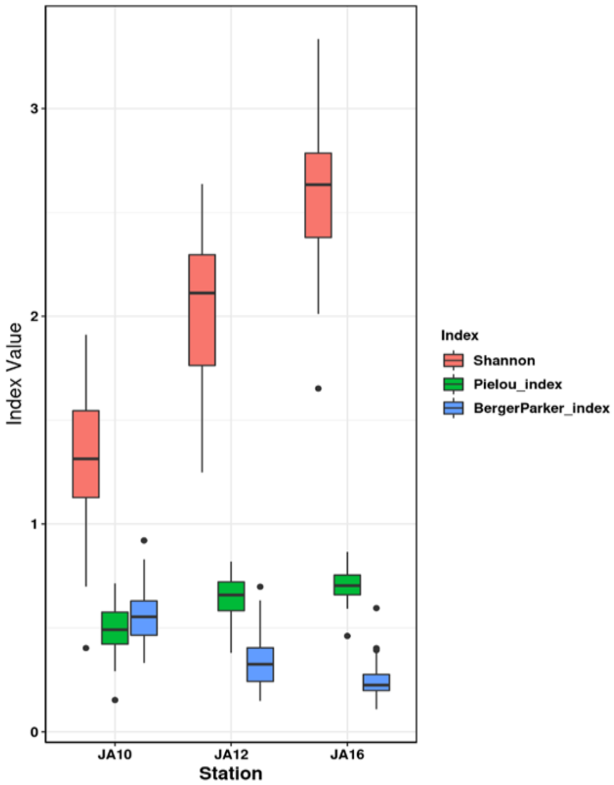

Figure 53: Alpha diversity indices at central Adriatic stations

The analysis of the copepod community led to the same results: The inshore station JA10 showed a lower diversity of copepods, a higher dominance in the community and a lower evenness than the offshore station JA1 (Figures 54 and 55). Interestingly, in both cases, autumn proved to be the season with the greatest diversity (Figures 56 and 57). In a next step, the influence of the underlying environmental conditions on the spatio-temporal distribution of the index values will be analysed.

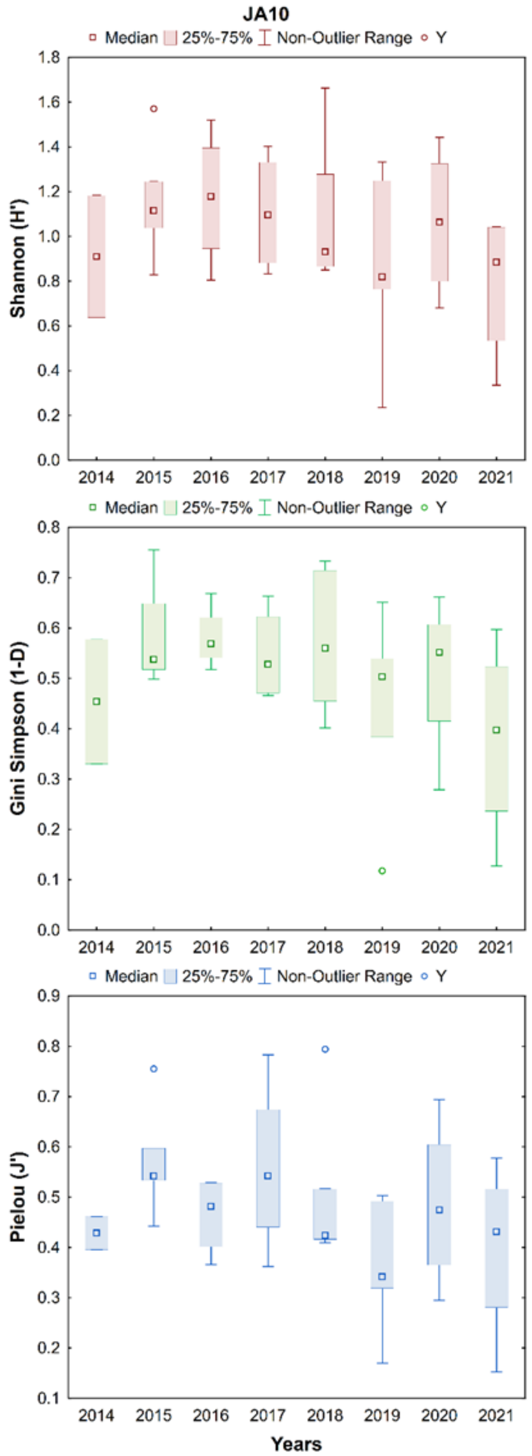

Figure 54: Interannual distribution of diversity indices in copepod community at coastal station JA10 in central Adriatic Sea.

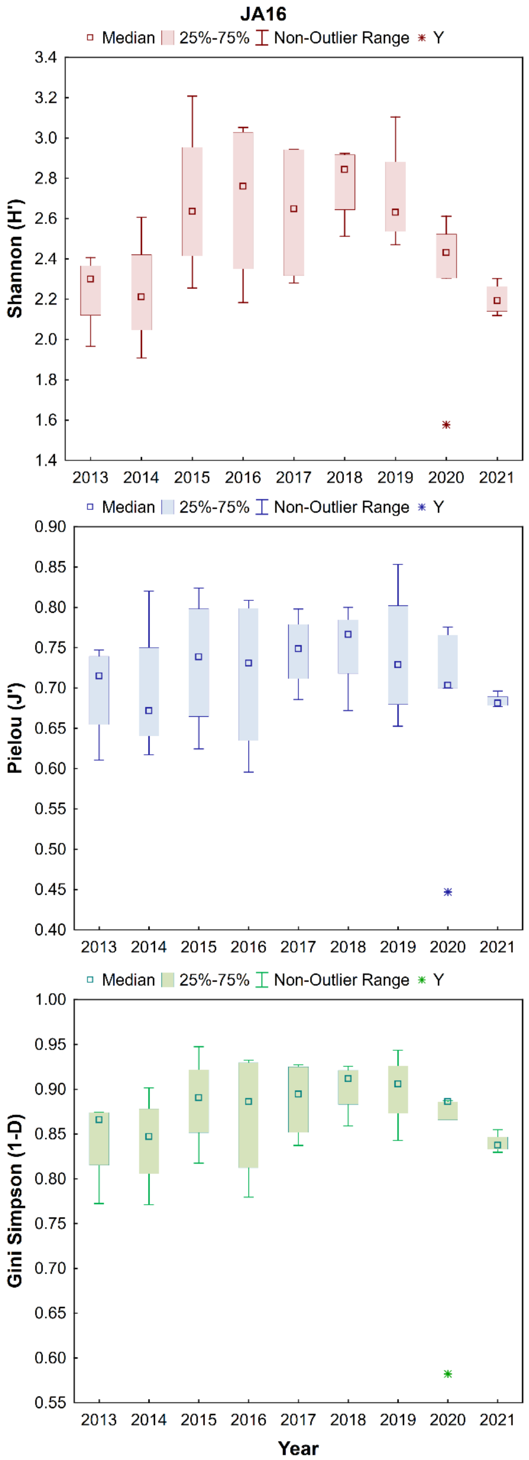

Figure 55: Interannual distribution of diversity indices at offshore station JA16 in central Adriatic Sea

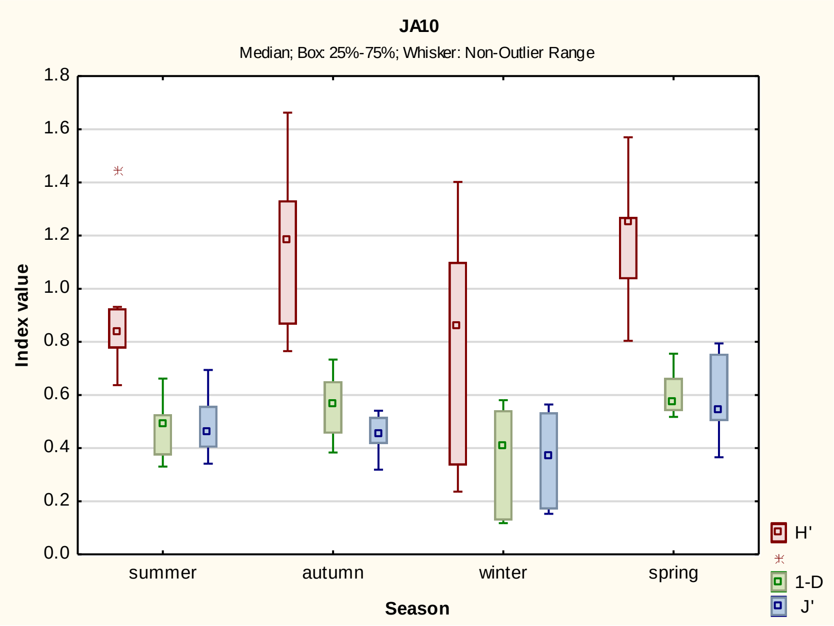

Figure 56: Seasonal distribution of diversity indices in copepod community at coastal station JA10 in central Adriatic Sea (Shannon, Pielou, Gini-Simpson).

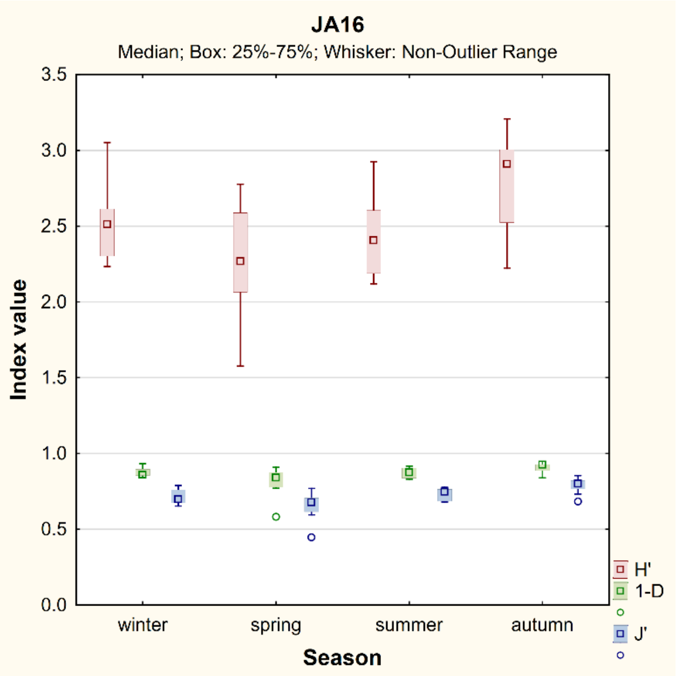

Figure 57: Seasonal distribution of diversity indices in copepod community at offshore station JA16 in central Adriatic Sea (Shannon, Pielou, Gini-Simpson).

Functional groups approach

Functional diversity is based on species traits rather than species identity (Santos et al., 2015). It is closely related to ecosystem functioning, as many ecosystem-level processes are influenced by the functional properties of coexisting species rather than their taxonomic identity (Naeem and Wright, 2003). Functional traits relate to species morphology, physiology, behavior, or life history (Litchman et al., 2013) and are often shared by closely related species, allowing for lower taxonomic resolution during sample analysis and more flexible data sets.

Using the project's catalogue of zooplankton indicators, which summarizes current global approaches to zooplankton indicators, we tentatively selected the most practical functional traits applicable to our data, relying on the life form pairs proposed by Ostle et al. (2017) and McQuatters Gollop et al. (2019) for the OSPAR region, as well as the information in Benedetti et al. (2016) on the functional traits of copepods in the Mediterranean Sea. We used four life pair combinations based on life history, morphology and physiology of zooplankton species to visualize interannual and seasonal variations in central Adriatic dataset: Relationships between holoplankton and meroplankton, between crustacean and gelatinous components, between small and large copepods, and between carnivorous and non-carnivorous zooplankton.

Holoplankton/meroplankton ratio

This ratio is considered an indicator of the coupling of benthic and pelagic organisms, sensitive to large-scale influences such as climate change and eutrophication (Kirby et al., 2008; Bedford et al., 2020). It is also influenced by the local coast-ocean gradient, relating to spatial changes in environmental variables such as Chl a, salinity and temperature (Michelsen et al., 2017). At the stations in the central Adriatic, meroplankton accounted for between 11.30 % and 38.14 % on an annual scale at JA10, between 7.83 % and 26.78 % at JA12 and between 3.04 % and 8.53 % at the offshore station JA16, with the highest seasonal variability observed at JA10 and lowest at JA16 (Figure 58).

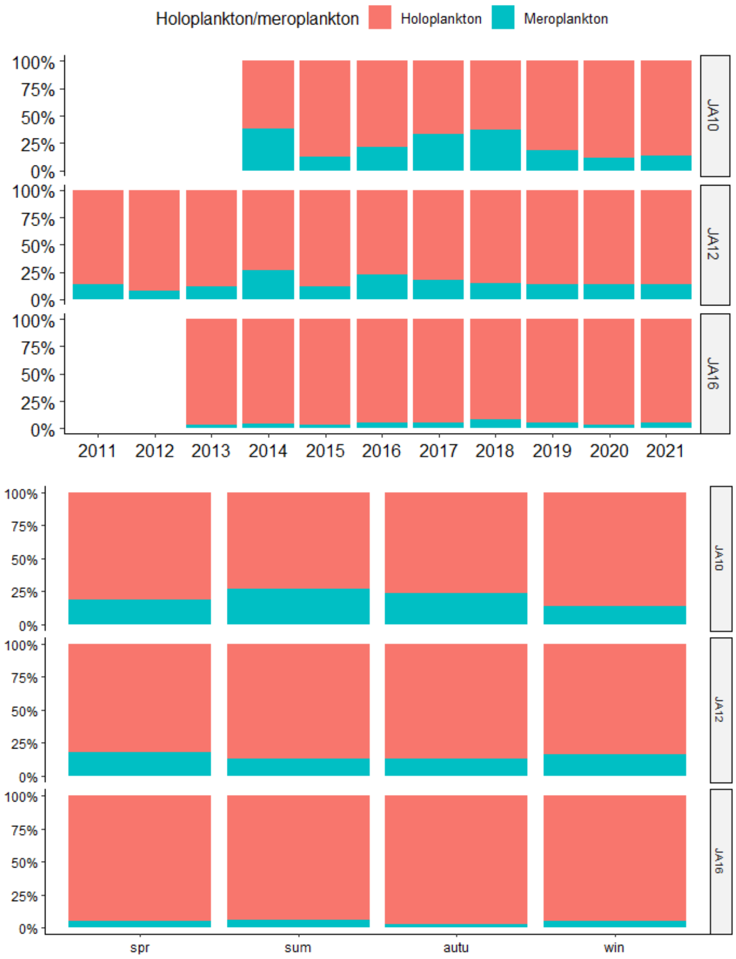

Figure 58: Interannual (upper panel) and seasonal distribution (lower panel) of holoplankton/meroplankton ratio at central Adriatic stations

Crustacean/gelatinous zooplankton ratio

The taxonomic groups of gelatinous zooplankton include scyphomedusae, siphonophores, ctenophores, pelagic tunicates and chaetognaths (Hamner et al., 1975; Nogueira Júnior et al. 2019). For ecological purposes, the most important relationship results from the increase of planktonic Cnidaria and Ctenophora over Copepoda and other crustacean groups, showing the gradual change between the two major trophic groups at the level of top predators: Fish and jellyfish (including ctenophores). The former represents a desirable outcome where fish are at the top, while the latter corresponds to the altered state of the ecosystem where energy is diverted to gelatinous macrozooplankton of non-commercial value (Schnedler-Meyer et al., 2016). This indicator is potentially related to large-scale pressures of anthropogenic origin, such as climate change, including warming, hypoxic and eutrophic conditions and acidification (Clerc et al., 2023). In our dataset, we considered all of the above groups within gelatinous zooplankton, with the exception of large jellyfish, for which there were no records. Large jellyfish are collected using a different methodology and quantified accordingly. Figure 59 shows an overwhelming dominance of crustaceans over gelatinous zooplankton in this mesozooplankton dataset. Interestingly, the contribution of gelatinous groups is quite similar at all three stations, ranging from 3.16 % to 7.38 % at JA10, from 2.82 % to 10.87 % at JA12 and from 2.78 % to 12.03 % at JA16. However, without the missing information on large jellyfish, it is not possible to fully capture the relevant changes in mesozooplankton using this indicator.

Figure 59: Interannual (upper panel) and seasonal distribution (lower panel) of crustacean/gelatinous zooplankton relationship at central Adriatic stations

Small copepods/large copepods

This size-based indicator examines the relationship between large copepods, representing a nutritional optimum for planktivorous fish, and small copepods, which are considered suboptimal for their survival (Beaugrand et al., 2004). A change in the proportion of large (≥2 mm in length) and small (<1.9 mm in length) adult copepods may therefore indicate changes in the food web structure (Capuzzo et al., 2018). In addition, the decrease in copepod community size composition has been attributed to the eutrophication effect (Uye, 1994). At central Adriatic stations, the contribution of small copepods is consistently large and with a slight decreasing gradient in offshore direction, ranging between 99.89% and 100% at JA10, between 99.27% and 99.94% at JA12 and between 97.16% and 98.61% at JA16.

Investigating the production and the trophic role of the copepod assemblages in the northern Aegean Sea, Zervoudaki et al. (2007) showed that in broad oligotrophic areas, the small-sized copepods were dominant in terms of biomass and production. Similarly, the relative abundances of small copepods and large copepods in the central Adriatic reflect the dominance of small species in the Mediterranean copepod assemblages (Zervoudaki et al., 2007; Mazzocchi et al., 2013) (Figure 60). Here, the prevalence of small-sized taxa is not an indication of the species' shift under the influence of warming climate, but the reflection of the natural communities of the warmer regions (Daufresne et al., 2009). This resource is optimally exploited in the food web, as sardine and anchovy, the most important small pelagic species in the Adriatic and throughout the Mediterranean, both feed on a variety of small prey, mainly copepods <1 mm (Borme et al., 2009; Nikolioudakis et al., 2012).

Figure 60: Interannual (upper panel) and seasonal distribution (lower panel) of relationship between small and large copepods at central Adriatic stations.

Carnivorus/non-carnivorous zooplankton

This life form pair is an indicator of the energy flow and the balance between primary and secondary consumers. Most non-carnivorous zooplankton species (herbivores and omnivores) are closely linked to phytoplankton cycles (Zhang et al., 2023). It is therefore expected that changes in primary producers will be rapidly followed by changes in primary consumers and propagate upwards in the food web (Gorokhova et al., 20216). It is also hypothesized that carnivorous zooplankton increase system stability when fish predation changes and mitigate the effects of algal blooms (Medvinskiĭ et al., 2007). However, it is still unclear how meaningful this indicator is in detecting anthropogenic environmental changes.

The mesozooplankton dataset of the central Adriatic is characterized by the clear dominance of non-carnivorous taxa (herbivores, omnivores and detritivores combined), ranging from 97.16 % to 99.68 % at JA10, from 97.59 % to 99.47 % at JA12 and from 95.20 % to 96.69 % at JA16 (Figure 61). The proportion of strictly carnivorous taxa increases slightly in the offshore direction, which is due to the greater diversity and contribution of large and small predatory copepods (e.g. Euchaeta, Haloptilus, Candacia and Corycaeidae, Sapphiniridae) and some gelatinous predators (e.g. Siphonophora, Chaetognatha) in open sea habitats. Seasonally, the relative proportion of carnivores is increased in autumn, which is the same at all stations and is due to the usual seasonal maximum of Chaetognatha and corycaeid copepods in this area.

Figure 61: Interannual (upper panel) and seasonal distribution (lower panel) of relationship between carnivorous and non-carnivorous zooplankton at central Adriatic stations.

Remarks and conclusions

The analysed mesozooplankton data from the Croatian Adriatic waters originate from the central Adriatic waters. However, the eastern Adriatic has pronounced longitudinal differences in terms of depth, nutrients, phytoplankton biomass and thermohaline conditions, which are reflected in mesozooplankton characteristics such as abundance and community structure (Hure and Kršinić, 1998). Therefore, it is important to extend the spatial coverage of the dataset to the two remaining sub-basins of the Adriatic Sea (northern and southern Adriatic) in order to obtain a more realistic picture of mesozooplankton dynamics and change patterns. Although both the northern and southern basins are also monitored for mesozooplankton in Croatia, the time span monitored is much shorter (generally from 2020 onwards), making it difficult to draw reliable conclusions regarding the use of mesozooplankton parameters for the purposes of the MSFD at the scale of the entire Croatian eastern Adriatic.

The analysis of the data set for the central Adriatic has shown that the mesozooplankton community is consistent with the prevailing natural conditions, as the stations are located along the distinct environmental gradient of depth and nutrient conditions. However, further work is needed to show the direct relationship with anthropogenic pressures and impacts.

Regarding the use of α-diversity indices, testing of further indices with different combinations of community composition (e.g. copepods and cladocerans; copepods, cladocerans and appendicularians, etc.) is planned for future work. So far, the conclusion is that there are clear changes in α-diversity along the coast-offshore gradient, highly driven by the changes in copepod community, but it is unclear whether more nuanced changes can be easily captured (e.g. between areas with less dramatic environmental differences and smaller differences in anthropogenic pressure). Functional diversity offers a promising approach, provided the right indicators are selected for the Adriatic mesozooplankton.

2.2.3 Greece

2.2.3.1 Case study: Aegean, Ionian and Levantine Seas

As a first attempt to apply selected indicators for the D1C6, we provide here results from the analysis of zooplankton data in Aegean, Ionian and Cretan passage-Levantine Seas (Figure 62). The data used came from multiple oceanographic cruises during the last 12 years (2009-2021) in Hellenic marine waters (Figure 63). Zooplankton have been collected using a 200 μm WP2 net by vertical tows from 100 m to the surface. In total 87 samples have been analysed. Among zooplankton groups, Copepoda and Cladocera were identified to species level (when possible) and Copepoda were classified according to sex and stage (females, males and juveniles, when possible). A thorough analysis of 18 ecological indices, expressing diversity (e.g., Menhinick index), evenness (e.g., Pielou's evenness J) and dominance (e.g., Berger-parker index), was performed to investigate structural changes of mesozooplankton communities. For this analysis only individuals belonging to the group "Copepoda" (adult females) and "Cladocera" were used, as those are the only groups in which detailed taxonomic analysis was performed (genus and/or species level). For the calculation of total abundance and number of taxa, all individuals found in a sample were used.

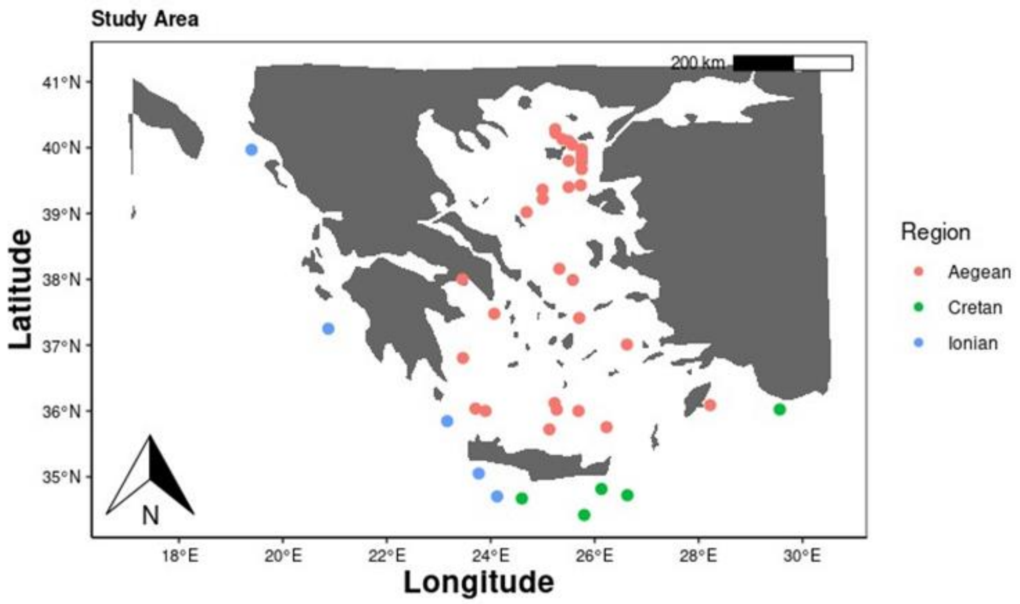

Figure 62: Zooplankton case study area in Greece.

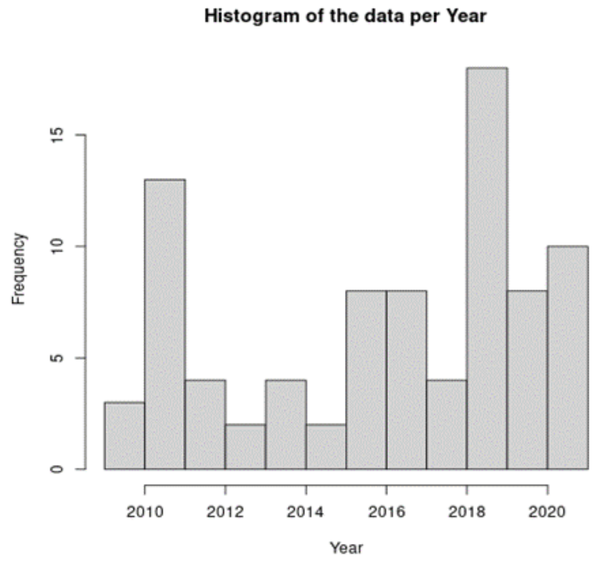

Figure 63: Frequency of zooplankton data collected per year in the Greek case study from 2009 until 2021.

For the three regions a clear pattern was observed and one-way ANOVA was performed, which further confirmed that there is a statistically significant differentiation between the regions both in regard to total abundance and number of taxa ($p < 0.001$) (Tables 5, 6 and Figures 64, 65, respectively). The stations in the Aegean Sea generally exhibited higher abundances in comparison to the other two regions.

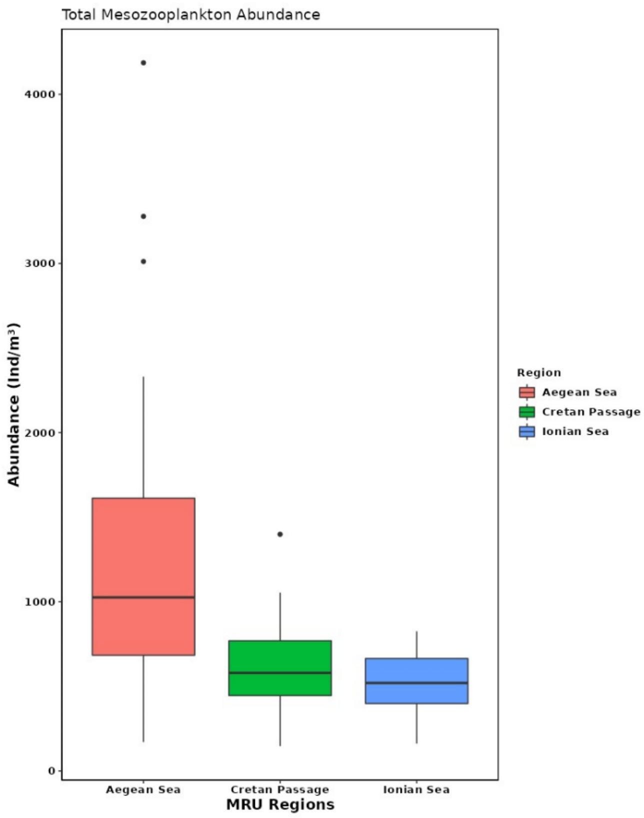

Figure 64: Mean Total abundance (ind m^{-3}) including sd, max and min values in the three different regions of the Greek case study from 2009 until 2021.

Table 5: Mean Total abundance (ind m^{-3}) including sd, max and min values in the three different regions of the Greek case study.

Region	Mean (ind m^{-3})	Sd (ind m^{-3})	Max (ind m^{-3})	Min (ind m^{-3})
Aegean Sea	1221	768	4186	170
Cretan Passage-Levantine Sea	626	349	1399	146
Ionian Sea	525	208	825	161

Table 6: Number of zooplankton taxa in the three different regions of the Greek case study.

Region	Mean (ind m^{-3})	Sd (ind m^{-3})	Max (ind m^{-3})	Min (ind m^{-3})
Aegean Sea	38	13	79	19
Cretan Passage-Levantine Sea	33	6	44	23
Ionian Sea	27	5	34	20

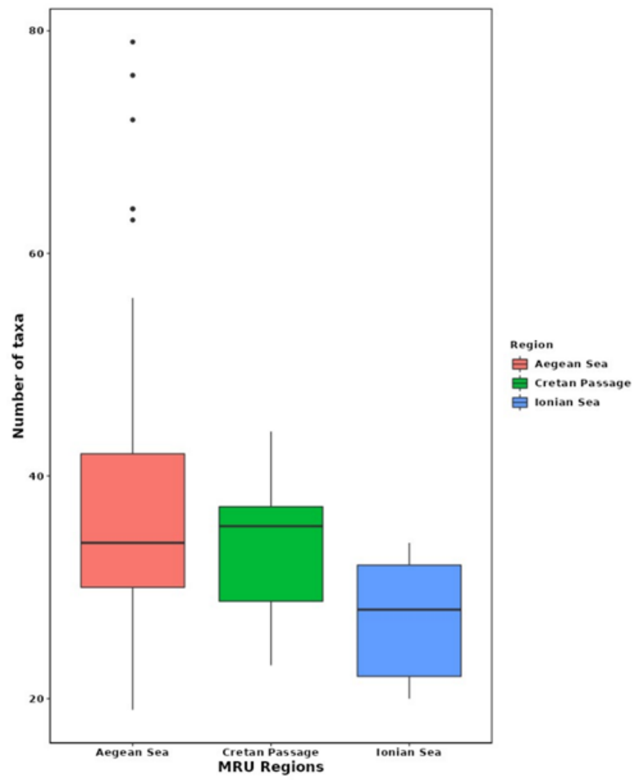

Figure 65: Number of zooplankton taxa in the three different regions of the Greek case study.

To summarize, the alpha-diversity indices and to better understand the community composition a PCA analysis was performed (Figure 66). A clear-cut pattern in alpha diversity was observed along the different areas, mainly due to differences in evenness, total abundance, dominance and phylogenetic diversity. Regarding the three diversity indices (Shannon, Pielou and Berger-Parker), the Ionian Sea is characterized by higher evenness values in comparison with the other two regions, the Aegean Sea is characterized by higher dominance values, which did not surpass 50%. Shannon's index had similar values for the stations belonging to the three regions but with greater SD for the Aegean Sea (Figure 61).

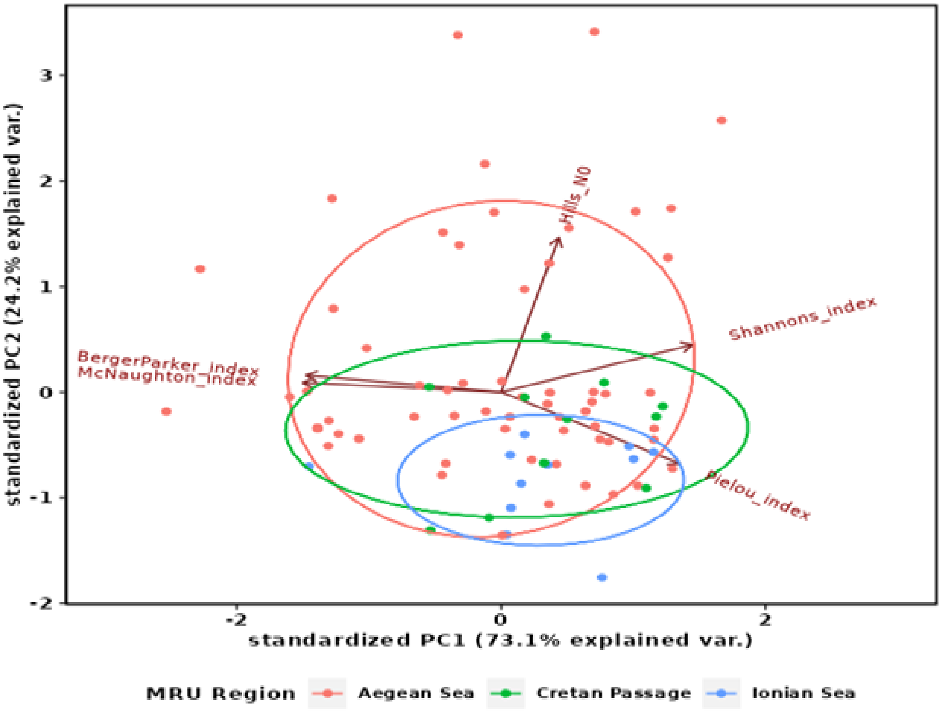

Figure 66: PCA analysis using alpha-diversity indices in the three different regions of the Greek case study.

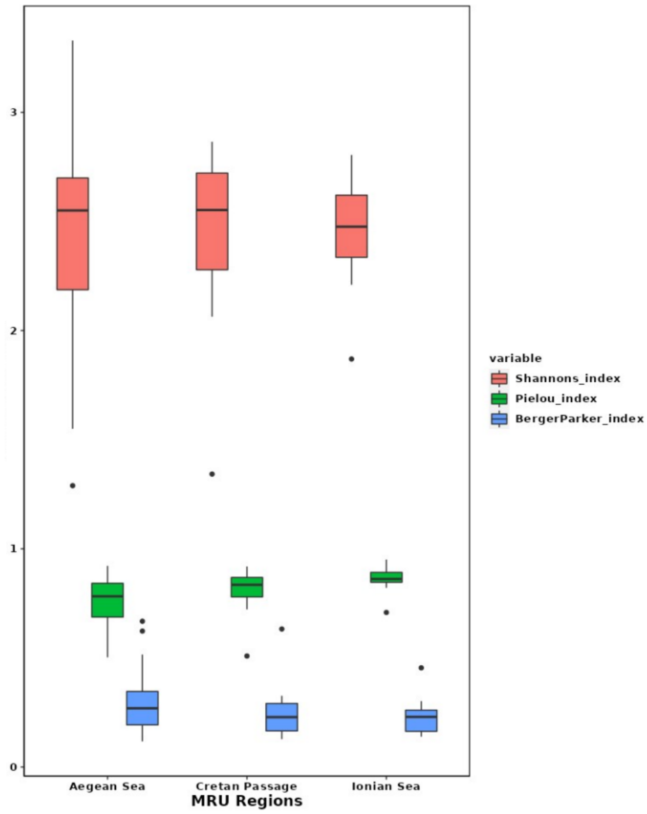

Figure 67: Alpha-diversity indices in the three different regions of the Greek case study.

Mesozooplankton biomass and abundance are generally lower in offshore waters. Epipelagic mesozooplankton communities in offshore waters are highly diversified in terms of taxonomic composition, but copepods represent the major group both in terms of abundance and biomass. Species-rich genera of the calanoids *(Clausocalanus, Calocalanus, Mecynocera, Haloptilus and Ctenocalanus*) and cyclopoids (*Oithona, Oncaea, Corycaeus, Farranula*) account for the bulk of copepod abundance and biomass in epipelagic layers of the offshore waters. Mesozooplankton Shannon diversity variations over year and during warm and cold periods in the three different offshore areas are shown in Figure 68. Alpha-diversity in the open sea remains almost stable throughout the years and seasons.

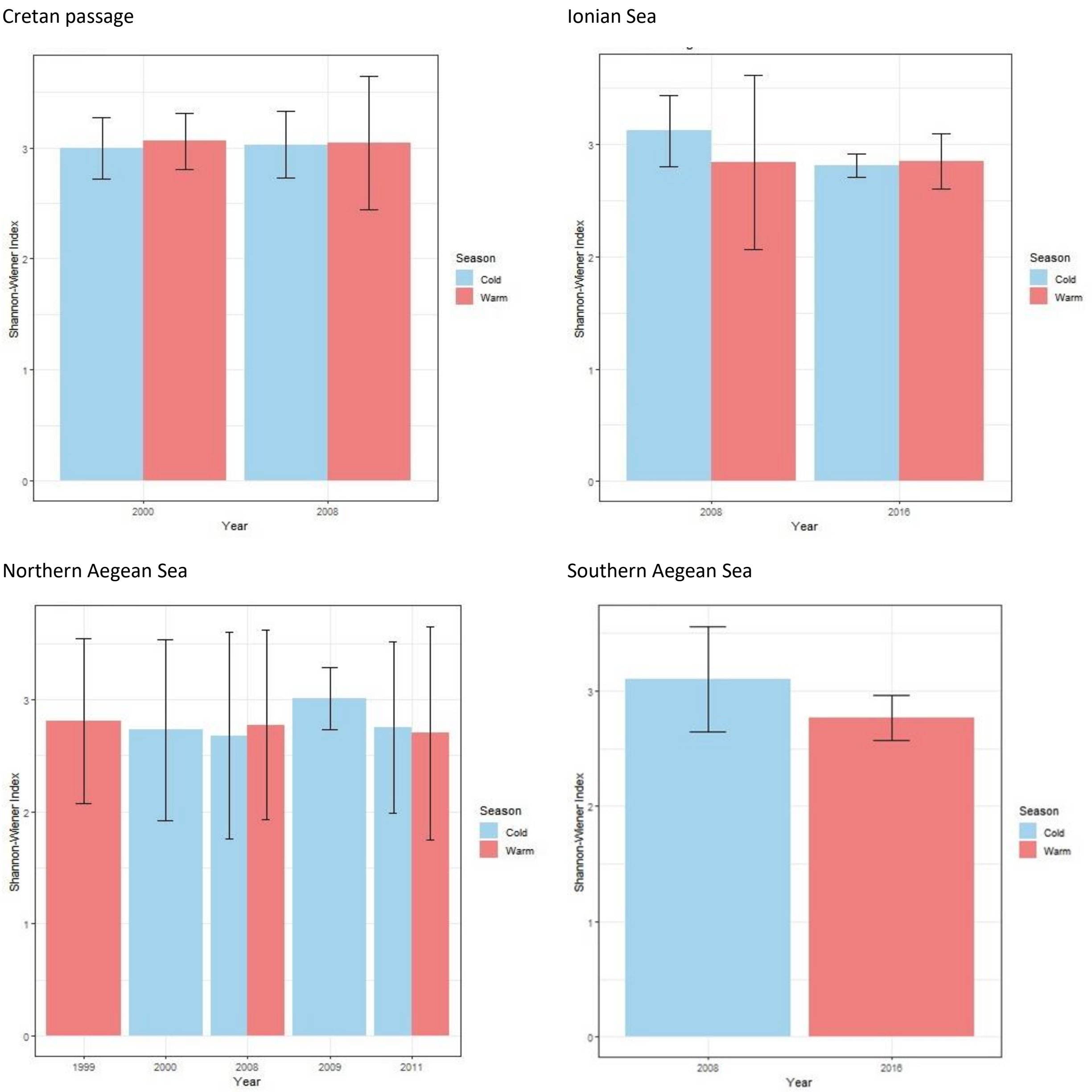

Figure 68: Aggregated mean values of the Shannon Wiener diversity index for the worm and cold periods with error bars indicating the 95 % confidence interval in the offshore waters of the different regions.

In addition, we examined the potential effect of biotic and abiotic variables in mesozooplankton assemblages' composition of the Aegean Sea in order to highlight the variables responsible for beta diversity changes in space and time. To recognize and interpret underlying patterns of community composition the Non-metric Multi-Dimensional Scaling (NMDS) method was used. The information on the potential parameters shaping that dissimilarity between samples were extracted by correlating vector and factor variables into the NMDS ordination. For the NMDS analysis a distance matrix is required as an input and in our case we chose the Bray-Curtis dissimilarity. Prior to the analysis the Hellinger's transformation method was applied to the abundance data to prevent the "double zero" problem. Also, a number of permutations was used in order to evaluate the significance of each fitted variable in the two methods. The non-metric multidimensional scaling (NMDS) analysis showed that the mesozooplankton communities analyzed can be clustered into two main groups based on their location: the NA communities in which distinct dissimilarities are observed between spring and autumn and the SA community in which season although plays a role in species composition, the differentiation is not as prominent. Temperature was found to have a statistically significant effect on assemblages' distribution in the 2D NMDS ordination with squared correlation coefficient $r^2 = 0.69$ (p-value = 0.001), followed by stations' bottom depth and salinity with $r^2 = 0.49$ (p-value = 0.001) and $r^2 = 0.37$ (p-value = 0.001) respectively. Generally, autumn is characterized by higher temperatures in the upper water column layer in comparison with spring. Moreover, temperature was identified as the main driver responsible for the seasonal assemblages' differentiation in the NA. Dissolved oxygen and chlorophyll-a concentrations were found to have no important effect on beta diversity in the Aegean Sea (Figure 69).

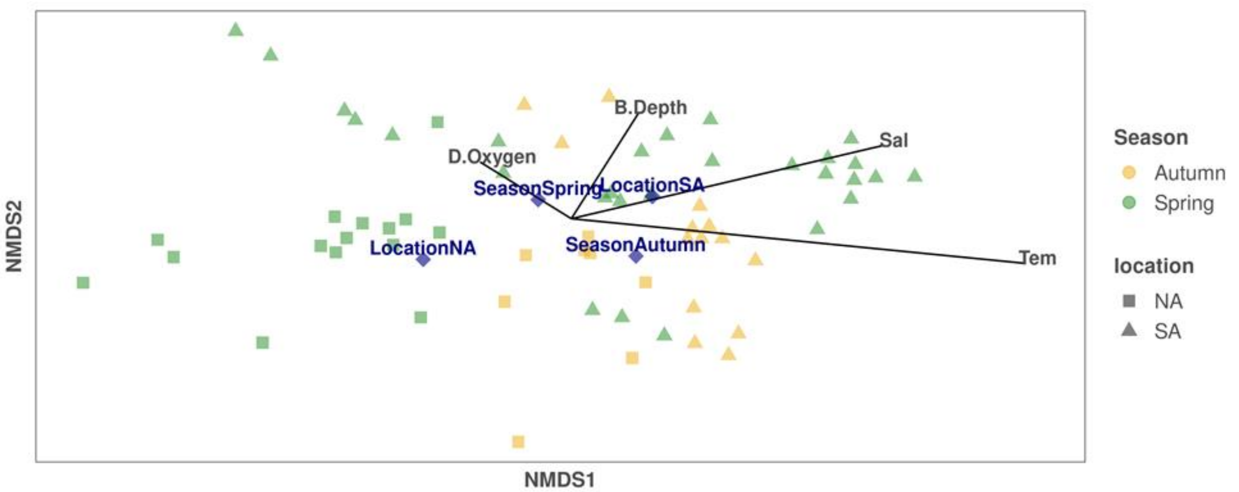

Figure 69: Distribution of stations according to the NMDS ordination analysis (stress value ≈ 0.18). The stations are color coded according to season and the shapes represent the stations location. The centroid values for all samples belonging to a certain category are plotted as well. The lines represent regression vectors for environmental variables with correlation to the mesozooplankton assemblages. Line length represents correlation strength and the line angle shows the direction of sample's increase with respect to that variable.

Conclusions

In this research, alpha diversity was generally higher in the more saline and oligotrophic waters of the South (SA) compared to the North Aegean (NA), whereas seasonal differentiations in the α-diversity patterns were more prominent for the NA, which is in accordance with previous research for this study area (Zervoudaki et al., 2020). Generally, mesozooplankton communities were dominated by copepod species in both the NA and SA. Although, during autumn in the NA a shift in the community composition was recorded, mostly caused by three cladoreran species (*Penilia avirostris, Evadne spinifera and Pseudevadne tergestina*). That shift is correlated with the increase in sea water temperature. For the SA the communities are mostly shaped by the salinity and seasonal differentiations in species composition were not as clear. This study is the first step into understanding how the biotic and/or abiotic parameters affect mesozooplankton alpha and beta diversity in the complex physicochemical environment of the Aegean Sea. Further research will shed more light to the mechanisms shaping mesozooplankton community structure. The potential use of mesozooplankton in ecological assessments that has been proposed in the context of the Marine Strategy Framework Directive (Ndah et al., 2022), is making the understanding of mesozooplankton dynamics and the parameters effecting their variability necessary.

2.2.4 Italy

2.2.4.1 Study area and sampling stations information

Zooplankton data collected along the Italian coastlines from 2015 to 2021 by the Italian Regional Environmental Protection Agencies (ARPAs) according to Marine Strategy Framework Directive (MFSD) monitoring protocols. The area covered by the monitoring regarded 15 Italian regions, 54 transects and 162 sites. Each transect included one coastal (3 nM), one intermediate (6 nM) and one offshore station (12 nM).

The division of the Italian seas into biogeographical areas (Figure 70) proposed by Bianchi (2004) has been followed, excluding a "microsector" (Strait of Messina). Therefore, for the assessment the Mediterranean marine region (Western Mediterranean, Adriatic Sea, Ionian Sea and Central Mediterranean Sea) was divided into eight (sub)region: Ligurian Sea, Northern Tyrrhenian Sea, Southern Tyrrhenian Sea, Strait of Sicily, Ionian Sea, Southern Adriatic Sea, Middle Adriatic Sea, and Northern Adriatic Sea.

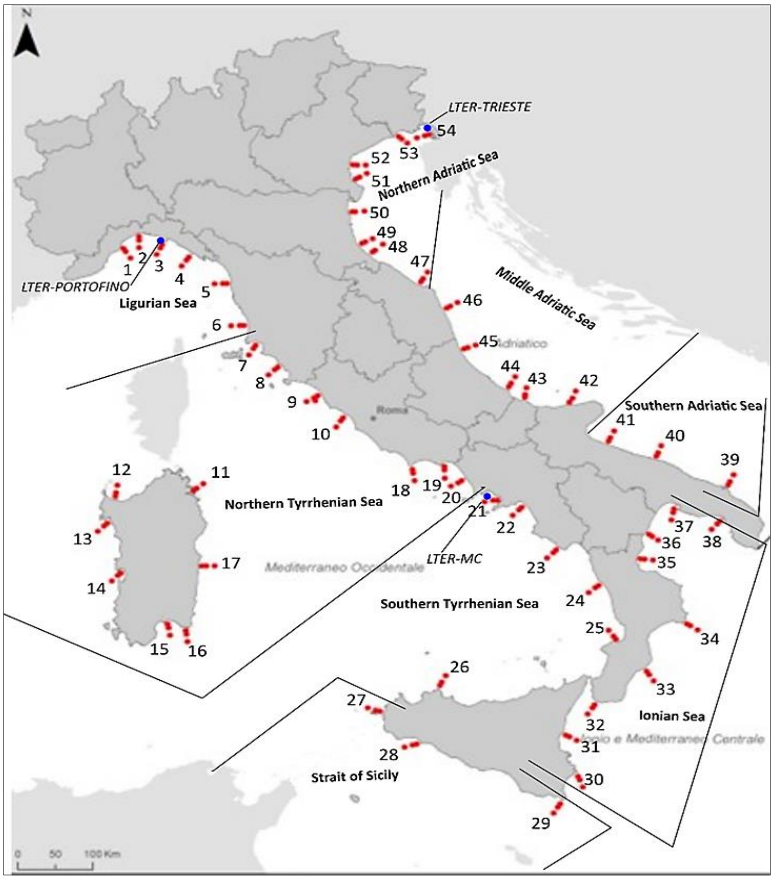

Figure 70: Map of the Italian coast showing stations sampled within the MSFD monitoring (red symbols), and marine regions and sub-regions considered. Zooplankton long-term series (LTER-sites) are also indicated (blue symbols).

2.2.4.2 Dataset

The collected abundance (Ind m^{-3}) of marine zooplankton, species composition and all metadata were organized into a dataset. All taxa have been verified as accepted species and given the currently accepted name as defined by the World Register of Marine Species database (WoRMS— https://www.marinespecies.org/). Considering the heterogeneous taxonomic expertise and competences in data compilation and quality check of the 15 regional Institutions implementing the MSFD in Italian waters, an important and considerable part of this study was to build a high-quality dataset to be able to use the validated data for the purposes of subsequent statistical processing (Figure 71).

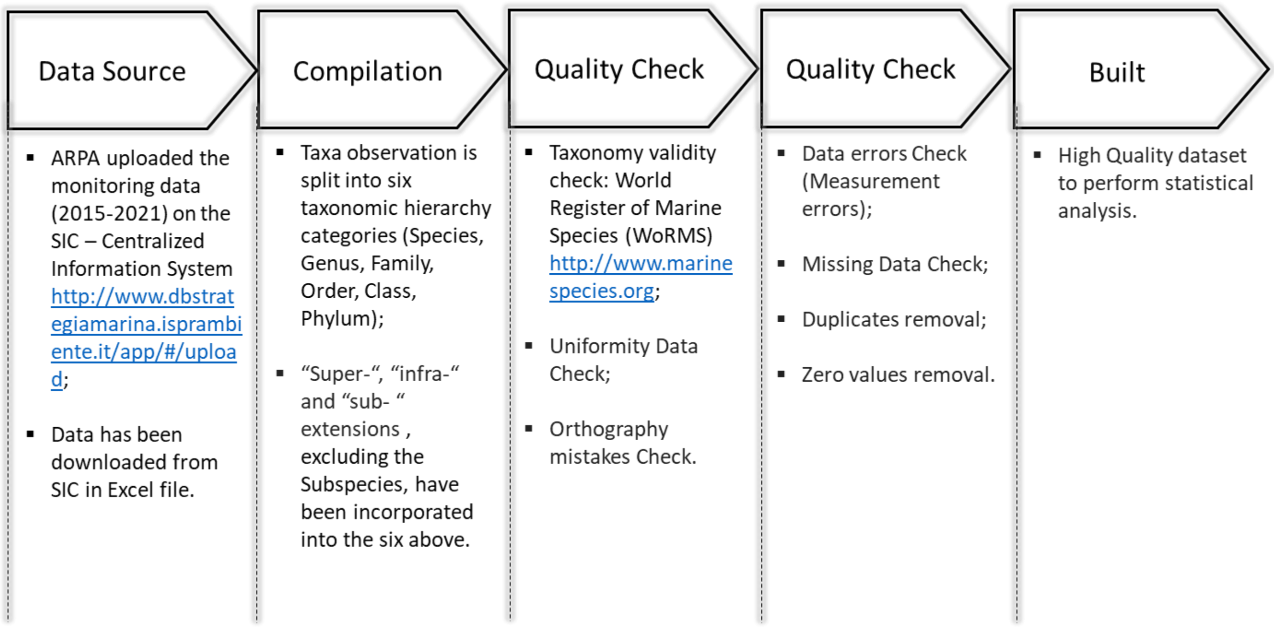

Figure 71: Data Quality Control steps, in order to deliver a coherent, comparable dataset that could be used to support the MSFD implementation.

Copepods constitute the main component of mesozooplankton biomass, making them particularly relevant to the indicator research framework that can be tested. Attention has only been paid to copepods and their adult stage; juveniles (copepodites) have not been included in this study.

The copepods data were assembled into dataset with the same methodology described previously. The list of copepod species was then carefully evaluated. The ‘’dubious taxa’’ (Table 7), i.e. copepod species not previously recorded in Italian waters based on the most recent copepod checklist (Relini, 2008) that it was not possible to verify because preserved specimens were missing, were assigned at genus level (e.g. “*Paracalanus* spp. Unidentified”). The same assignment was used for the specimens that were identified in their family or order levels (i.e. scientific name + spp. Unidentified). Lastly, the copepod dataset made up on 63080 records and 22 columns.

Table 7: Copepod species not previously recorded in Italian waters (according to Relini, 2008, Biol. Mar. Mediterr., 15 (suppl. 1).

SCIENTIFIC NAME	DISCOVERY LOCALITY
Calocalanus equalicauda	Veneto
Candacia catula	Sicilia
Centropages brachiatus	Sicilia
Clytemnestra gracilis	Marche, Veneto
Copilia lata	Sicilia
Copilia mirabilis	Sicilia
Distioculus minor	Lazio
Ditrichocorycaeus lubbocki	Puglia, Veneto
Ditrichocorycaeus minimus	Lazio
Eurytemora affinis	Puglia
Ferranula curta	Veneto
Goniopsyllus clausi	Sardegna, Veneto
Labidocera detruncata	Sicilia
Monstrilla grandis	Friuli Venezia Giulia, Puglia
Monstrilla longiremis	Puglia, Veneto
Oculosetella gracilis	Lazio
Oithona simplex	Sicilia
Oncaea waldemari	Campania, Sicilia
Paraeuchaeta tumidula	Veneto
Pareucalanus sewelli	Sicilia

2.2.4.1 Variability of phenology: Copepod dynamics in the period 2017-2021

Copepods abundance data were used for the period 2017-2021 as this period has the highest sampling frequency, although data coverage is generally inconsistent across locations and years and has several gaps. In the period 2017-2021, the average total abundance of copepods in the different subregions (Figures 72 and 73) does not show great interannual variability within the same marine subarea considered, but presents differences between the areas, and specifically, the average abundances are higher extensive were recorded in the Middle Adriatic Sea (4469 ± 407 Ind m^{-2} during 2018), while the lowest abundances were detected in the Strait of Sicily 417 ± 24 Ind m^{-2} during 2019 to 292 ± 23 Ind m^{-2} during 2021.

The annual dynamics of the Copepoda group, averaged for the period considered, shows a certain variability in each marine sub-region (Figure 74). Since the data coverage is heterogeneous, the annual peaks of abundance do not necessarily reflect the typical seasonal trend of each marine subregion.

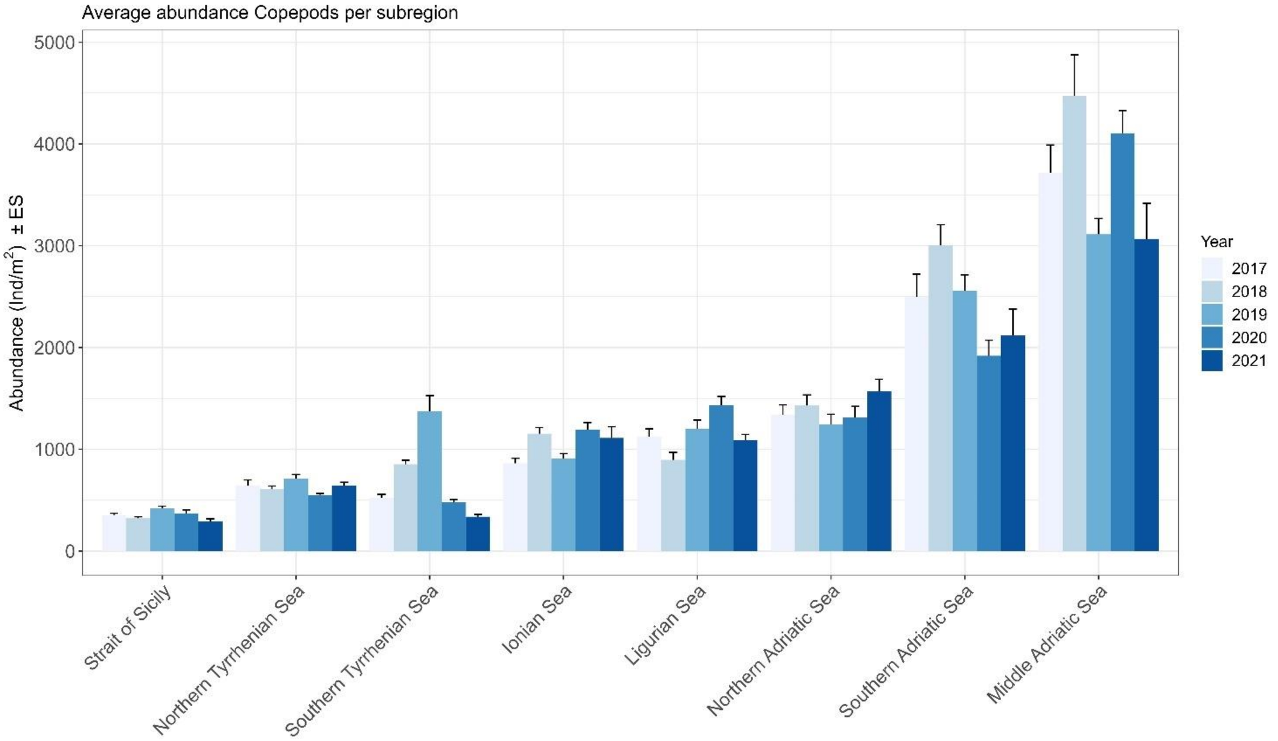

Figure 72: Average annual copepods abundance (Ind m^{-2} ± SE) in eight (sub)regions of the Italian case study.

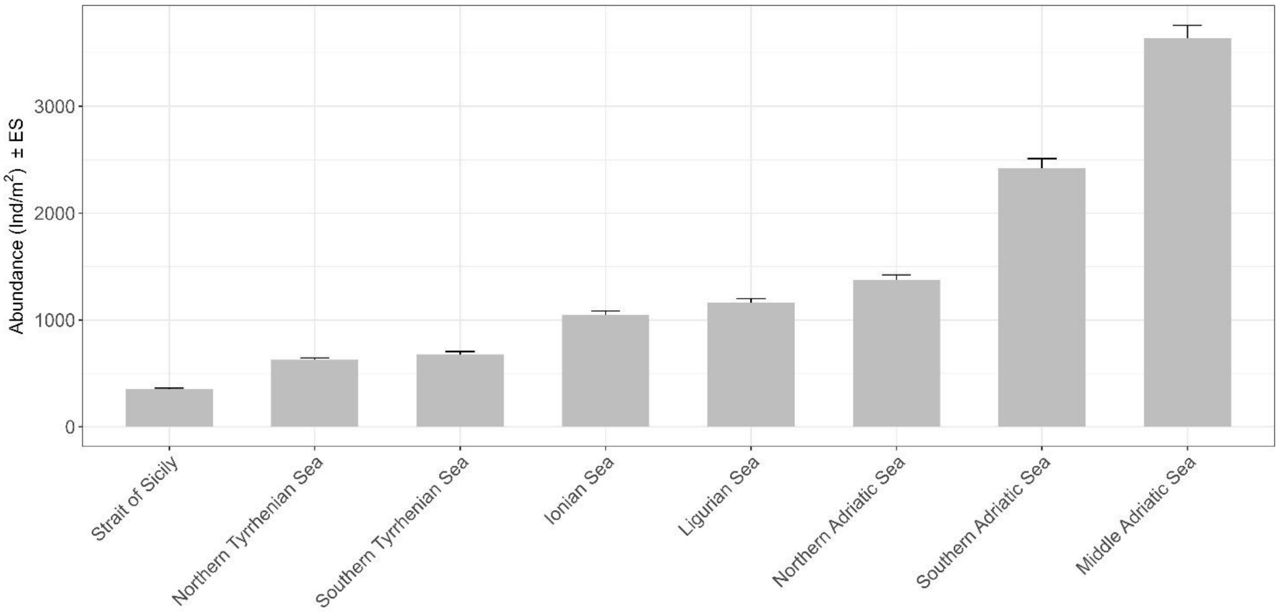

Figure 73: Overall average annual copepods abundance (Ind m^{-2} ± SE) in eight (sub)regions of the Italian case study.

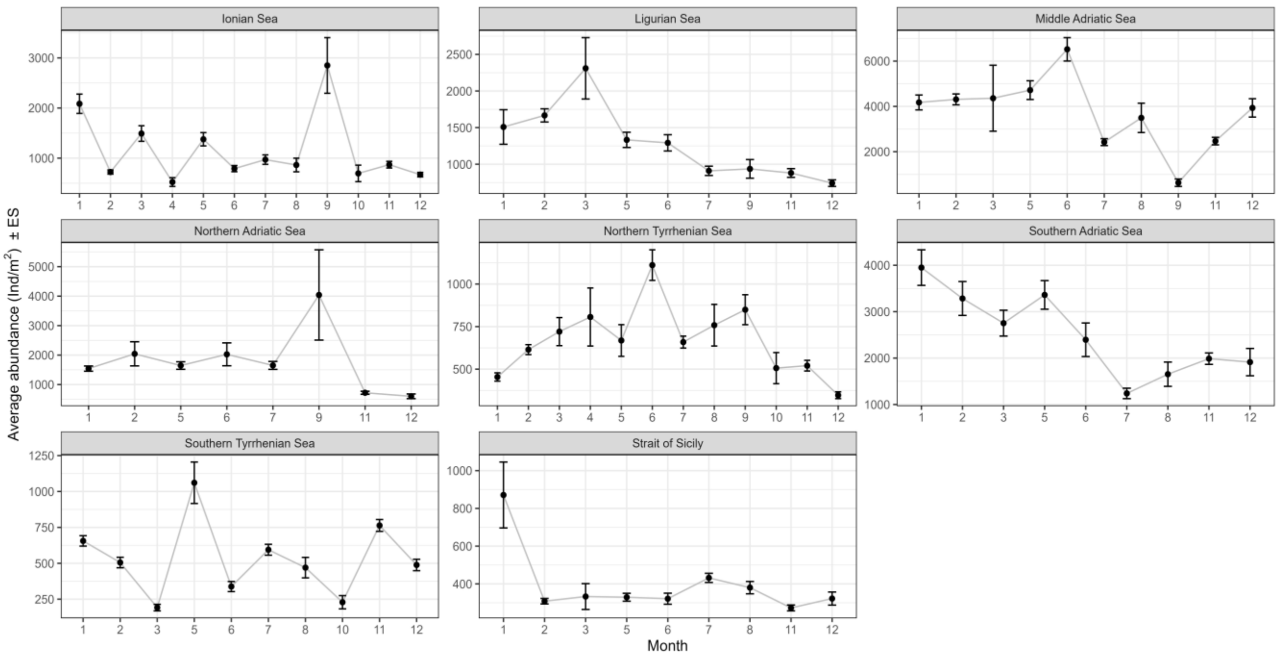

Figure 74: Annual dynamics of copepods abundance (Ind m^{-2}) in eight (sub)regions of the Italian case study. Note the different scales on y axes.

2.2.4.2 Indicator testing: Diversity indices to assess the state of the mesozooplankton community

There are a total of 255 taxa of the Copepoda Class (Figure 75), of which 179 were identified at Species level. Greatest number of Copepods taxa (including the level of Order, Family, Genus and Species) is present in the Northern Tyrrhenian Sea (192), followed by the Ionian Sea (184), while the smallest number of taxa detected are in the Middle Adriatic Sea (105) and Southern Adriatic Sea (90).

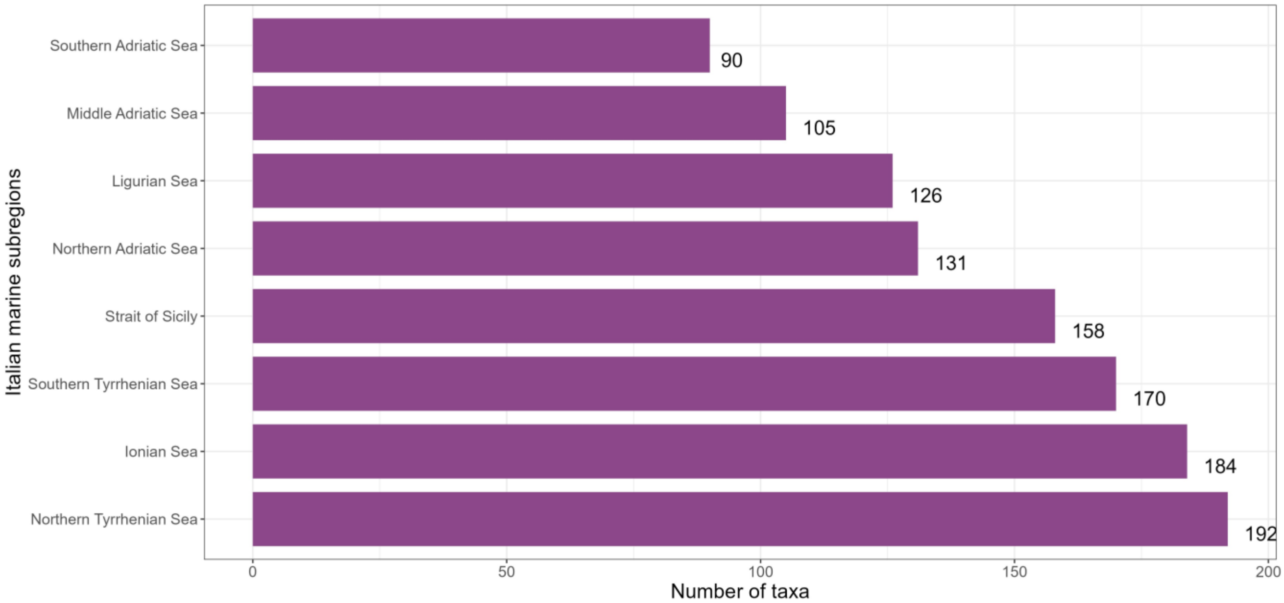

Figure 75: Total number of copepod taxa identified in eight (sub)regions of the Italian case study.

Alpha diversity indices

Diversity indices were calculated to examine the seasonal and annual variability in mesozooplankton community composition in the marine sub-regions. Four seasons were thus determined: winter (January-March), spring (April-June), summer (July-September) and autumn (October-December). Alpha diversity indices were calculated at the species level and abundance calculated as individuals per square meters (Ind m^{-2}) to normalize the depth values, which reach a maximum of 100 m.

Shannon, Gini-Simpson, and Rao indices were calculated to indicate the alpha diversity. The statistical analyses of the research were analyzed in R (version 4.3.2).

The Shannon–Wiener index (Shannon, 1949) was used as follows:

$H' = -\Sigma_i \, p_i \log(p_i)$

where pi is the proportion of the i-th species in the sample, to determine the abundance distribution among the various species;

The Gini-Simpson index (Simpson, 1949) was calculated as:

$D_{\text{Gini-Simpson}} = 1 - \Sigma_i \, (n_i/N)^2$

where ni is the number of individuals in species i, N = total number of individuals of all species, and ni/N = pi (proportion of individuals of species i);

The Rao's quadratic entropy index (Rao, 1982) based on the proportion of the abundance of species present in the community and some measure of dissimilarity among them. When the species are completely different in terms of their traits, Rao quadratic entropy is equivalent to the Gini-Simpson index (where d ij is the dissimilarity).

The index values calculated on the copepod data matrix are shown in the Figures 76-81. Box plots reports the data distribution with the mean (●), the median (line), the interquartile range (box), the non-outlier range (vertical bars), the outliers (point smaller).

Sub-regional reference values of biodiversity indices were defined based on the 25-75% distributional range of indices observed within a sub-region, aggregated by season and by years (excluding the years with poor sampling coverage). Results of the analysis showed that thresholds varied depending on the season and the marine sub-region considered, with the Tyrrhenian and Ionian seas characterised by a more diversified mesozooplankton community than the other marine sub-regions.

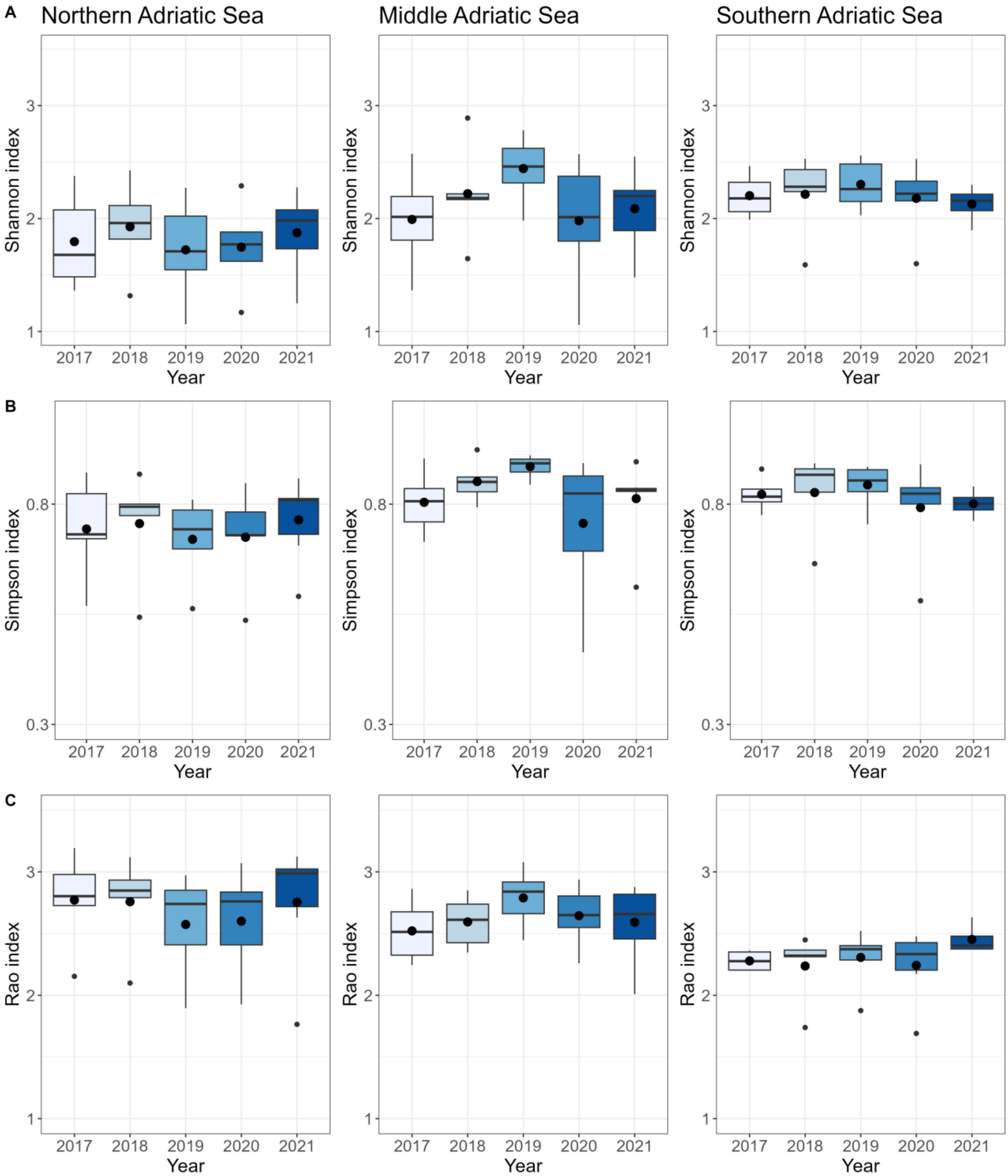

Figure 76: Shannon index (A), Gini-Simposn index (B), Rao index (C), calculated for each year, are shown for Adriatic basin (Northern Adriatic Sea, Middle Adriatic Sea, Southern Adriatic Sea).

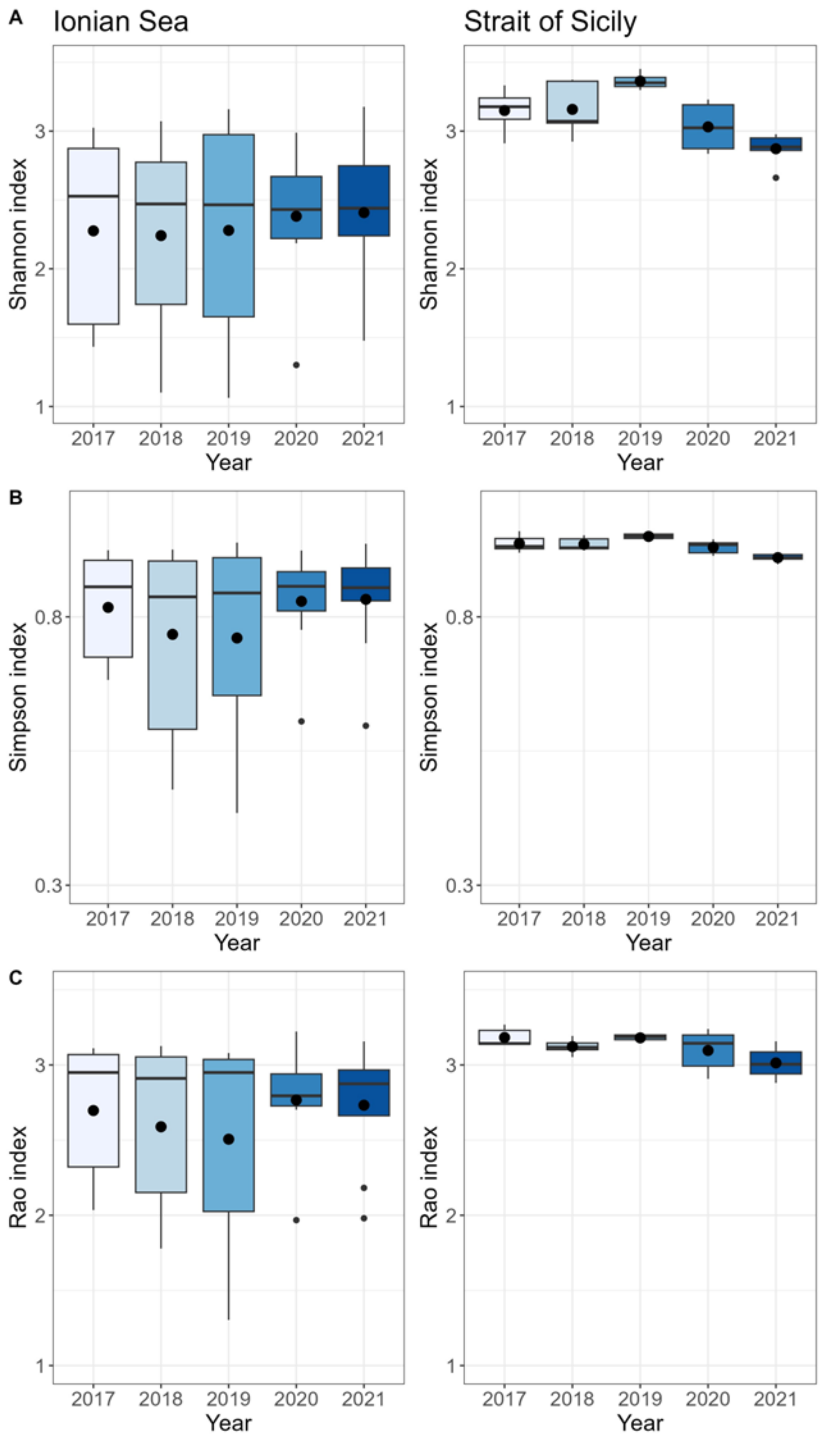

Figure 77: Shannon index (A), Gini-Simpson index (B), Rao index (C), calculated for each year, are shown for Ionian basin (Ionian Sea and Strait of Sicily).

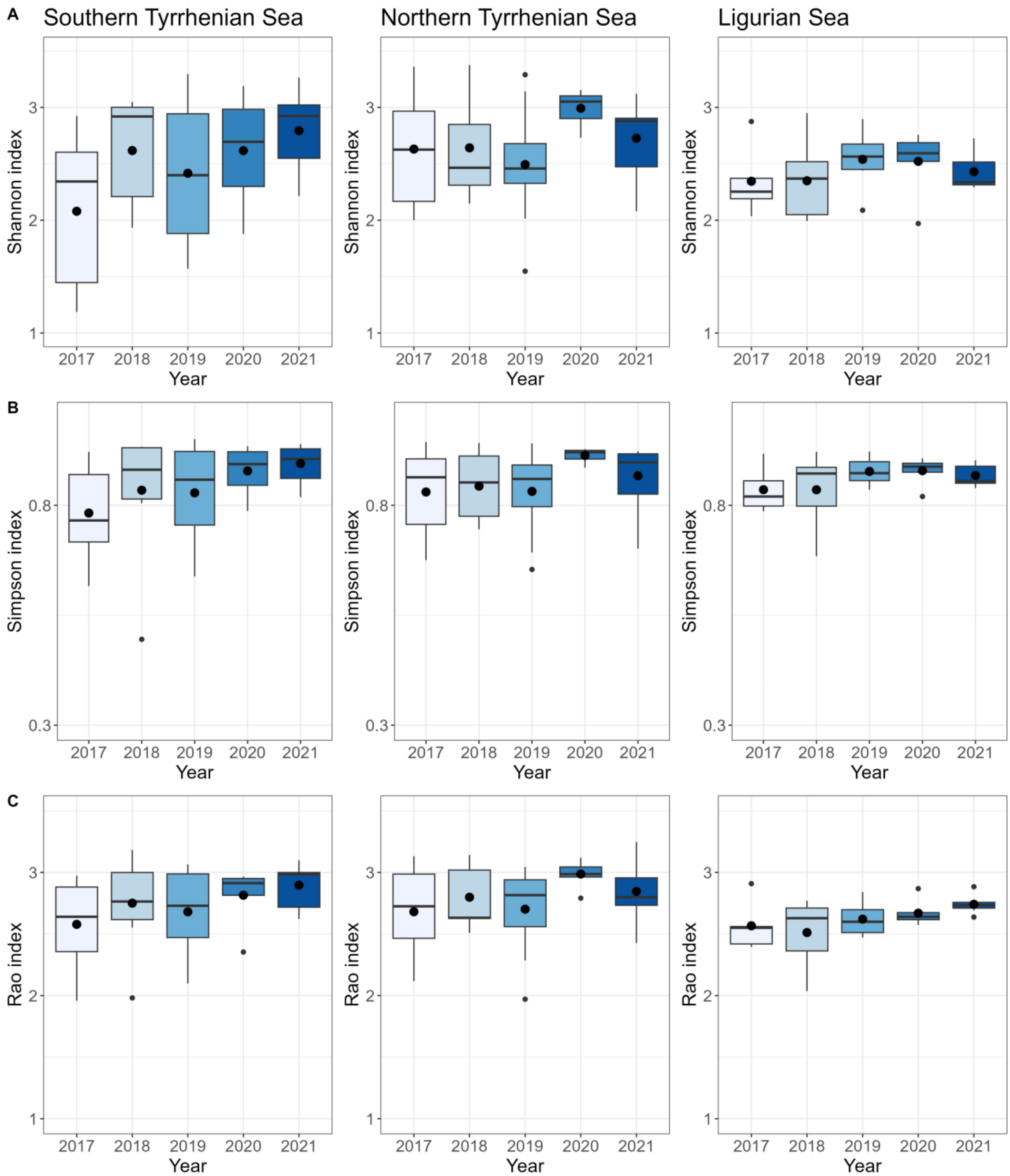

Figure 78: Shannon index (A), Gini-Simpson index (B), Rao index (C), calculated for each year, are shown for Tyrrhenian basin (Southern Tyrrhenian Sea, Northern Tyrrhenian Sea, Ligurian Sea).

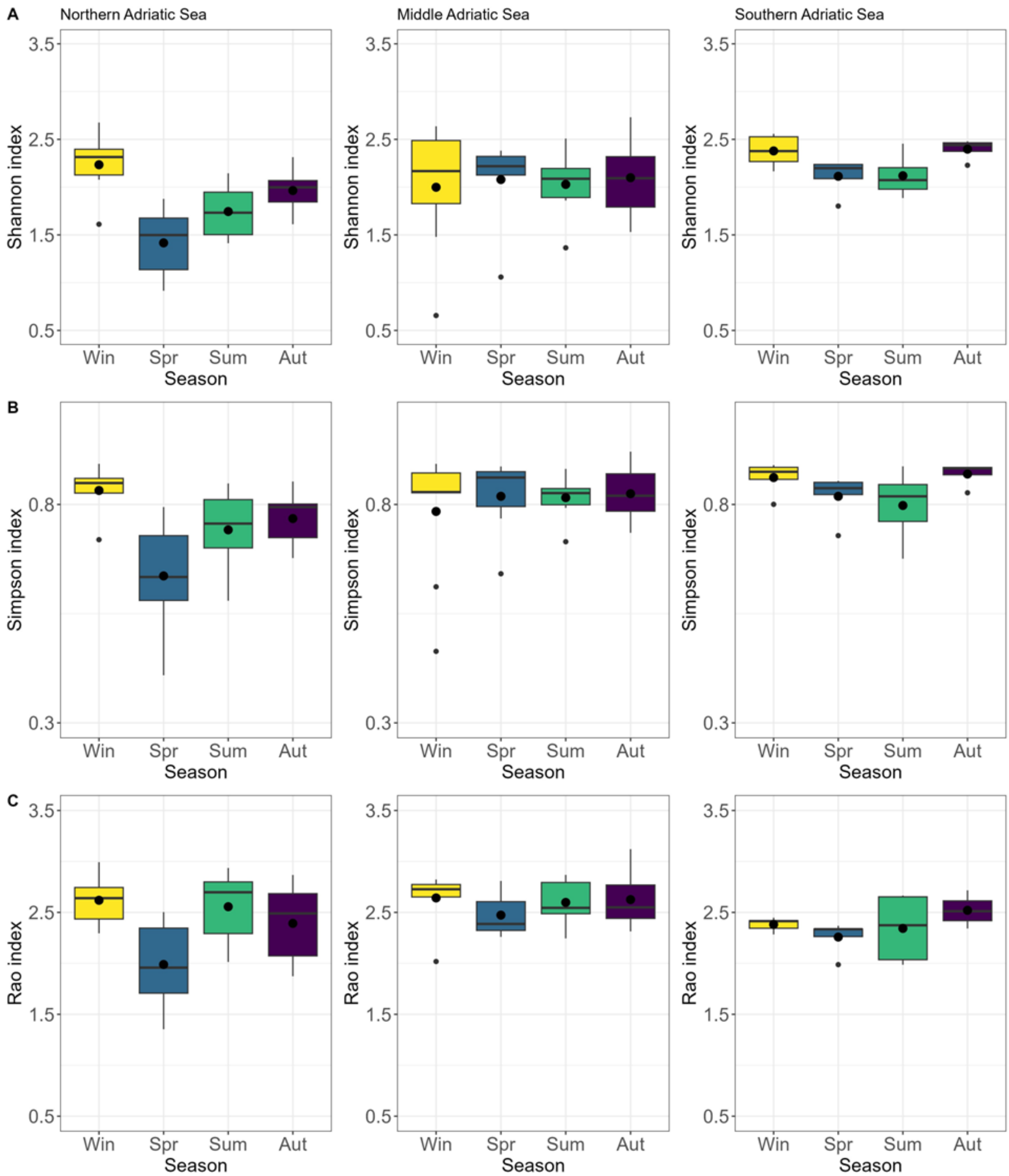

Figure 79: Shannon index (A), Gini-Simpson index (B), Rao index (C), calculated for each season, are shown for Adriatic basin (Northern Adriatic Sea, Middle Adriatic Sea, Southern Adriatic Sea).

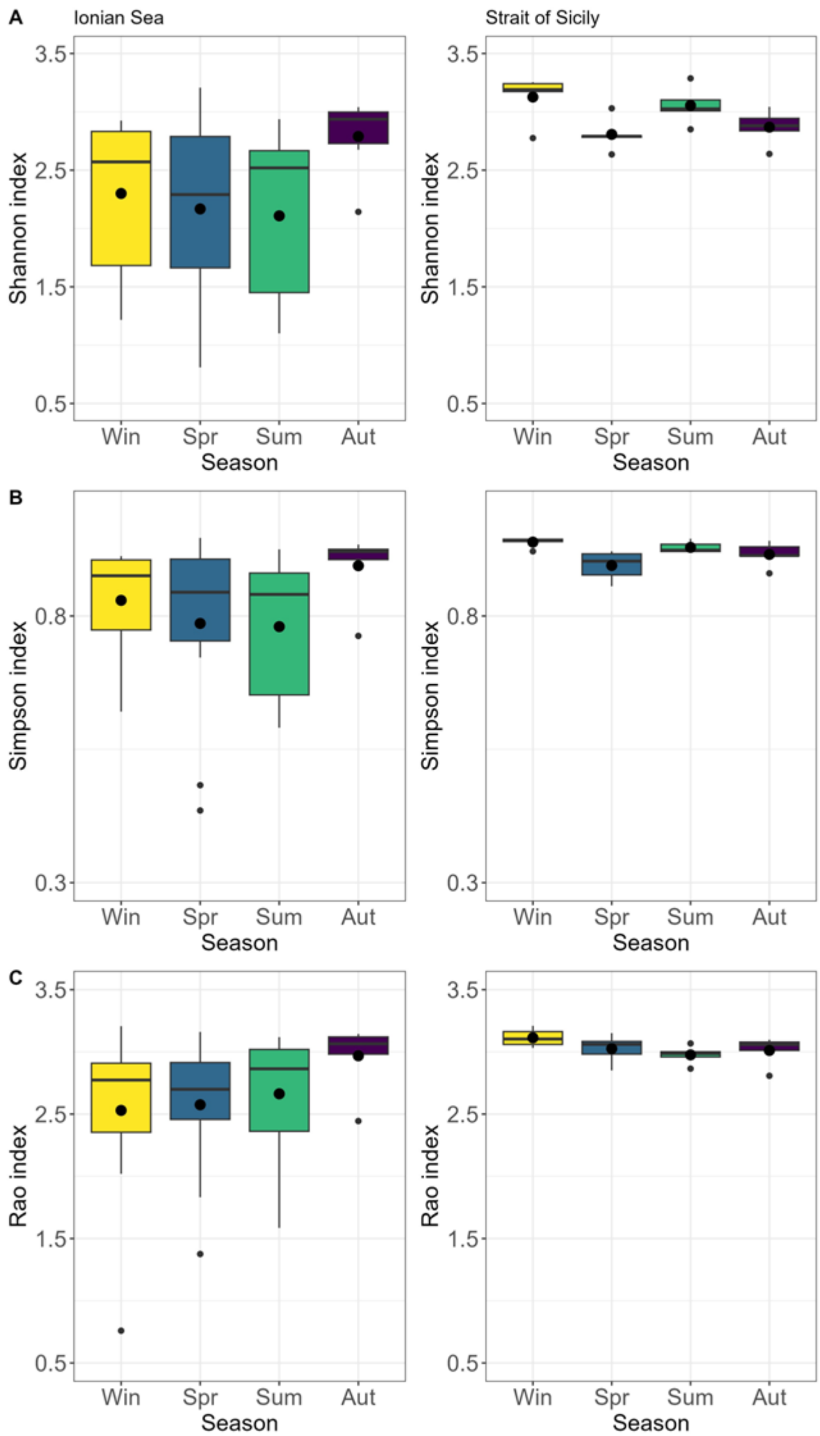

Figure 80: Shannon index (A), Gini- Simpson index (B), Rao index (C), calculated for each season, are shown for Ionian basin (Ionian Sea and Strait of Sicily).

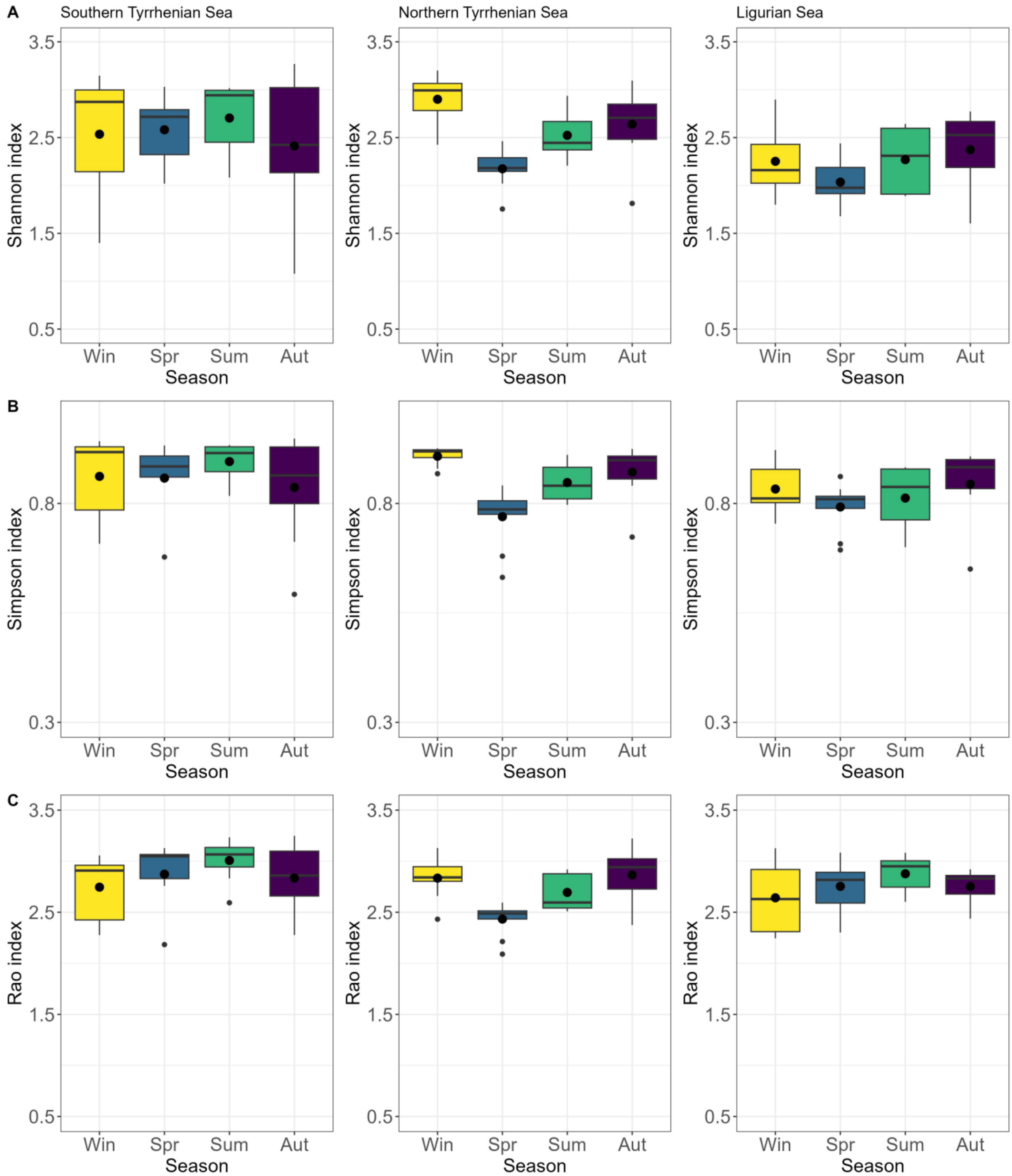

Figure 81: Shannon index (A), Gini-Simpson index (B), Rao index (C), calculated for each season, are shown for Tyrrhenian basin (Southern Tyrrhenian Sea, Northern Tyrrhenian Sea, Ligurian Sea).

Beta diversity index

It was applied a beta diversity measure to assess the change in community structure from one sampling unit to another along a temporal gradient, from year to year. Local Contribution to Beta Diversity (LCBD) was computed as the method described in detail by Legendre and De Cáceres (2013). According to Rombouts et al. (2019) LCBD values indicate how much each observation contributes to the total variance of the community over time. Values range from 0 to 0.5, with low values meaning sites poor in species or degraded or high conservation over time, high values indicate particular ecological conditions or disturbance of invasive species.

LCBD results show few inter- and intra-annual difference in mesozooplankton communities along the coastal-broad gradient of each transect, in each locality (political region) (Figures 82-89).

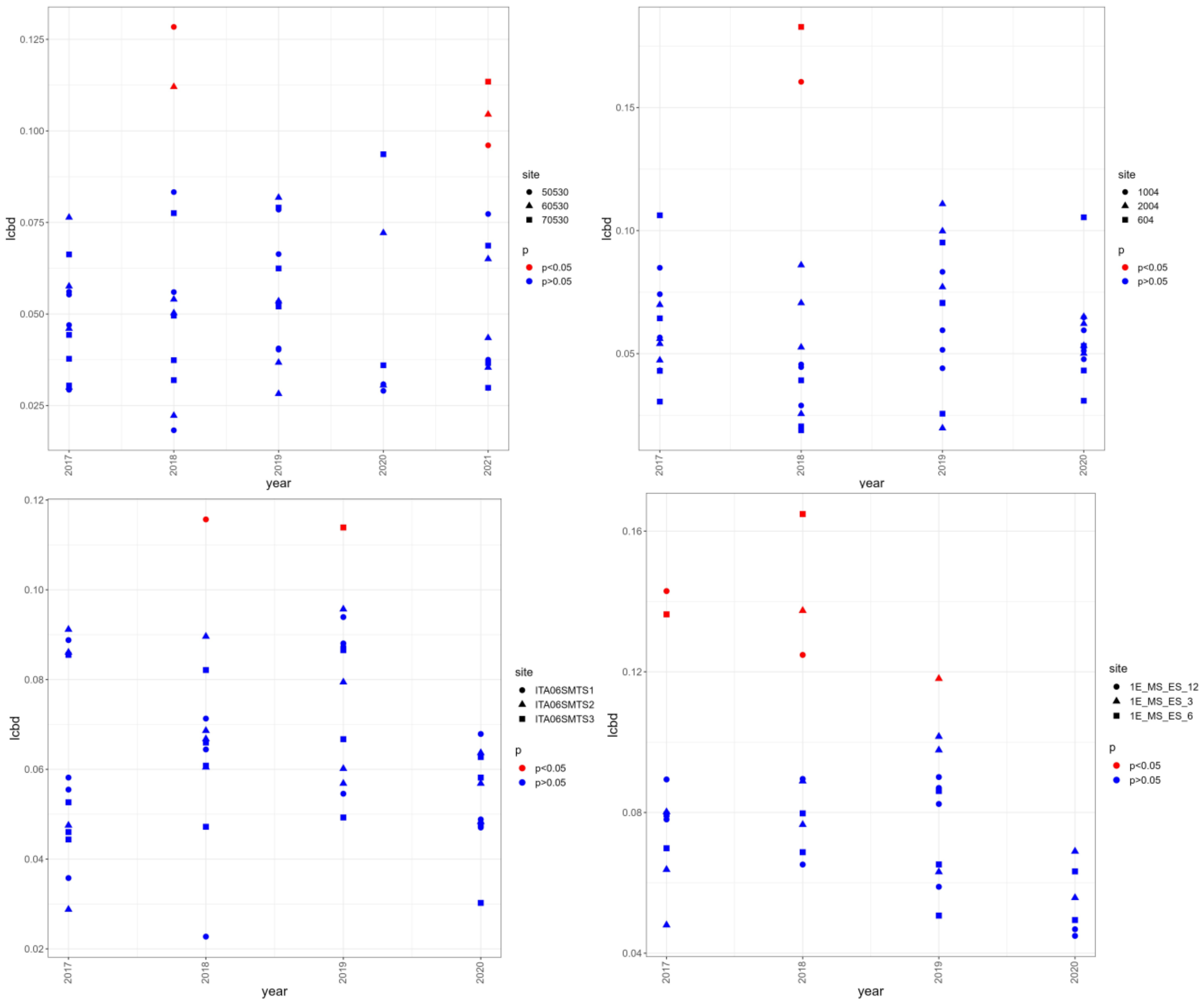

Figure 82: Local contributions to beta diversity (LCBD) in time (2017-2021) for the transects in Veneto, Emilia-Romagna, Friuli Venezia Giulia and Marche in the Northern Adriatic Sea sub-region. Colours indicate the significance level α = 0.05 (blue p>0.05, red p<0.05).

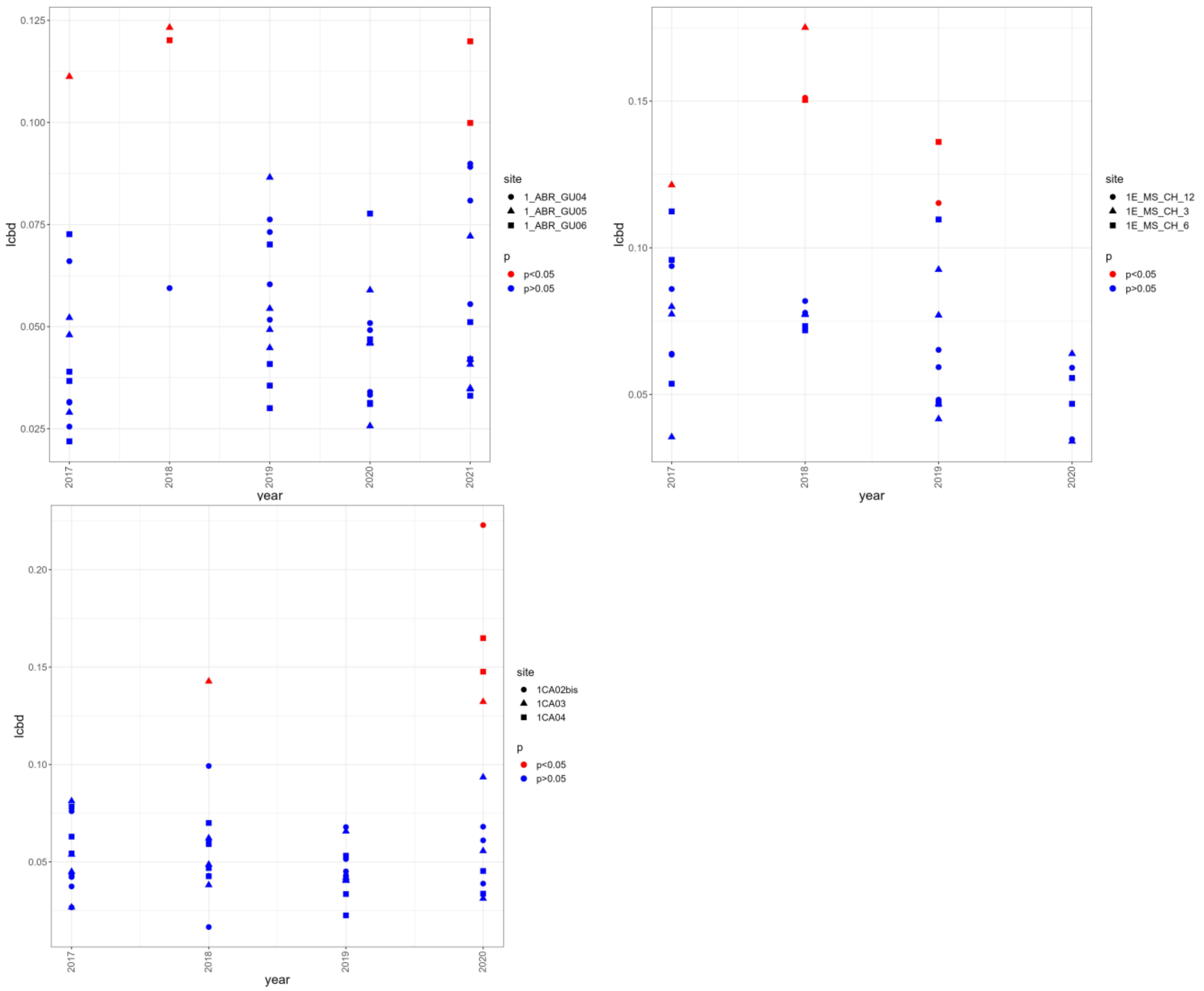

Figure 83: Local contributions to beta diversity (LCBD) in time (2017-2021) for the transects in Abruzzo, Marche and Puglia in the Middle Adriatic Sea sub-region. Colours indicate the significance level α = 0.05 (blue p>0.05, red p<0.05).

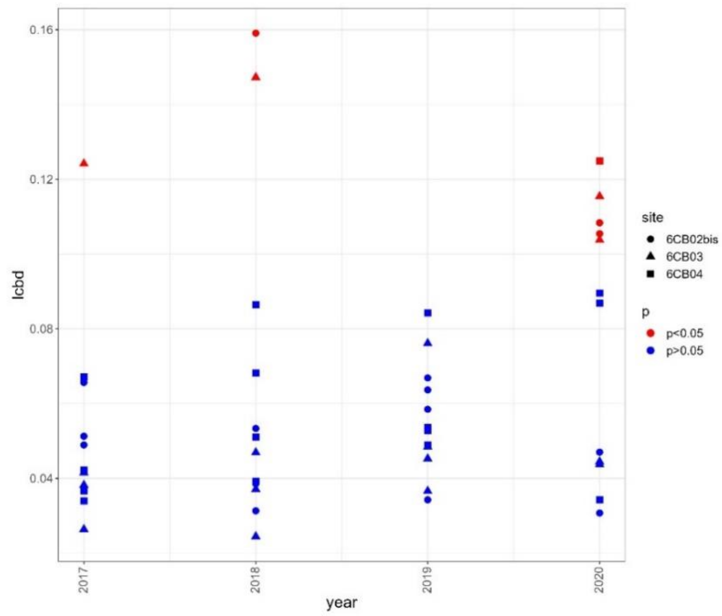

Figure 84: Local contributions to beta diversity (LCBD) in time (2017-2021) for the transect in Puglia in the Southern Adriatic Sea sub-region. Colours indicate the significance level α = 0.05 (blue p>0.05, red p<0.05).

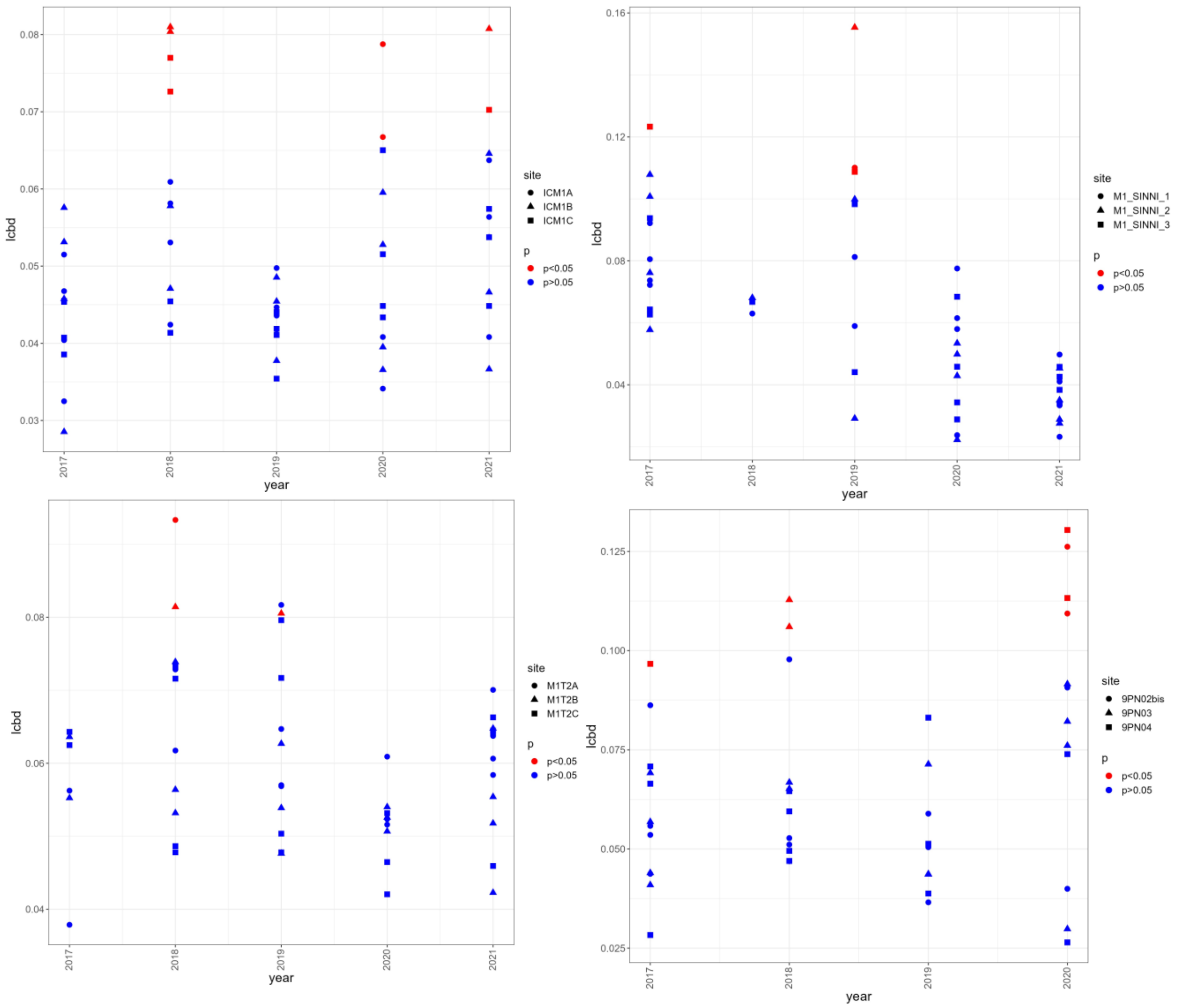

Figure 85: Local contributions to beta diversity (LCBD) in time (2017-2021) for the transects in Sicilia, Basilicata, Calabria, Puglia in the Ionian Sea sub-region. Colours indicate the significance level α = 0.05 (blue p>0.05, red p<0.05).

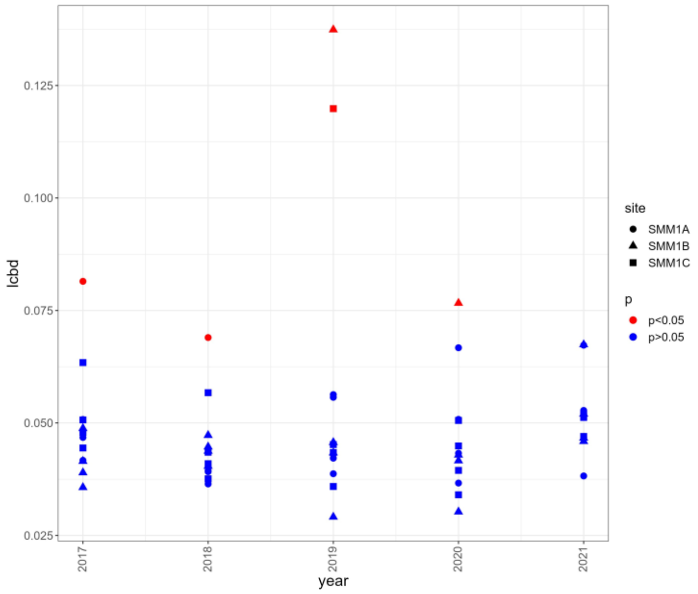

Figure 86: Local contributions to beta diversity (LCBD) in time (2017-2021) for the transect in Sicilia in the Strait of Sicily sub-region. Colours indicate the significance level α = 0.05 (blue p>0.05, red p<0.05).

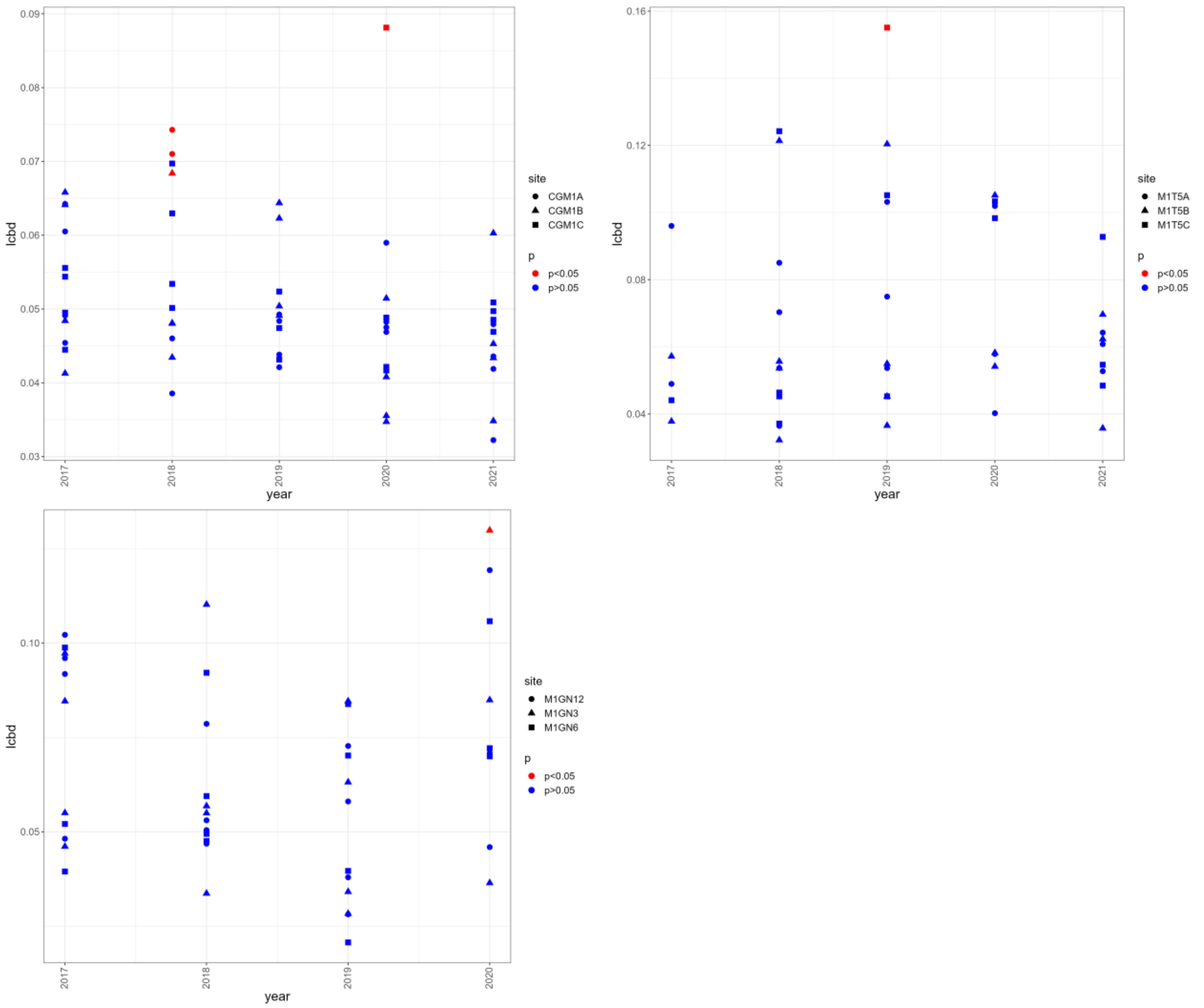

Figure 87: Local contributions to beta diversity (LCBD) in time (2017-2021) for the transects in Sicilia, Calabria and Campania in the Southern Tyrrhenian Sea sub-region. Colours indicate the significance level α = 0.05 (blue p>0.05, red p<0.05).

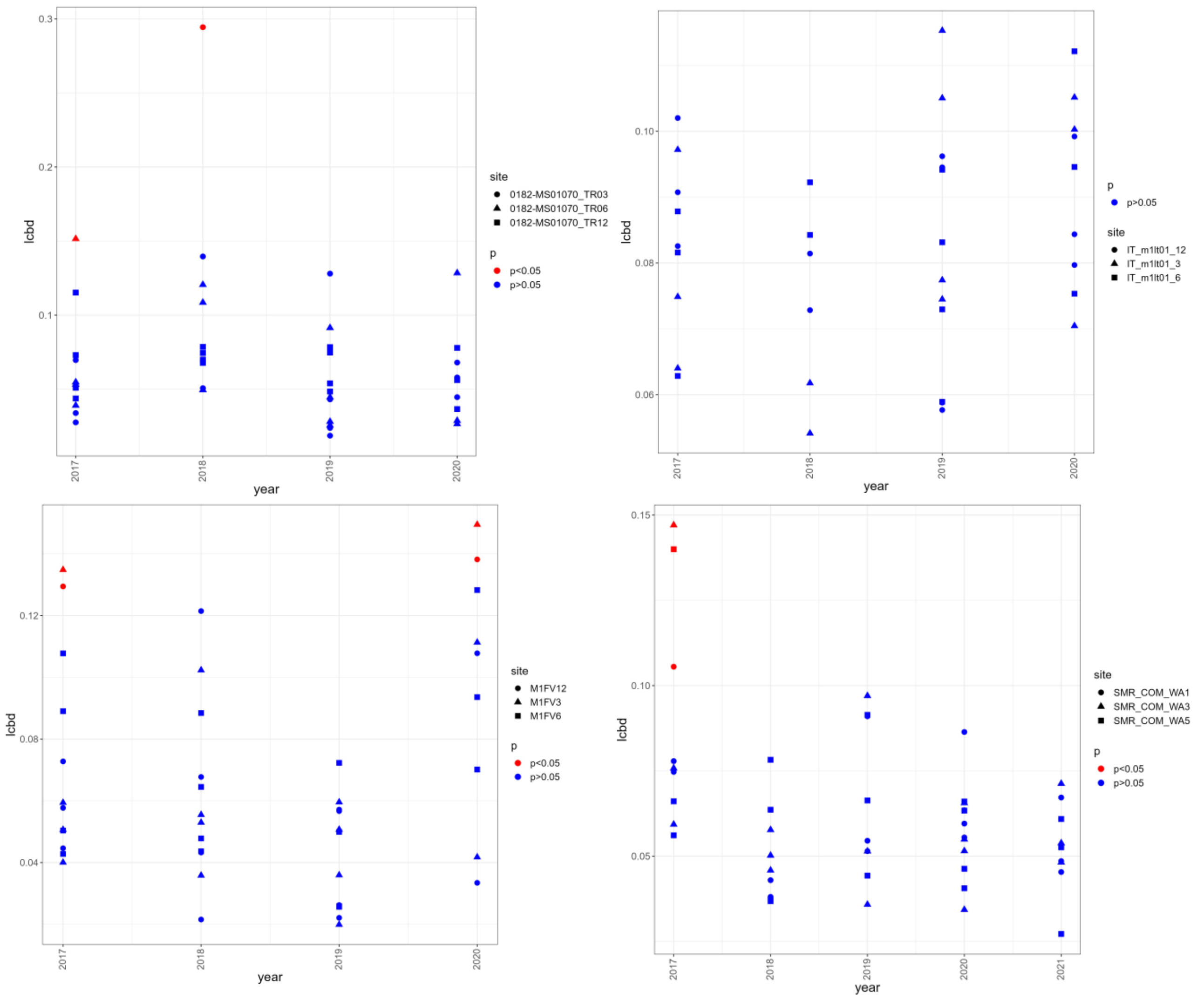

Figure 88: Local contributions to beta diversity (LCBD) in time (2017-2021) for the transects in Sardegna, Campania, Lazio and Tuscany in the Northern Tyrrhenian Sea sub-region. Colours indicate the significance level α = 0.05 (blue p>0.05, red p<0.05).

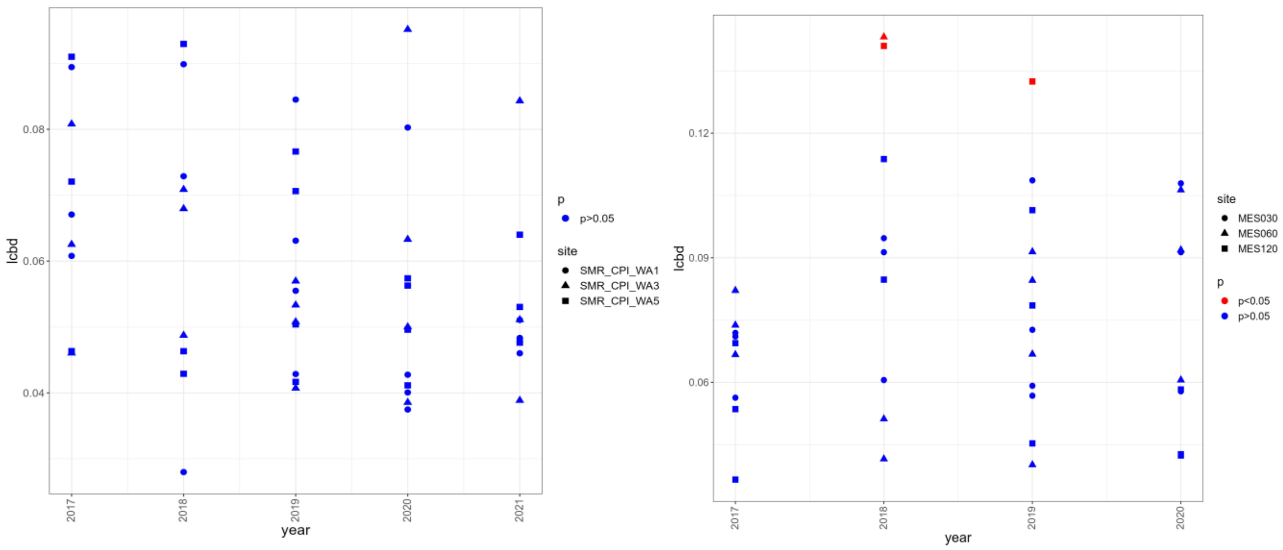

Figure 89: Local contributions to beta diversity (LCBD) in time (2017-2021) for the transects in Tuscany and Liguria in the Ligurian Sea sub-region. Colours indicate the significance level α = 0.05 (blue p>0.05, red p<0.05).

Importance Value Index

The Importance Value Index (IVI) can be used to indicate the overall importance of a taxa/species into community. IVI (= RDi + RFi) was calculated as the sum of the relative density (RDi = (ni/N) ∗ 100), where ni is the number of individuals of the taxa i and N is the total number of individuals of all the taxa, and the relative frequency (RFi = (fi/F) ∗ 100) of the taxonomic units in the community, where fi is the number of occurrence of the taxa i and F is the total number of occurrence of all the taxa. For these analyses, annual abundance time-series data, at the species level, from each Italian region were considered (Table 8).

Table 8: The first five species with the highest values of the Important Value Index (IVI; expressed as a percentage) for the eight marine subregion in the Italian case study.

Subregion	Scientific Name	importance.value
Northern Adriatic Sea	*Paracalanus parvus complex*	26,67597571
	Acartia (Acartiura) clausi	24,36577281
	Oncaea curta	13,33748174
	Oithona similis	11,86639251
	Oithona nana	7,104371293
Middle Adriatic Sea	*Paracalanus parvus complex*	26,86127391
	Acartia (Acartiura) clausi	14,66352521
	Centropages typicus	14,48121591
	Clausocalanus furcatus	11,61370948
	Ctenocalanus vanus	8,066089986
Southern Adriatic Sea	*Paracalanus parvus complex*	30,79803326
	Oithona similis	14,29709617
	Clausocalanus furcatus	13,3305449
	Oncaea media	9,174824567
	Oithona plumifera	8,859733763
Ionian Sea	*Paracalanus parvus complex*	21,29290735
	Clausocalanus furcatus	11,04267819
	Oithona similis	10,23434969
	Acartia (Acartiura) clausi	9,924875599
	Temora stylifera	6,536322943
Strait of Siciliy	*Oithona similis*	9,565955916
	Farranula rostrata	9,424209586
	Mecynocera clausi	8,275876797
	Clausocalanus furcatus	7,812770078
	Calocalanus contractus	6,396041145
Southern Tyrrhenian Sea	*Paracalanus parvus complex*	12,85580804
	Temora stylifera	12,37432373
	Clausocalanus furcatus	9,541182559
	Farranula rostrata	9,00082066
	Mecynocera clausi	8,69476986
Northern Tyrrhenian Sea	*Paracalanus parvus complex*	27,81075476
	Clausocalanus furcatus	9,679869958
	Centropages typicus	8,326665553
	Temora stylifera	7,944878884
	Farranula rostrata	5,801762657
Ligurian Sea	*Paracalanus parvus complex*	29,24229099
	Clausocalanus furcatus	13,12206482
	Centropages typicus	11,03280864
	Clausocalanus pergens	10,95612664
	Clausocalanus paululus	8,589753288

2.2.4.3 Remarks and Conclusion

Regarding the scale of assessment, for spatial scale the data analysis and indices were performed on eight Italian (sub)regions (assessment areas), in the marine region Mediterranean Sea, but it is not excluded that it will be necessary to test the other indices on a different scale, e.g. local scale, reducing to one transect for each Italian regions (political region); for temporal scale it will be necessary a homogeneous sampling effort during each year of monitoring by the ARPAs to compare the data collected. For this purpose, it would be useful to increase the frequency of monitoring per month.

For the future, the dataset must be updated with last data recorded (2022-2024) and the aim will be to calculate other common or candidate indicators (indices), as the current assessment of the status of pelagic habitat types has little consistency, coordinating more our approaches at Regional Sea Conventions (e.g. OSPAR, UNEP-MAP). Finally, for GES purposes environmental parameters and local incident pressures will also be integrated into the analyses and it will be useful to compare the results with the historical data series of the Long-Term Ecological Research (LTER-Italy) sites to draw a temporal baseline with which to better understand the changes in zooplankton communities over time as species composition time series provide more information to assess the nature of the change and the biological and environmental mechanisms responsible.

2.2.5 Survey for mesozooplankton monitoring in the Mediterranean Sea

In the framework of the Activity 2, we also performed a survey available at the following link:

https://ec.europa.eu/eusurvey/runner/Mesozooplankton_Monitoring.

The survey was launched in order to gather general information from Mediterranean and Black Sea zooplankton experts regarding the current status of zooplankton-targeted activities in the context of MSFD monitoring of the marine environment. During the survey, we had in total 14 responses (Slovenia, Bulgaria, Croatia, Tunisia, Israel, France, Italy and Greece) on the 13 posed questions (Figure 90).

Figure 90: Results of the survey for Mediterranean and Black Sea zooplankton experts on the current status of zooplankton-targeted activities in the context of MSFD monitoring.

Is your country actively engaged in the implementation of the Marine Strategy (Directive 2008/56/EC)?

		Answers	Ratio
Yes		12	85.71 %
No		2	14.29 %
No Answer		0	0 %

Does your country conduct national monitoring specifically for mesozooplankton parameters under the Marine Strategy Framework Directive (MSFD) and /or Regional Sea Conventions?

		Answers	Ratio
Yes		12	85.71 %
No		2	14.29 %
No Answer		0	0 %

Which mesozooplankton parameters do you include in your national monitoring?

		Answers	Ratio
None		1	7.14 %
Abundance		14	100 %
Biomass		7	50 %
Taxonomic composition		12	85.71 %
No Answer		0	0 %

When conducting mesozooplankton sampling for monitoring, do you focus on:

		Answers	Ratio
Coastal waters		6	42.86 %
Open waters		4	28.57 %
Specific transects encompassing both		9	64.29 %
No Answer		0	0 %

What is the frequency of mesozooplankton sampling in your coastal monitoring activities

		Answers	Ratio
Monthly		4	28.57 %
Seasonally		2	14.29 %
Biannually		0	0 %
Longer time scales		0	0 %
No Answer		8	57.14 %

What is the frequency of mesozooplankton sampling in your open waters monitoring activities

		Answers	Ratio
Monthly		0	0 %
Seasonally		2	14.29 %
Biannually		2	14.29 %
Longer time scales		0	0 %
No Answer		10	71.43 %

What is the frequency of mesozooplankton sampling in your transect monitoring activities

		Answers	Ratio
Monthly		0	0 %
Seasonally		8	57.14 %
Biannually		1	7.14 %
Longer time scales		0	0 %
No Answer		5	35.71 %

What is the designated depth for mesozooplankton sampling in your monitoring efforts?

		Answers	Ratio
Water column (near bottom-surface)		7	50 %
Surface layer (within upper 100 m)		4	28.57 %
Multiple depth layers		4	28.57 %
No Answer		0	0 %

What specific tools are utilized for the collection of mesozooplankton samples in your monitoring processes?

		Answers	Ratio
Zooplankton Nets		13	92.86 %
Continuous plankton recorder		0	0 %
Other		1	7.14 %
No Answer		1	7.14 %

At what taxonomic level do you typically analyse mesozooplankton samples for monitoring?

		Answers	Ratio
Genus/species level		12	85.71 %
Copepods at species level		6	42.86 %
Higher groups (family, order, class, phyllum)		6	42.86 %
No Answer		0	0 %

Have you incorporated omics technologies for in-depth analysis of mesozooplankton samples in your research or monitoring initiatives?

		Answers	Ratio
Yes		2	14.29 %
No		12	85.71 %
No Answer		0	0 %

Have you utilized imaging techniques such as Zooscan or Zoolmage for the analysis of mesozooplankton samples in your monitoring programs

		Answers	Ratio
Yes		4	28.57 %
No		10	71.43 %
No Answer		0	0 %

In parallel to mesozooplankton, do you collect data on other environmental parameters during your monitoring activities?

		Answers	Ratio
No		1	7.14 %
Yes, physical (Temperature and Salinity)		6	42.86 %
Yes, physical and chemical		12	85.71 %
Yes, phytoplankton/chl-a		13	92.86 %
No Answer		0	0 %

3 Discussion

3.1 Lessons learnt from case studies

Phytoplankton case studies were designed to cover different aspects of temporal and spatial scales of phytoplankton communities and pelagic habitat in general. In brief, Croatian case study in the eastern Adriatic Sea and Slovenian case study in the northernmost part of the Adriatic Sea were performed in the long-term perspective with trend analyses as a crucial step, while Italian case study covered the spatial gradient of prevailing natural conditions more in detail, since it encompassed all Italian regions covering Adriatic, Ionian, Tyrrhenian and Ligurian Seas and transect of three sampling stations at an on shore-off shore gradient. A case study was also performed in the Saronikos Gulf (Aegean Sea, Greece), but provided no solid conclusions because of the very limited sampling frequency of the data.

Summarizing the results of case studies, both Croatian and Slovenian time-series showed an increasing trend of phytoplankton genera richness with a concomitant decrease in phytoplankton abundance, though the latter was much weaker in the northernmost part of the Adriatic Sea as compared to the more oligotrophic eastern Adriatic. The diversity and evenness also showed a more or less consistent long-term increase at both sites with a concomitant decrease of dominance. These trends were slightly more pronounced at the open-sea stations as compared to coastal ones. As expected, longer time series provided more reliable results, while the shorter data series still showed similar patterns, suggesting that the effects of environmental changes on phytoplankton communities are the same at all sampling stations, regardless of the different anthropogenic influences.

In both eastern Adriatic and northernmost Gulf of Trieste, the observed changes in the phytoplankton community characteristics are in line with recent changes toward more oligotrophic conditions and the lessened pollution impact due to better wastewater management (e.g., Mozetič et al., 2010, 2012). This is also mirrored in the fact that there were no considerable differences in the temporal dynamics of the studied indices between impacted or non-impacted sites, despite the different hydrodynamic conditions in the studied areas. All this suggests that the increasing diversity observed in the studied areas cannot be attributed solely to the different degree of anthropogenic pressure, but that other large-scale trends such as climate change (e.g. Henson et al., 2021; Benedetti et al., 2021) should also be taken into account.

The Italian case study highlighted the very high temporal (seasonal and between years) and spatial (within regions, subbasins, seas) variability of phytoplankton communities, in terms of abundances, diversity and community composition even at the genus level. However, no differences were observed between the stations of each transect, concordant with the results of other two case studies but complicating the understanding of diversity patterns in response to environmental changes. While alpha diversity indices did not show the potential to identify important difference, a beta diversity index, namely Local Contribution to Beta Diversity (LCBD) identified as a potentially useful tool for the identification of changes, together with the Importance Value Index (IVI).

Different results were obtained with the analyses of the eLTER site Senigallia Susak dataset covering a much longer period of time (1988-2022). Several studies (Totti et al., 2019a; Neri et al., 2022, 2023) highlighted that differences occur between the coastal and offshore stations in terms of phytoplankton seasonal cycle, diversity values and relevant species, but in relation to the circulation regime and to the different water masses, more than to a direct anthropogenic impact. In the offshore station, the main seasonal driver is the vertical structure of the water column (i.e., mixing vs stratification) which influences the conditions for the phytoplankton community at the surface and in the whole mixed layer, in terms of light and nutrient availability. On the contrary, in the shallower coastal station (10 m depth), where the water column is mixed almost throughout the year, the main constrain is the outflow of riverine waters (itself related to the precipitation regime) carried by the Western Adriatic Current (Totti et al., 2019a; Neri et al., 2022, 2023). Therefore, the observed differences between the coastal and offshore stations are considered as 'physiological', and the higher biomass/lower biodiversity recorded onshore reflect a very moderate human impact, that is mainly exerted through the river runoff. In this perspective, the measurements of anthropogenic impact could be biased by the superimposition of meteoclimatic events. Totti et al. (2019a, b) highlighted that anomalous phytoplankton blooms (either harmful or not) were enhanced after intense rains following long periods of drought, requiring a lot of caution when interpreting data.

As compared to rather the predictable seasonal phytoplankton dynamics observed in the past (e.g. Totti et al., 2001; Cabrini et al., 2012), a switch to a more erratic community dynamics, probably triggered by climatic and hydrological factors at the mesoscale was observed at two LTER stations, i.e. LTER Senigallia-Susak and LTER Slovenia Gulf of Trieste (sampling station 000F) (Totti et al., 2019a and Vascotto et al., 2021, respectively).

In contrast to the phytoplankton diversity case studies, which were addressed in the framework of previous projects, the zooplankton case studies presented here represent a valuable first step towards understanding the diversity of zooplankton in the Mediterranean Sea on a broader temporal and spatial scale. Although there have been some attempts to develop indicators based on zooplankton diversity (Serranito et al., 2016; Villarino et al., 2020), none have yet been developed or have defined thresholds for the Mediterranean (sub)region. Also in the case of zooplankton, the three case studies covered either greater temporal variability, with the Croatian and Greek datasets covering more than a decade, or greater spatial coverage by the Italian case study, which (like the phytoplankton) included the Mediterranean sub-regions of the Adriatic, Ionian, Tyrrhenian and Ligurian Seas. In this way, five MSFD Marine Reporting Units (MRUs) were studied (Tyrrhenian, Adriatic, Ionian, Aegean and Levantine Seas).

The extensive zooplankton datasets showed considerable subregional differences in the frequency, duration and spatial coverage of monitoring, as well as in the zooplankton groups and parameters included, making it difficult to compare the results. For example, the Croatian case study included an analysis of microzooplankton in the central eastern Adriatic and highlighted that total abundance of ciliates/protozoans and micrometazoans could serve as potential indicators together with diversity, evenness and dominance indices for the tintinnid community. The mesozooplankton community, whose diversity was investigated in all three case studies, showed spatial and temporal differences that were more or less consistent with the different prevailing natural conditions along environmental gradients such as depth, salinity and trophic gradient. The results of the Croatian case study made it clear that the spatial coverage of the study needs to be extended to the northern and southern eastern Adriatic in order to gain a more comprehensive understanding of mesozooplankton dynamics. The Italian case study, which already covers a spatially extended dataset, also underlined the potential need to test other indices on a different, i.e. even more localised scale, by focusing on only one transect per Italian region to reveal differences due to anthropogenic influences. For this purpose, a more homogeneous sampling frequency, possibly on a monthly basis, would be recommended.

Together, the three case studies underlined the complexity of zooplankton community dynamics, the need for standardized methods and the importance of appropriate spatial and temporal consideration. In addition, further research is needed to shed more light on the mechanisms that shape the structure of (meso)zooplankton communities in the context of anthropogenic pressures and impacts. It is still unclear whether more nuanced changes can be easily detected, for example between areas with smaller differences in either anthropogenic pressures or prevailing natural conditions. Functional diversity could be a promising approach, provided that the right indicators for (meso)zooplankton are selected and a relationship to environmental (natural or human) pressures is established. Understanding the dynamics of (meso)zooplankton and the parameters that influence its variability seems necessary, as the use of mesozooplankton in the assessment of ecological status has been proposed under the Marine Strategy Framework Directive (Ndah et al., 2022).

In summary, significant differences were observed for both phytoplankton and zooplankton between areas with different prevailing conditions that could not be linked to anthropogenic influences. Furthermore, it was difficult to link the observed trends to other processes that were not related to hydrological and climatic influences on a larger scale. However, the observation and interpretation of trends in plankton diversity dynamics is extremely important to link these changes to large-scale influences related to climate change, which is predicted to have a massive impact on plankton abundance and diversity (e.g. Beaugrand et al., 2015; Ibarbalz et al., 2019). In this respect, too, the importance of data from long-term ecological research (LTER) is enormous, because only with historical data series is it possible to better understand the changes in plankton communities over time and the biological and environmental mechanisms responsible for them, also in relation to large-scale trends such as climate change.

3.2 Recommendations for more harmonized approach towards GES definition for pelagic habitat in the Mediterranean Sea

To foster a more harmonized approach towards defining Good Environmental Status (GES) for pelagic habitats in the Mediterranean Sea, this chapter includes some recommendations, which derived from the comprehensive synthesis of the work done within all the tasks of the ABIOMMED Activity 2. It has to be stressed, that not all the Mediterranean member states participated in the Activity, nor have the case studies undertaken encompassed all the sub-regions of the Mediterranean Sea. But we believe that these recommendations could help to direct future effort for incorporation of pelagic habitat components into the assessment systems of biodiversity descriptor not only for the member states but also for non-European countries contracting parties of Barcelona convention.

- <u>Importance of the assessment scales</u>

The results of the phytoplankton and zooplankton case studies advocate for a comprehensive spatial coverage of monitoring stations that would ensure representation in different subregions and/or marine reporting units (MRUs) and adequately cover environmental gradients. For example, in the Italian case study, the transect of three stations along the coast-offshore gradient had an inadequate sampling resolution (both missing samples, short period of time) to reveal the decreasing influence of coastal processes. Moreover, no differences in phytoplankton community characteristics were detected in the sampling stations within the same Italian region. On the contrary, the zooplankton studies in the eastern Adriatic pointed to the need to extend the spatial coverage of monitoring in order to improve the understanding of mesozooplankton dynamics and to promote the use of the corresponding promising indicators.

The study on the distribution of phytoplankton functional types and size classes in the Mediterranean Sea (Deliverable D2.1b) also supported the recommendation to assess pelagic habitats across various temporal scales, but at the same time emphasised the importance of including climate regimes in the assessment process. The study encouraged the use of satellite data and associated modelling products, as well as regionalizations already available, to track phytoplankton trends at the scale of the entire Mediterranean Sea, which is practically impossible when using only monitoring data from member states. However, distinguishing between anthropogenic influence and natural variability remained a challenge.

- <u>Importance of studying relevant phytoplankton and zooplankton groups/size classes</u>

The results of the study on assessment scales (Deliverable 2.1b) led to the conclusion that not only the temporal and spatial scale is important, but also the scale of the plankton itself. Examining other phytoplankton and zooplankton groups (size classes) would perhaps make it easier to detect changes in environmental status related to human and other influences, for example pico-size fraction of phytoplankton or microzooplankton (as in the case of the Croatian case study).

National monitoring programmes are mainly dedicated to microphytoplankton (and to a limited extent nanophytoplankton) in the coastal waters of the Mediterranean and less frequently in coastal waters. This limitation results from the tradition of monitoring and research methods, which mainly include light microscopy and the so-called Utermöhl phytoplankton, while research and even less monitoring are much less focused on picophytoplankton, especially at the large basin scale. The picophytoplankton size class typically represents the dominant fraction under oligotrophic conditions and indeed dominates most of the open sea in the Mediterranean. Regionally limited studies of picophytoplankton provide interesting insights into the characteristics of the picoplankton community in specific areas, albeit with a limited understanding of the temporal and spatial components.

- Long term ecological research stations

Long-term ecological research stations (LTER) have played a pivotal role in advancing the understanding of pelagic habitats, also in the case of the assessment of phytoplankton and zooplankton components. We would therefore recommend to integrate monitoring data with LTER information, both acquired with a reasonable frequency, which would ensure a comprehensive understanding of environmental changes. While LTER stations may have initially been designed for diverse research purposes, their wealth of valuable data and auxiliary information can significantly contribute to environmental status assessments.

Maintaining and potentially expanding the network of LTER sites for future data use is a reasonable and strategic approach. The adaptability of LTER sites to incorporate new and emerging methodologies, such as those based on omics, further enhances their relevance. Instead of relying solely on a spatially extended network of monitoring stations, a more effective strategy may involve combining data from these stations with LTER sites even with a reduction of monitoring stations if it was previously evidenced that they provide redundant data.

One notable advantage of LTER stations lies in their ability to establish connections between biological data and the physical, chemical, and human impact characteristics of the environment over extended periods. As we have also seen through our case studies, the long-term data series of both phytoplankton and zooplankton are extremely important to capture trends and decipher responses to environmental changes, including the impacts of climate change. Moreover, promoting the use of historical data from LTER facilitates providing context and valuable insights into plankton community changes over time, forming a foundation for informed decision-making and sustainable management of pelagic habitats.

- Importance of appropriate sampling frequency

The importance of an appropriate sampling frequency cannot be overstated in the assessment of phytoplankton and zooplankton, considering the temporal dynamics inherent to these pelagic communities. Establishing a consistent and well-defined temporal scale is critical, with suggested frequencies of at least monthly for phytoplankton and at least seasonal for zooplankton, varying depending on the ecosystem's characteristics, be it open sea or coastal regions. The maintenance of long-term stations, even with fewer monitoring stations but ensuring longevity, emerges as a strategic approach. A more homogeneous sampling frequency across member states, such as monthly, is advocated to facilitate meaningful comparisons and unveil potential variations resulting from anthropogenic influences. In doing so, the scientific community can achieve a more comprehensive understanding of the temporal patterns, contributing to informed management strategies.

- Evaluation of trends as a foundation of assessments and trust in expert judgement

Given our knowledge of the diversity of phytoplankton communities in the Mediterranean, even after extensive case studies within the ABIOMMED project, and given that studies of zooplankton as a possible indicator are still in the early stages, it remains virtually impossible to define the conditions of plankton communities that would correspond to reference conditions. Instead, the observed trend, especially at larger scales, can be associated with overarching environmental alterations, such as increasing temperatures and the related modifications in water column conditions and currents. In light of this complexity, proposing threshold values for Good Environmental Status (GES) calculations becomes impractical. Rather, a more prudent approach involves evaluating regional trends and changes, considering their manageability, and assessing their consistency across different areas. In this way, the absence of specific thresholds in the Mediterranean Sea, characterized by intricate and highly dynamic pelagic diversity, necessitates a reliance on comprehensive trend evaluations.

Similar approach was also developed and proposed recently by OSPAR (2022) and McQuatters-Gollop et al. (2022), which categorized biodiversity status based on temporal changes of plankton functional types and expert judgment, providing a valuable avenue for GES definition. These categories, whether tied to indicator thresholds or informed solely by temporal changes, offer an alternative approach to understanding and assessing the impacts on pelagic habitats. It is crucial to acknowledge, however, that the assignment of indicators to these categories currently lacks a formal link to policy regulations. Nevertheless, given the unique challenges presented by the Mediterranean Sea's pelagic diversity, characterized by intrinsic high variability and limitations of traditional microscopy methods, such expert-driven approach offers a pragmatic means of assessing and managing the environmental status of these habitats. Embracing the expertise of researchers and acknowledging the evolving nature of our understanding becomes paramount in the absence of rigid thresholds.

- Establish connections to Descriptor 4 and focus on changes in food webs

The necessity of establishing connections to Descriptor 4 (D4) becomes evident when considering observed trends during case studies, such as the noteworthy decline in phytoplankton abundance coupled with an increase in diversity in specific regions, the trend towards a strengthened picophytoplankton dominance at the basin level, along with changes in zooplankton communities across the Mediterranean basin. These trends suggest potential alterations in the trophic interactions of the pelagic food web, possibly indicating a decrease in trophic efficiency, a phenomenon supported by prior studies. To comprehensively capture and understand the consequences of phytoplankton and zooplankton changes, we strongly support to focus on the broader context of the pelagic food web that will, with the consideration of multiple trophic guilds, enhance the detection of cascading effects at different levels of the food web.

Studying pelagic food webs not only aids in detecting and understanding changes at the primary and secondary producer levels but also facilitates the development of more manageable and plausible measures if a good ecological status is not achieved. As highlighted earlier, long-term ecological research (LTER) studies are pivotal for identifying crucial trends and regime shifts in the pelagic habitat, providing a foundation for researching changes in food webs. It was recommended that a D4 assessment would include at least three trophic levels, with representation from a primary producer and at least one consumer from a non-fish guild, thereby supporting the inclusive use of phytoplankton and zooplankton in the D4 framework.

- Continuation of the collaboration through a working group of multidisciplinary experts

The collaboration initiated within the ABIOMMED framework, bringing together Mediterranean phytoplankton and zooplankton experts, has proven invaluable in defining outstanding issues related to pelagic habitat diversity assessment. We recognize the importance of continuity of collaboration and a structured framework for future collaboration, for which a significant step has been taken with the establishment of a multidisciplinary group of experts for pelagic habitat, nominated by the Contracting Parties of the Barcelona Convention (UNEP/MAP/SPA/RAC, 2023). We express strong support for the continuation of this collaborative effort and advocate for the group's future work to be undertaken either at the level of European member states under the Marine Strategy Framework Directive (MSFD) umbrella or within the broader context of all Mediterranean countries under the Barcelona Convention.

The envisaged work of the group would include the development and implementation of standardized monitoring protocols for phytoplankton and zooplankton across the Mediterranean Sea. This effort aims to address observed differences in monitoring frequency, duration, and parameters to ensure consistency in data collection, a critical aspect for meaningful cross-regional comparisons. Furthermore, the group would work on recommendations for a harmonized monitoring plan at the Mediterranean level. This harmonization is essential to establish a cohesive and comprehensive understanding of pelagic habitats, facilitating informed decision-making and sustainable management practices.

4 Conclusions

The extensive case studies on phytoplankton and zooplankton in the Mediterranean Sea, conducted within the ABIOMMED framework, have provided valuable insights into the temporal and spatial dynamics of pelagic habitat diversity. The results underline the complexity and variability of these communities, emphasizing the need for a comprehensive approach in monitoring and assessment.

The phytoplankton case studies, ranging from the eastern Adriatic to the Aegean Sea, revealed consistent trends in increasing phytoplankton richness, diversity and evenness coupled with a decrease in abundance. These changes were tentatively linked to broader environmental alterations, such as rising temperatures and modifications in water column conditions, emphasizing the influence of large-scale factors like climate change. Moreover, the zooplankton case studies, despite their subregional differences, represented a crucial first step in understanding the broader dynamics of zooplankton diversity in the Mediterranean, highlighting the need for standardized methods and a more in-depth examination of relevant groups and size classes.

Recommendations derived from the ABIOMMED project emphasize the importance of appropriate spatial and temporal assessment scales, encouraging integration of information derived from MSFD monitoring stations with Long-Term Ecological Research (LTER) data to cover different subregions and environmental gradients. The focus on relevant phytoplankton and zooplankton groups and detection of relevant trends is crucial for a holistic understanding of pelagic habitats and the integration of the findings into the assessment of food webs (Descriptor 4). The significance of appropriate sampling frequency and the evaluation of trends, supported by expert judgment, emerge as foundational elements for effective environmental status assessments.

Moreover, a higher taxonomical resolution in the identification of both phytoplankton and zooplankton taxa is highly advocated, as any evaluation of biodiversity indices theoretically requires the information on the whole species composition in a community, while a major portion of nanoplanktonic community is identified as ‘undetermined’.

To foster a more harmonized approach, there is a need for continued collaboration through a multidisciplinary group of experts, which could operate under the MSFD umbrella or within the broader context of the Barcelona Convention. This group's future work includes developing standardized monitoring protocols, addressing differences in monitoring frequency, and recommending a harmonized monitoring plan at the Mediterranean level. The longevity of collaboration and advancement of our understanding of the pelagic habitat in the Mediterranean Sea will fostering a comprehensive and forward-thinking approach to pelagic habitat assessment.

Gaps in knowledge and outstanding issues

The current implementation of pelagic indicators for D1C6 criterion is clearly behind the degree of development of other D1 criteria and other descriptors of the MSFD. General constraints to assessing criterion D1C6 relate to the nature of the pelagic domain, the biology and ecology of planktonic organisms, and the methodologies used for their monitoring. Other constraints include the lack of experts in plankton taxonomy, the expert-dependent precision in taxonomic analysis and the lack of understanding of the drivers of diversity characteristics and dynamics constrain the development of specific diversity indicators and to some extent of functional-groups indicators.

To establish reference conditions, as mandated by the WFD, phytoplankton communities must be described based on their state under completely or nearly completely undisturbed conditions, with little or no impact from human activities. The WFD also assumes that the nature of phytoplankton communities reflects the "memory" of sustained pressure. However, as noted above, even in the absence of anthropogenic pressure, phytoplankton communities are highly dynamic. Marine phytoplankton communities respond to the physical and chemical constrains of their environment and, therefore, do not temporally integrate environmental changes. Indeed, even within a single seasonal cycle, phytoplankton communities will be highly variable (Garmendia et al., 2013; Borja et al., 2012) and will not give rise to a climax community. As stressed by Camp et al. (2016), an effective and accurate assessment of the status of marine ecosystems and their disturbances requires recognition of the dynamic nature of plankton. The state of a plankton community should not, and cannot, be evaluated by comparing its composition and relative abundance to a static "reference" species assemblage which, even if it existed, would be far from representative (Camp et al., 2016).

Nevertheless, detailed information on taxonomic analysis of plankton samples will always be necessary to correctly interpret the patterns of other variables such as biomass or abundance of general plankton components (e.g. phytoplankton and mesozooplankton) or functional groups on which other indicators may be based.

5 References

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SUPPORT COHERENT AND COORDINATED ASSESSMENT OF BIODIVERSITY AND MEASURES ACROSS MEDITERRANEAN FOR THE NEXT 6-YEAR CYCLE OF MSFD IMPLEMENTATION

6 Supplement

Phytoplankton

<u>Case study: Croatia, E Adriatic - Mali Ston</u>

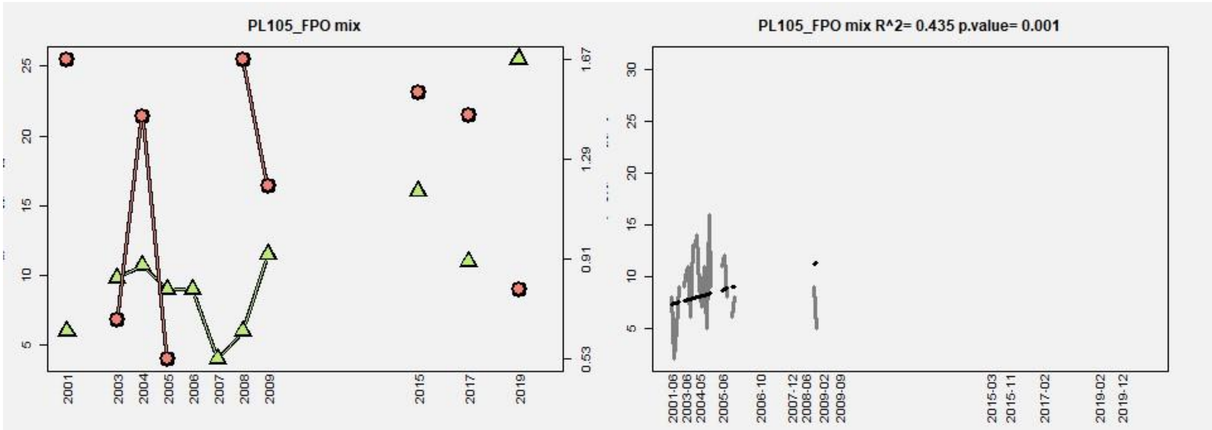

Figure S1: Genus richness in the middle layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panel: linear model

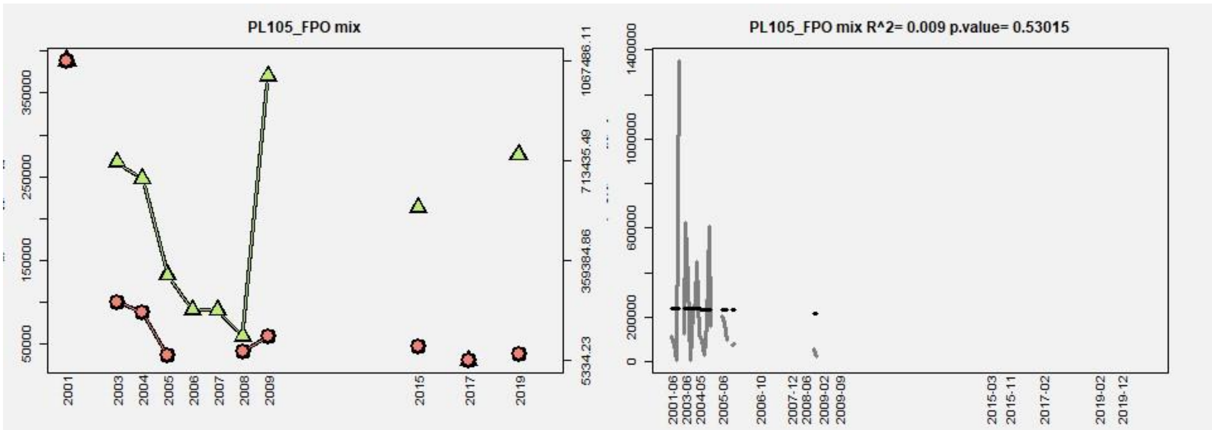

Figure S2: Phytoplankton abundance in the middle layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panel: linear model

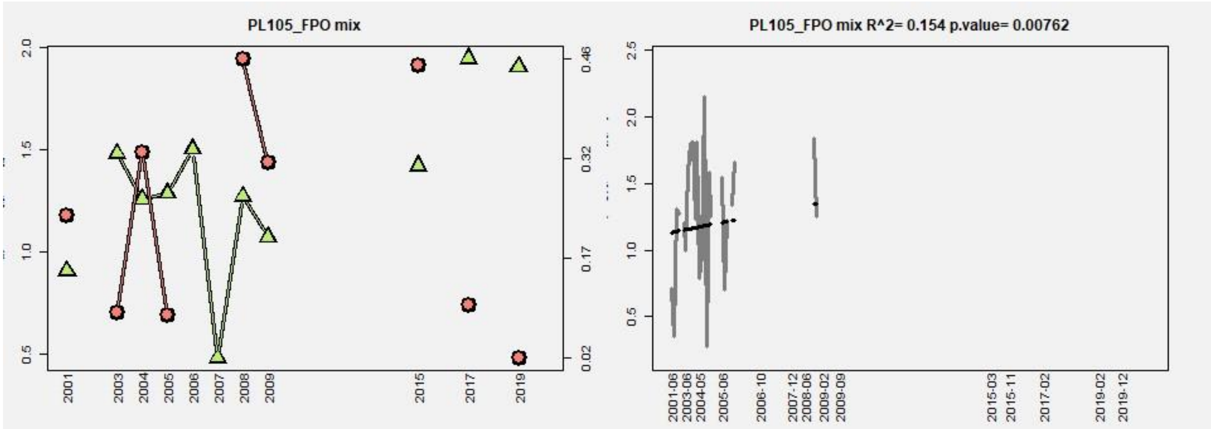

Figure S3: Shannon - Wiener's diversity index in the middle layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panel: linear model

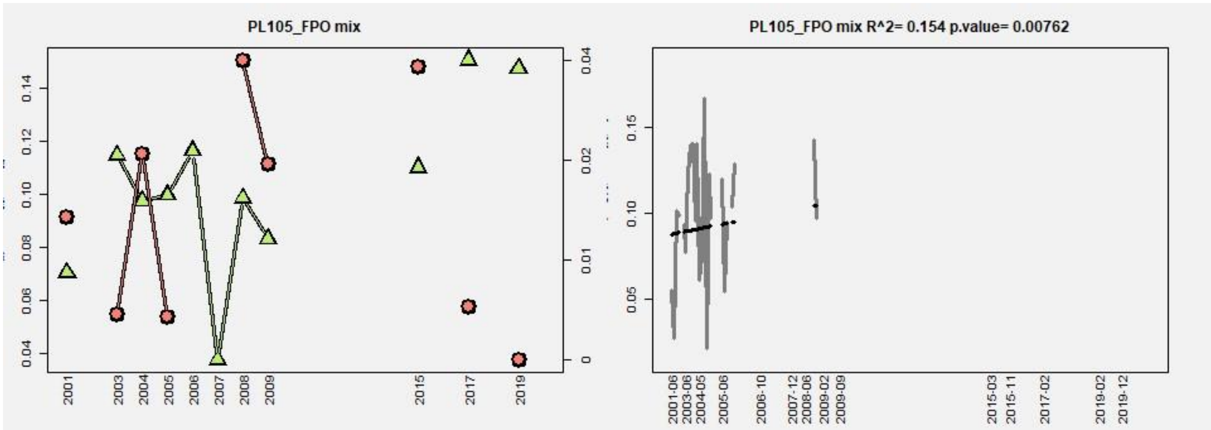

Figure S4: Pielou's evenness in the middle layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panel: linear model

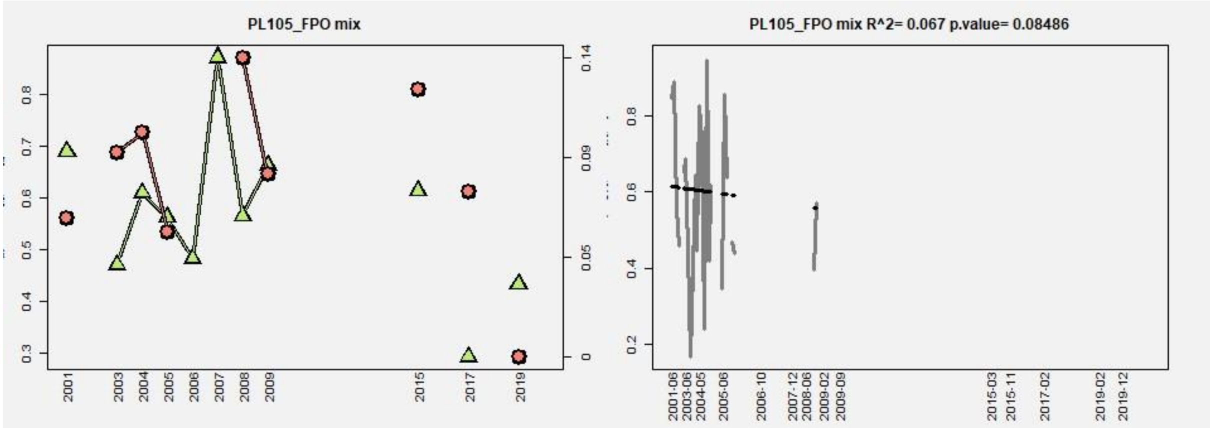

Figure S5: Berger-Parker's dominance in the middle layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panel: linear model

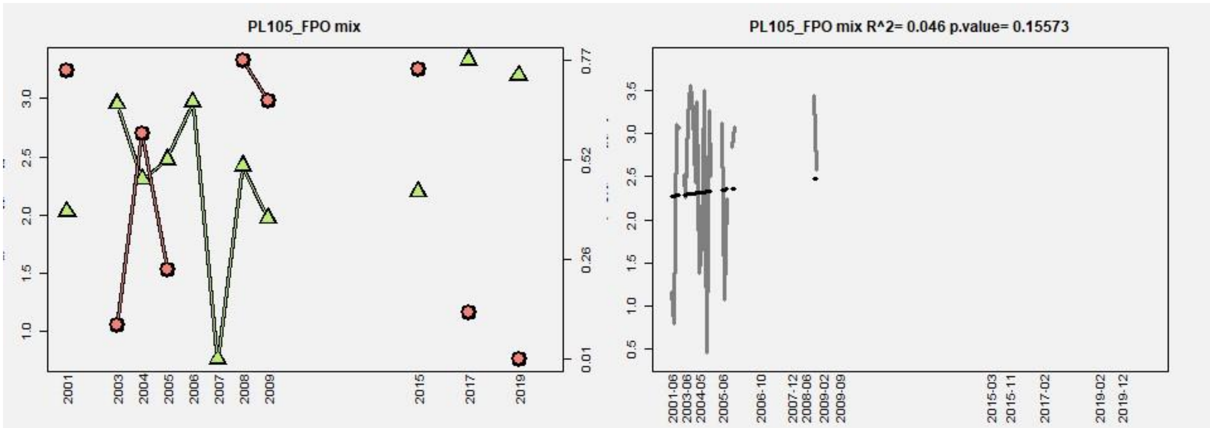

Figure S6: Rao's index in the middle layer of stations PL105/FP05 in the period 2001-2019. Left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panel: linear model

Case study: Croatia, E Adriatic, Kaštela – Hvar – Vis transect

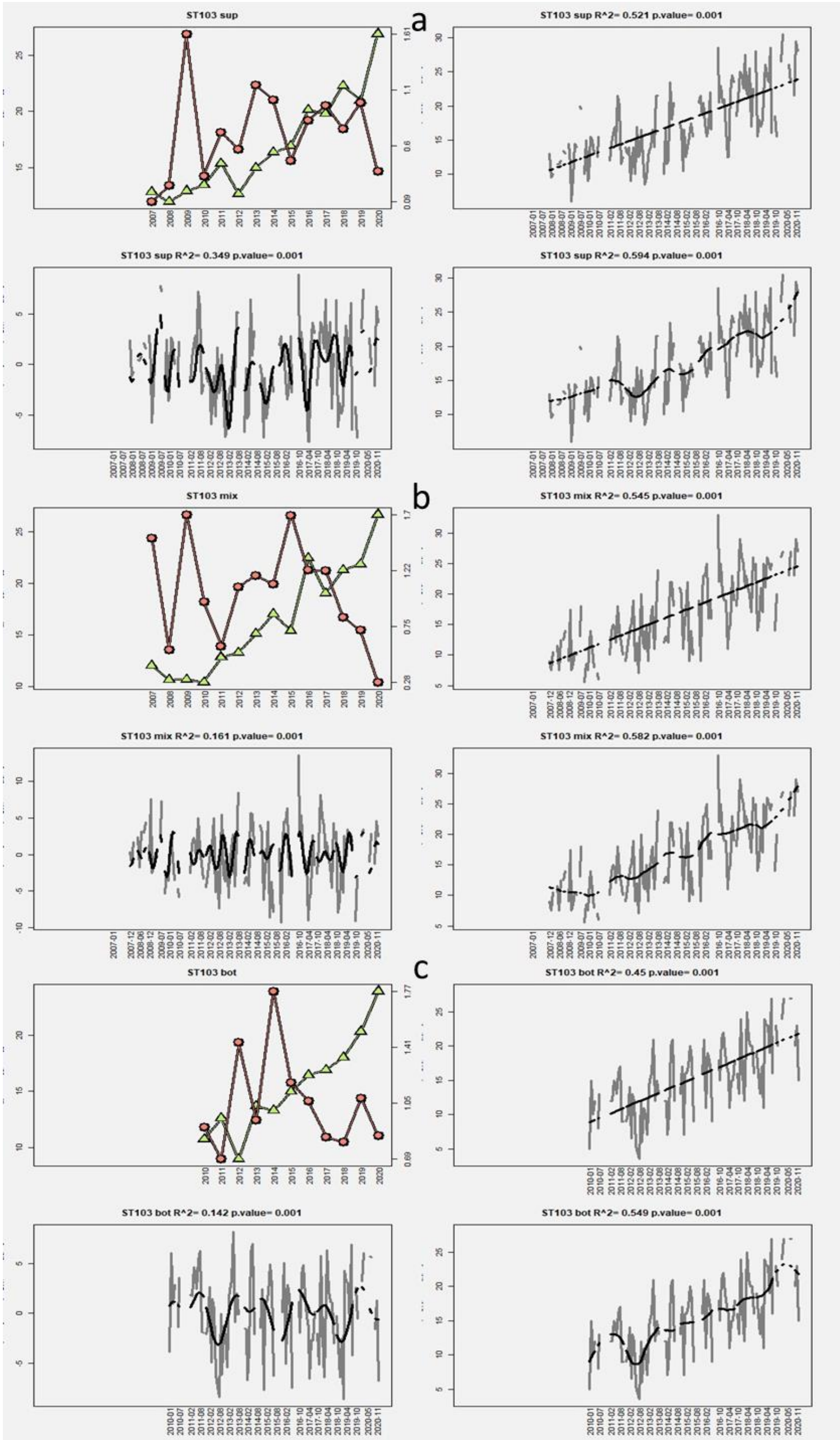

Figure S7: Genus richness in the (a) surface, (b) middle and (c) bottom layer of station ST103 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

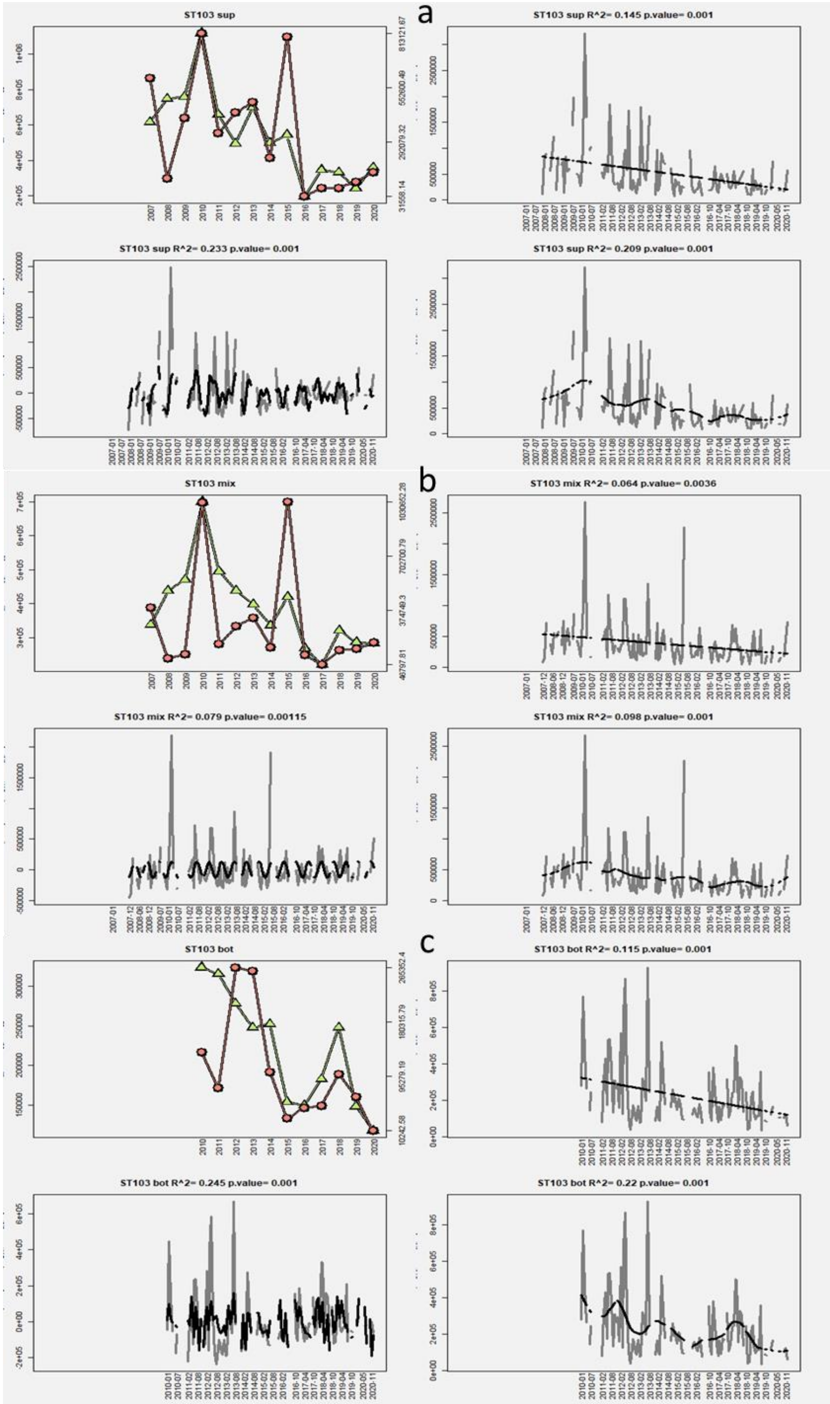

Figure S8: Phytoplankton abundance in the (a) surface, (b) middle and (c) bottom layer of station ST103 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

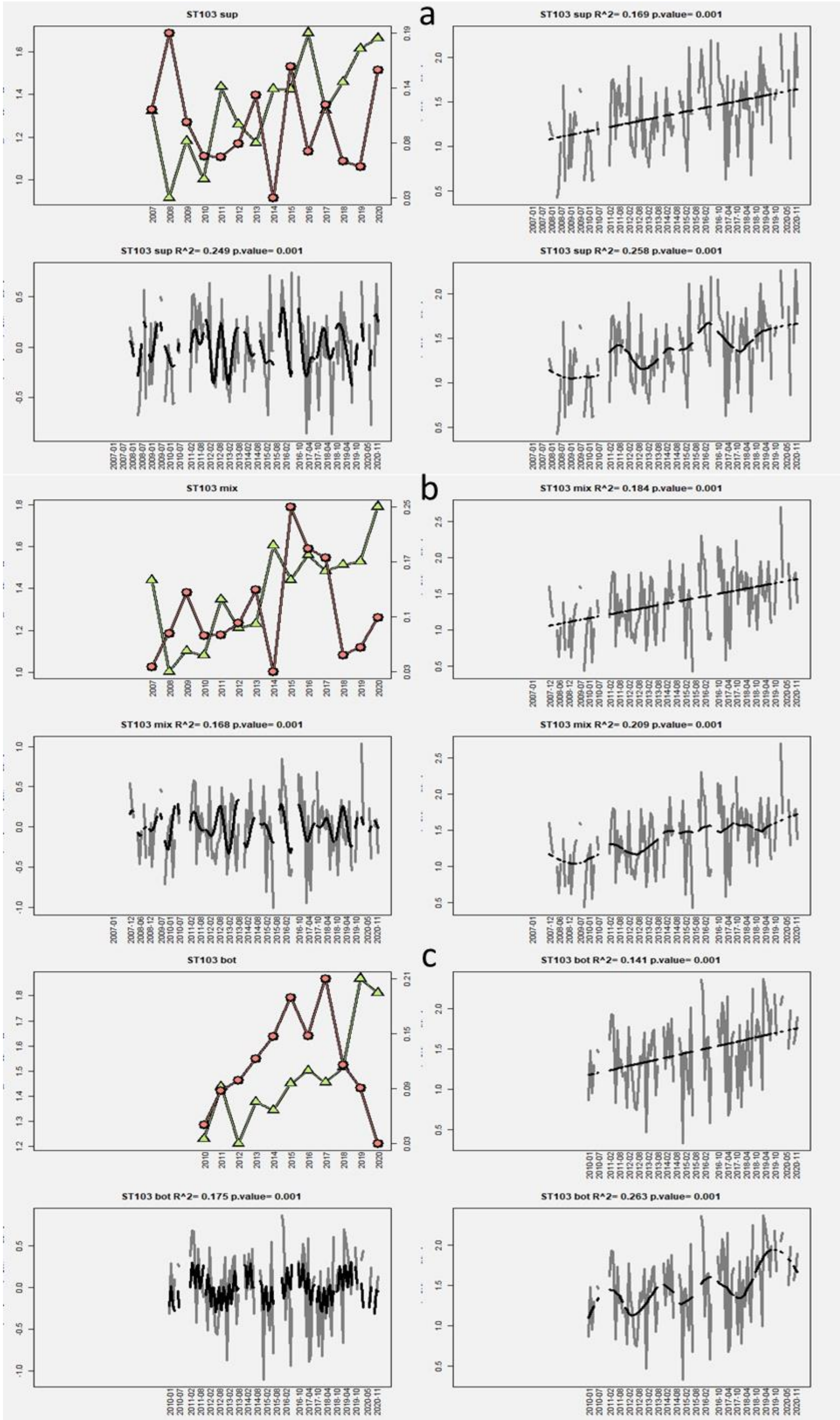

Figure S9: Shannon - Wiener's diversity index in the (a) surface, (b) middle and (c) bottom layer of station ST103 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

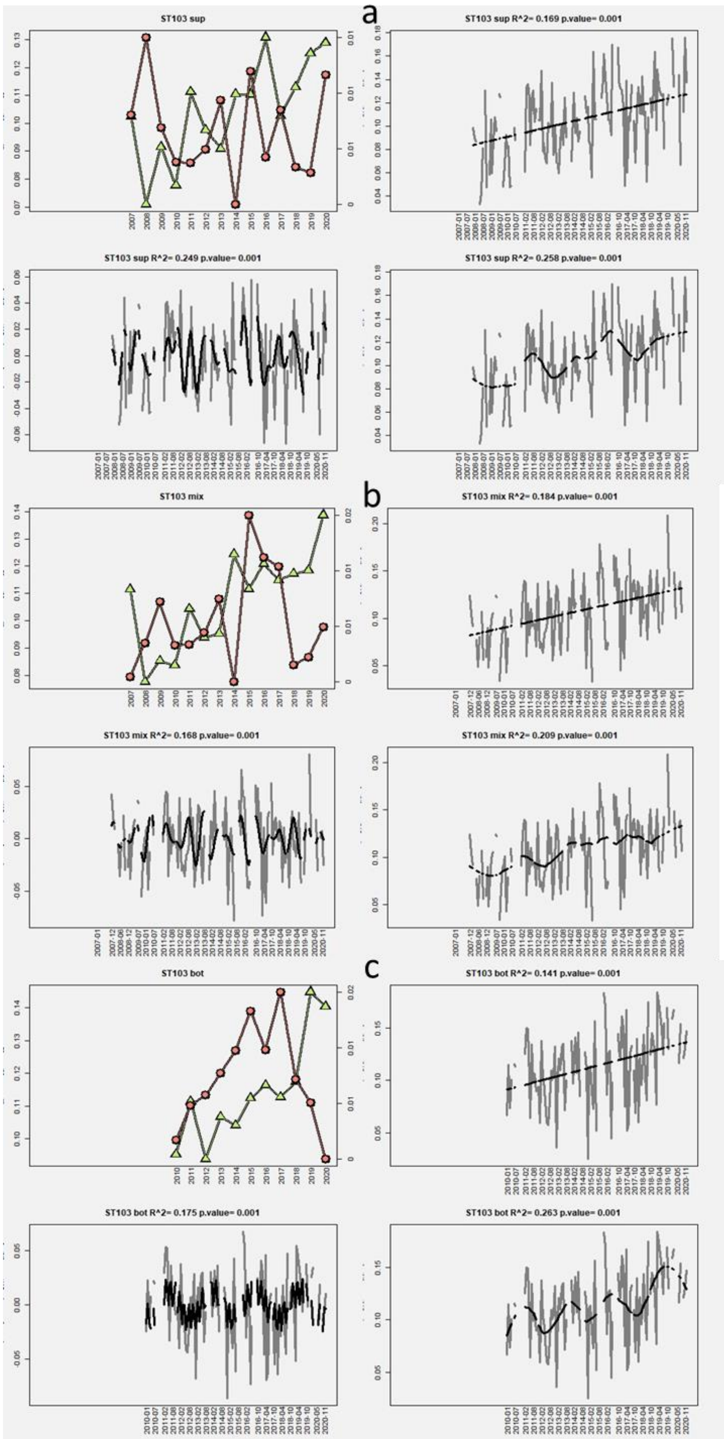

Figure S10: Pielou's evenness in the (a) surface, (b) middle and (c) bottom layer of station ST103 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

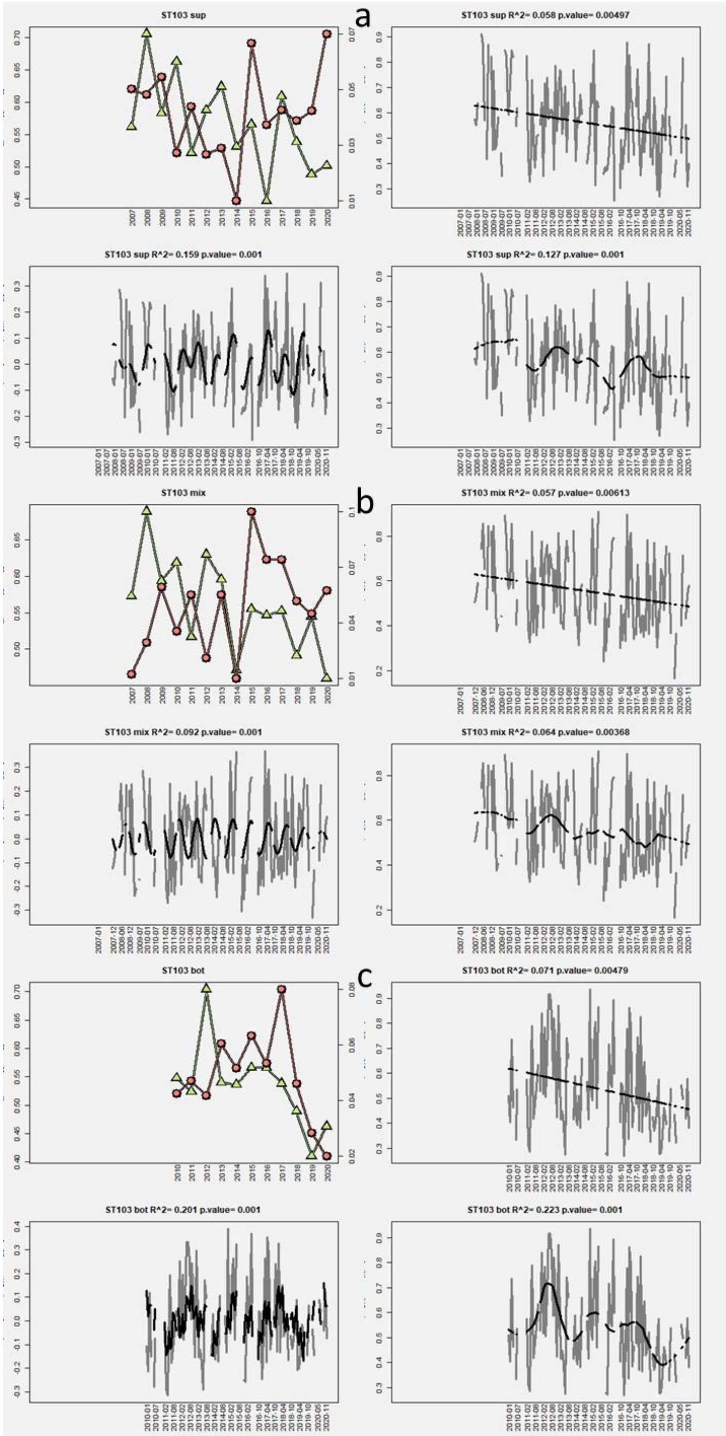

Figure S11: Berger-Parker's dominance in the (a) surface, (b) middle and (c) bottom layer of station ST103 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

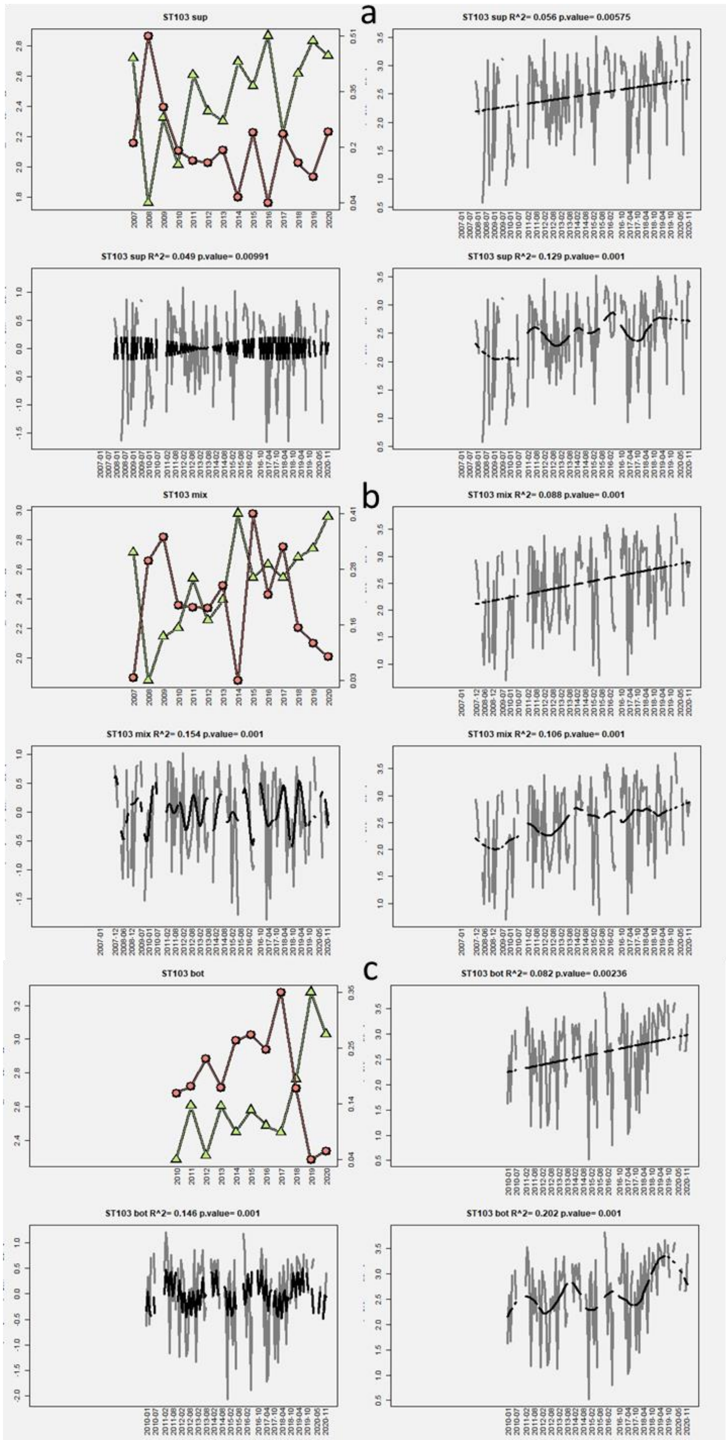

Figure S12: Rao's index in the (a) surface, (b) middle and (c) bottom layer of station ST103 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

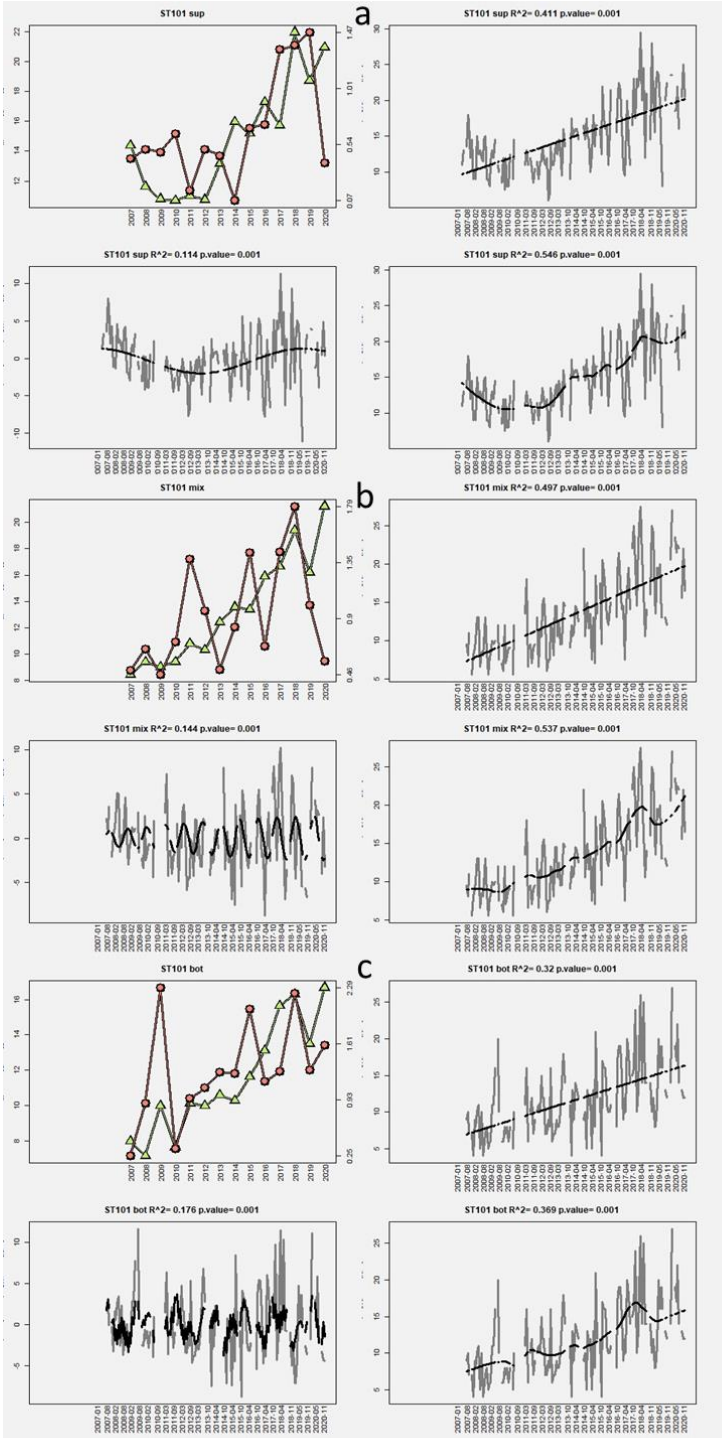

Figure S13: Genus richness in the (a) surface, (b) middle and (c) bottom layer of station ST101 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

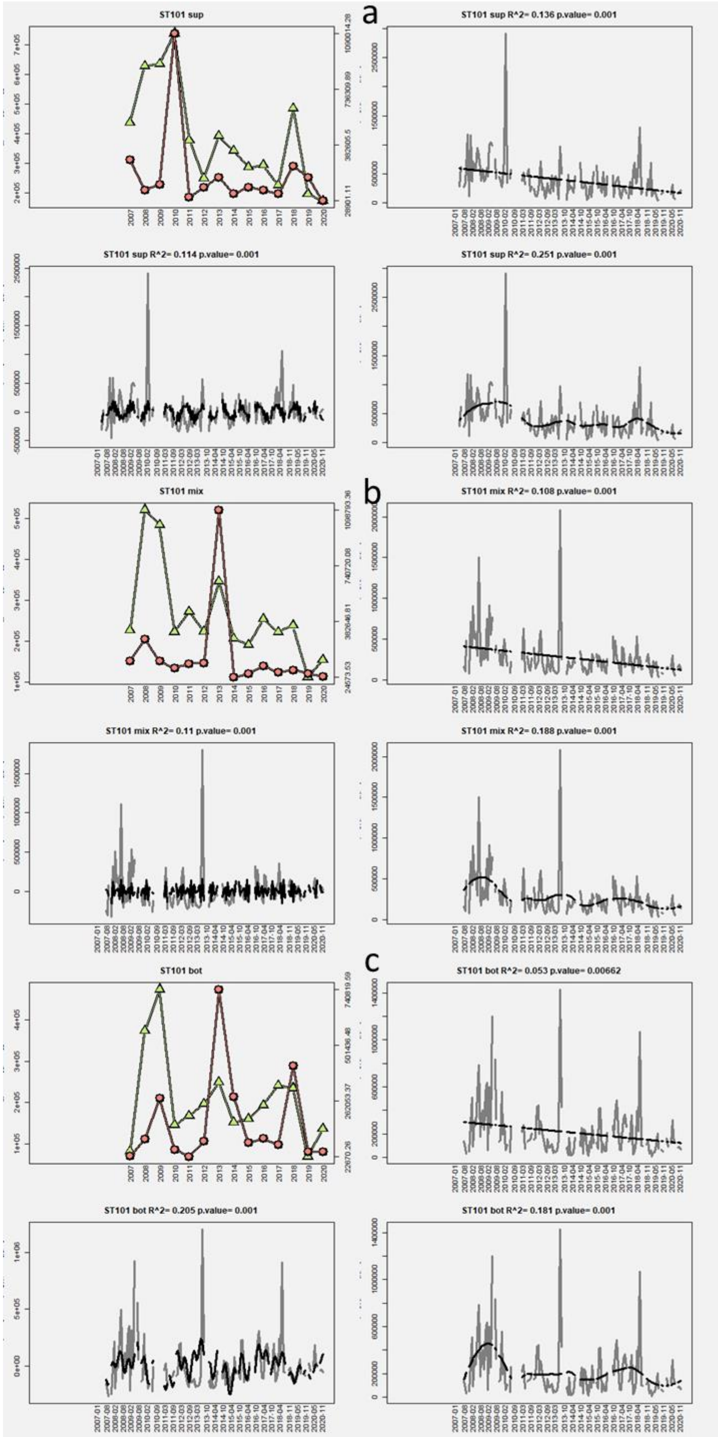

Figure S14: Phytoplankton abundance in the (a) surface, (b) middle and (c) bottom layer of station ST101 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

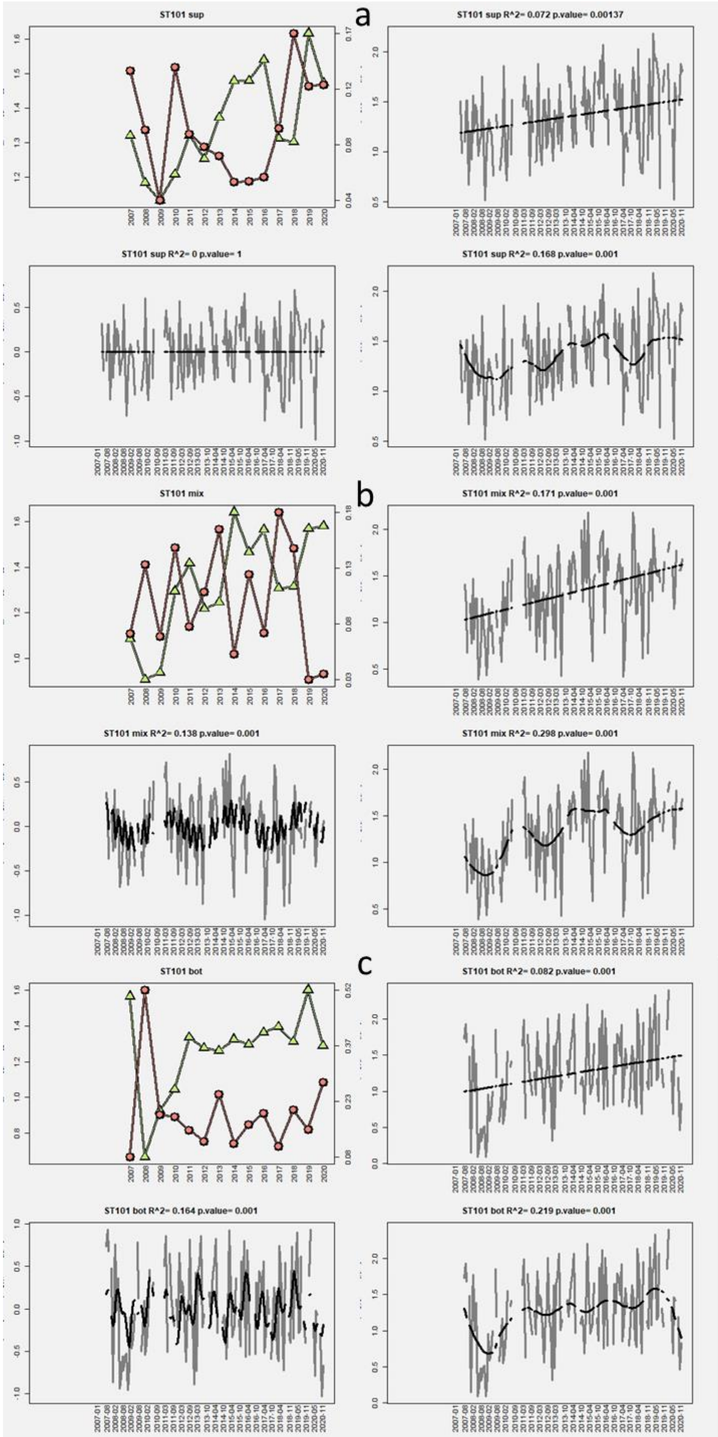

Figure S15: Shannon - Wiener's diversity index in the (a) surface, (b) middle and (c) bottom layer of station ST101 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

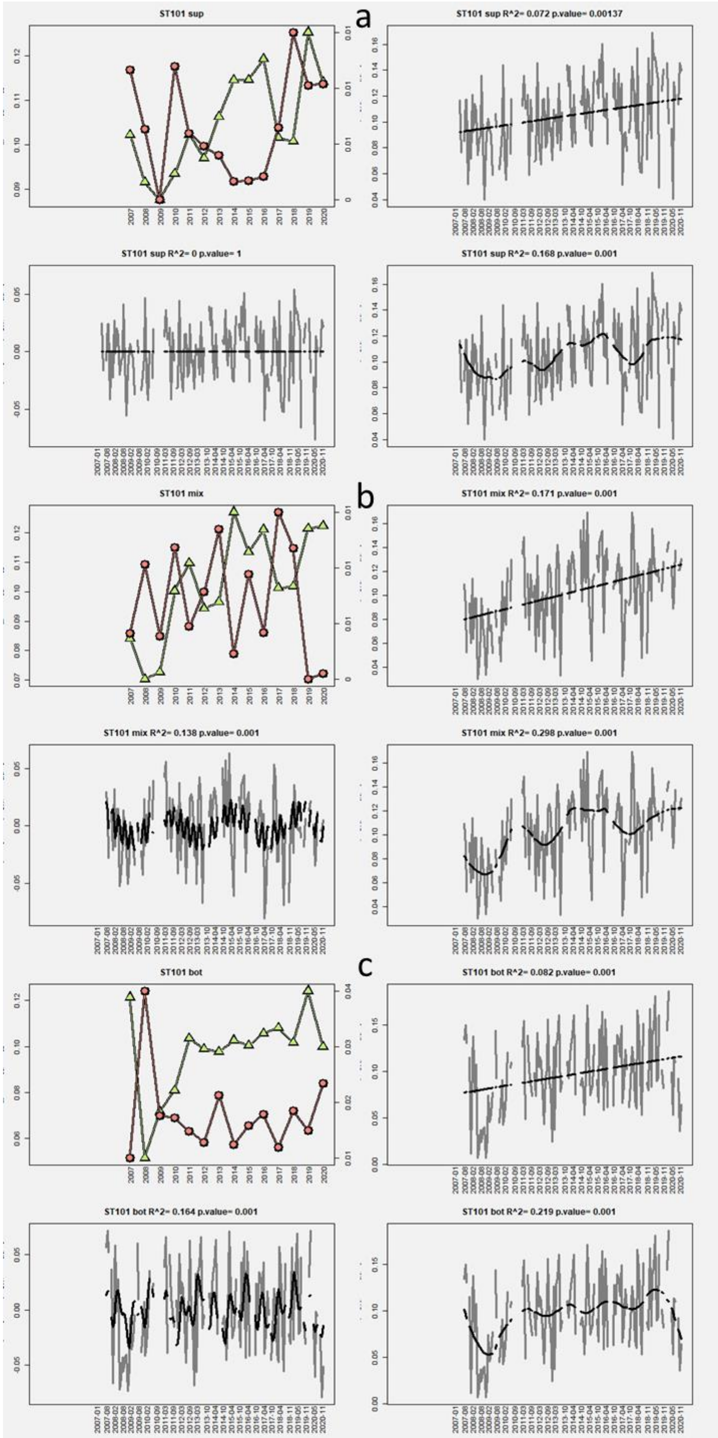

Figure S16: Pielou's evenness in the (a) surface, (b) middle and (c) bottom layer of station ST101 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

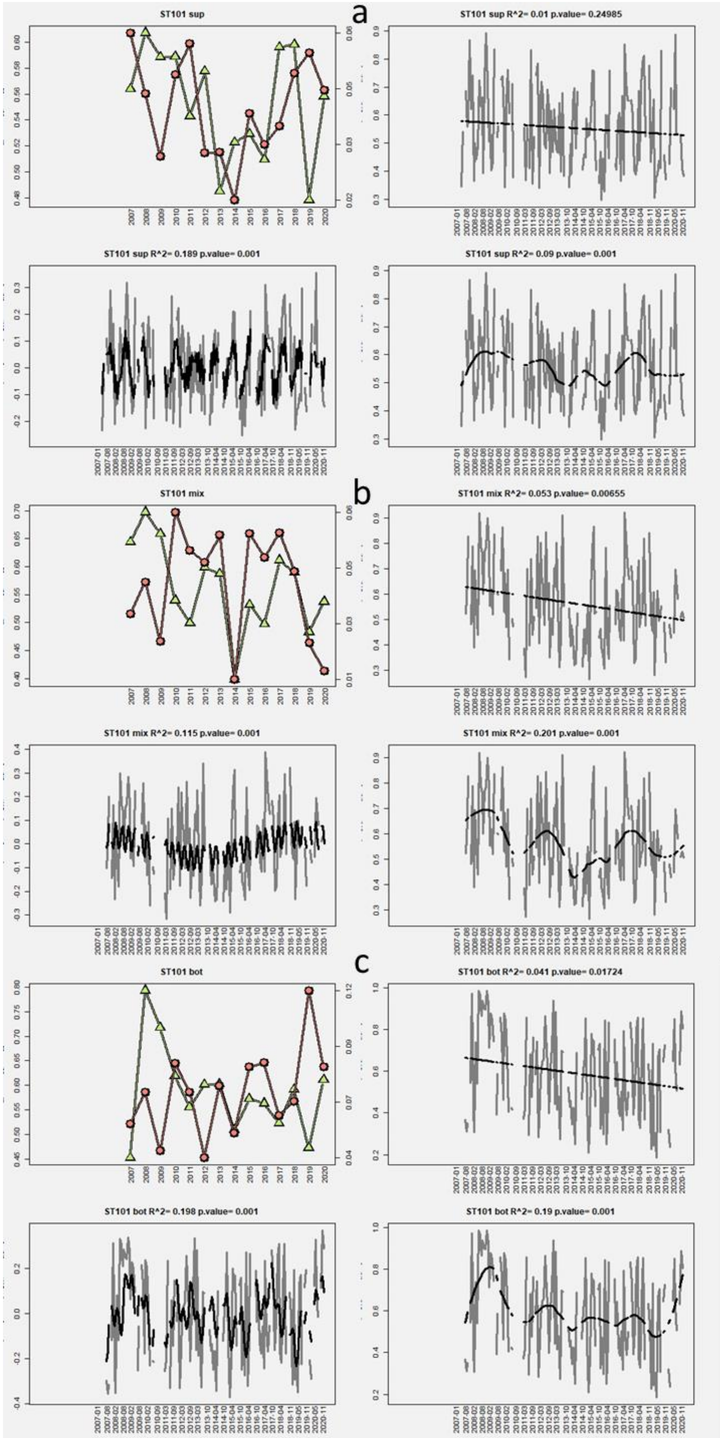

Figure S17: Berger-Parker's dominance in the (a) surface, (b) middle and (c) bottom layer of station ST101 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

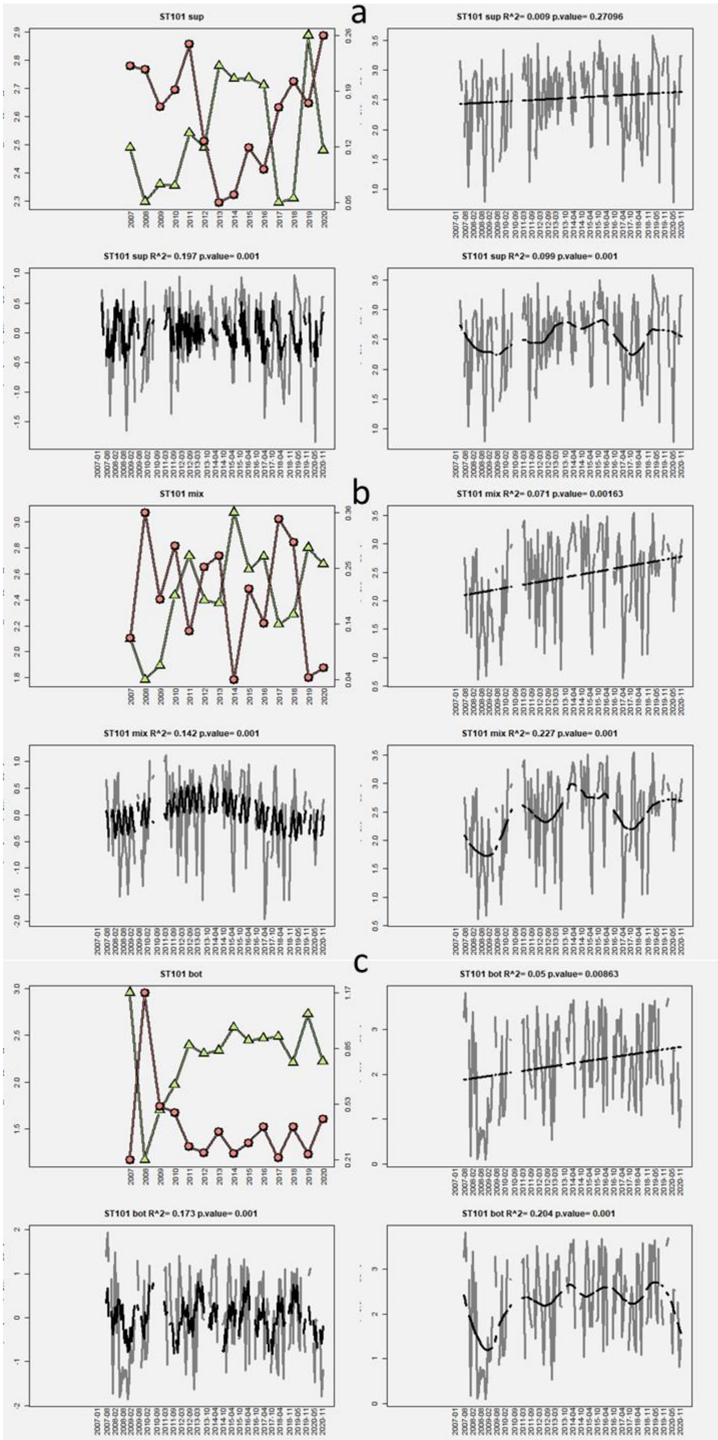

Figure S18: Rao’s index in the (a) surface, (b) middle and (c) bottom layer of station ST101 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

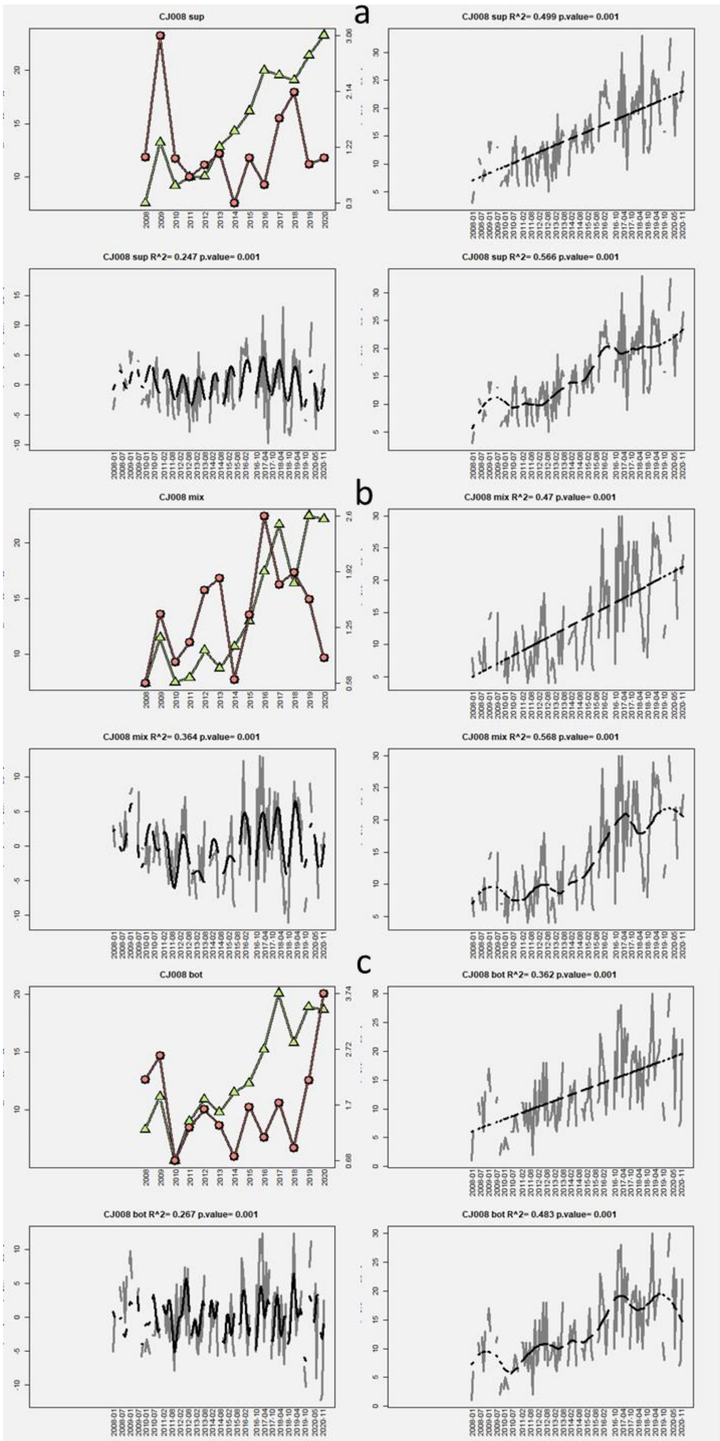

Figure S19: Genus richness in the (a) surface, (b) middle and (c) bottom layer of station CJ008 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

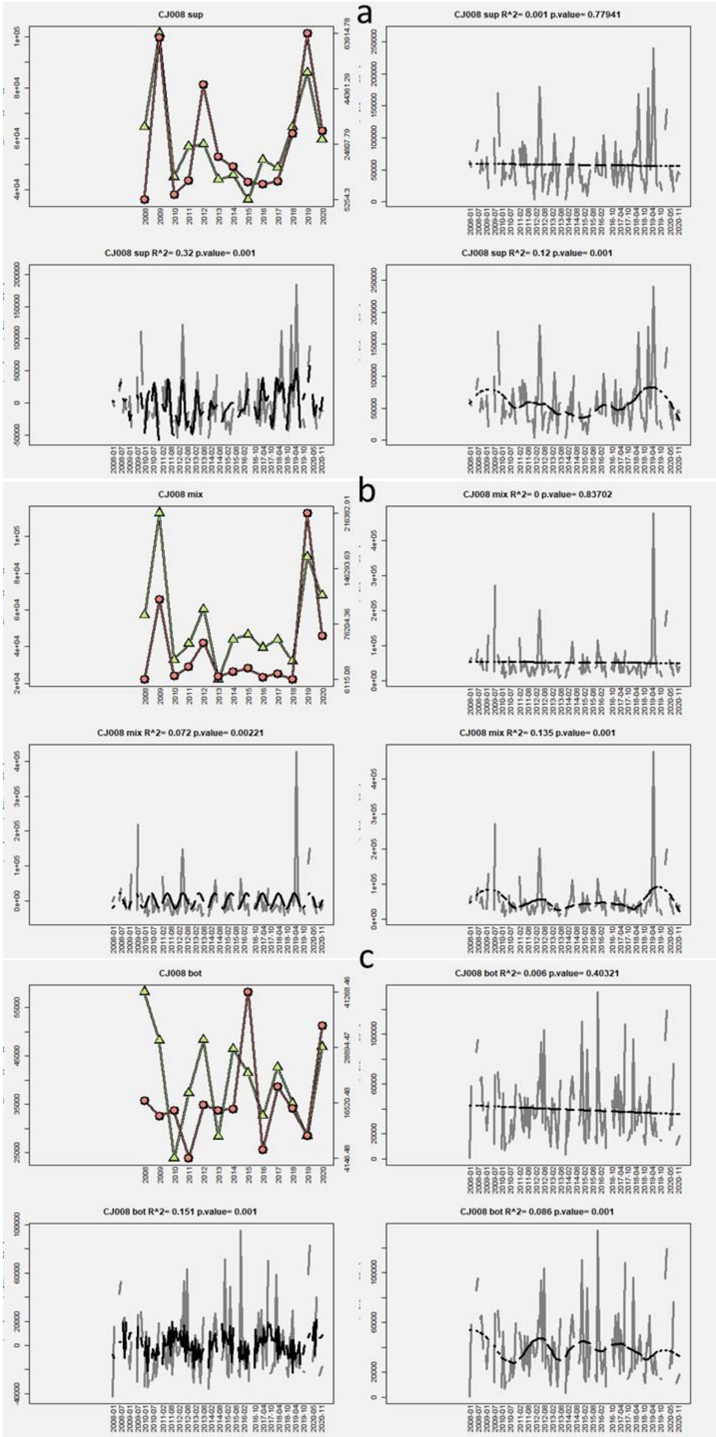

Figure S20: Phytoplankton abundance in the (a) surface, (b) middle and (c) bottom layer of station CJ008 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

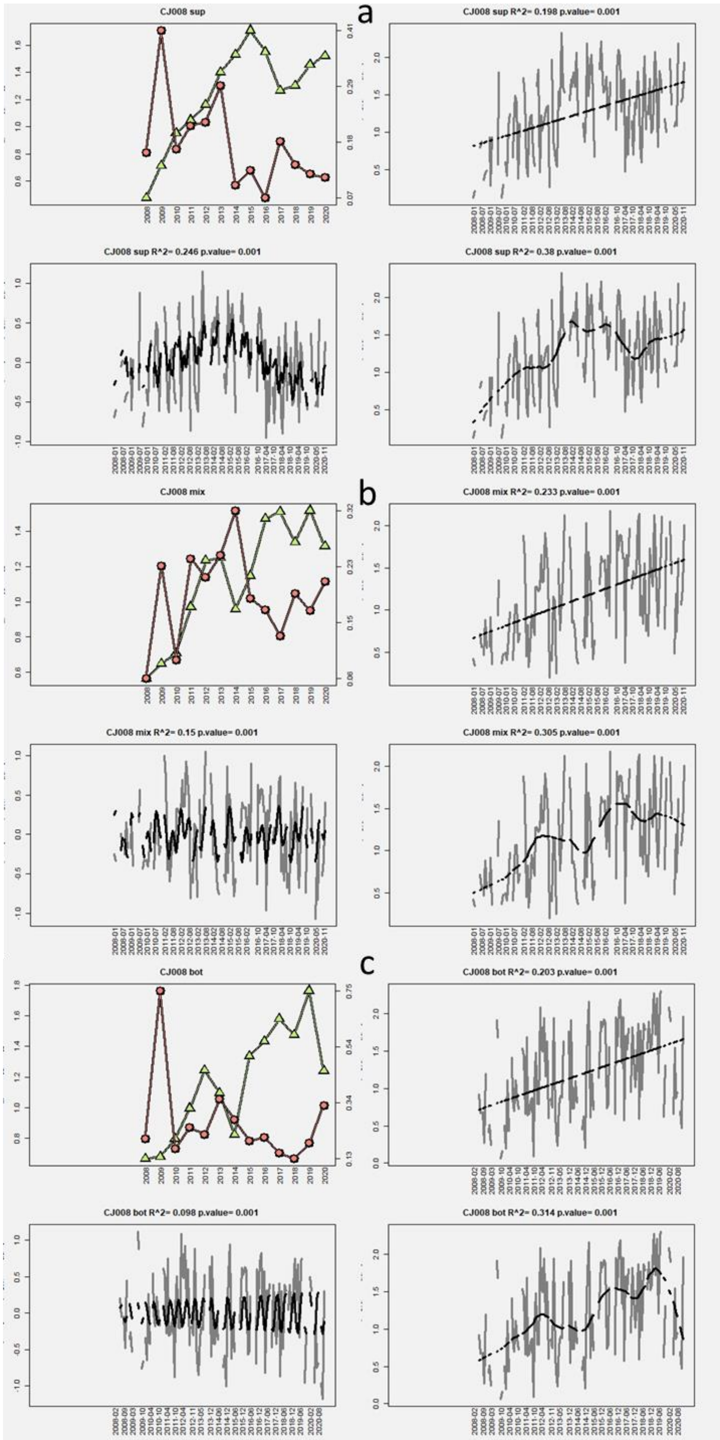

Figure S21: Shannon - Wiener's diversity index in the (a) surface, (b) middle and (c) bottom layer of station CJ008 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

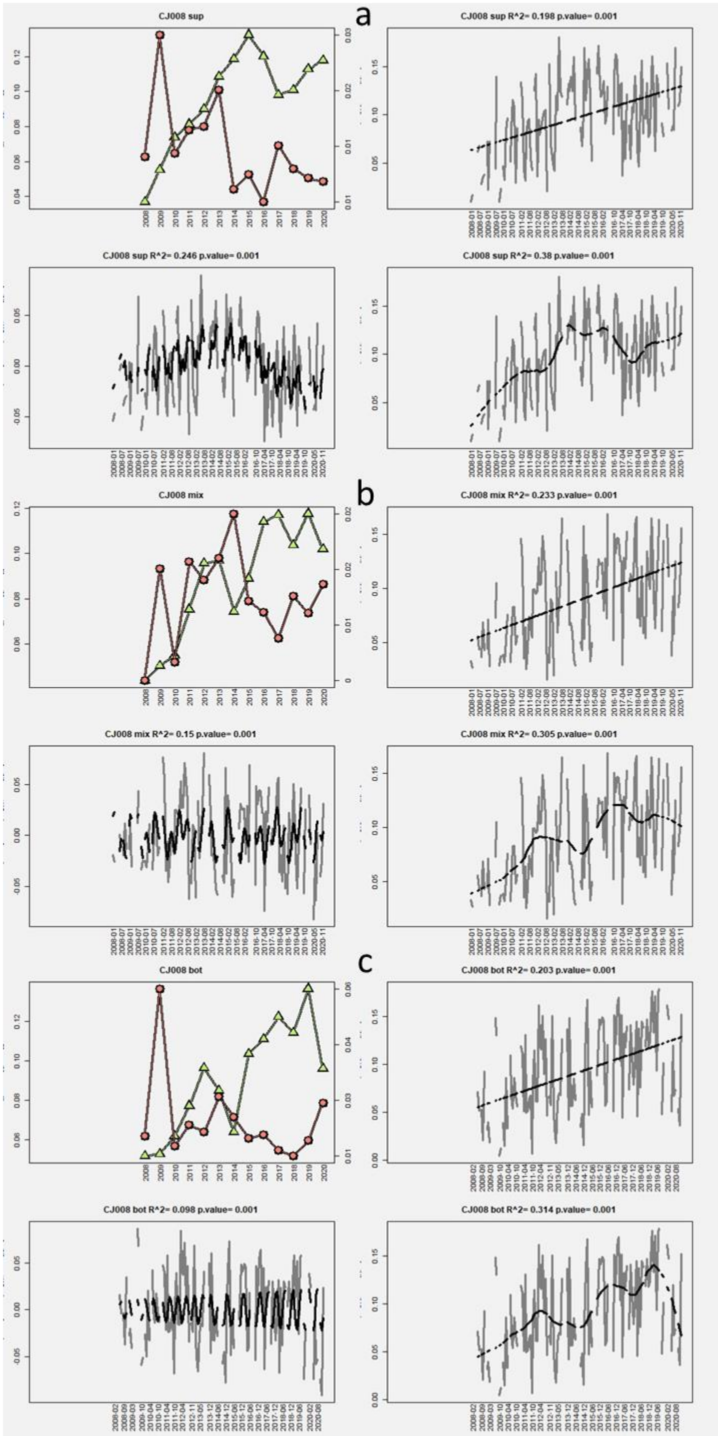

Figure S22: Pielou's evenness in the (a) surface, (b) middle and (c) bottom layer of station CJ008 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

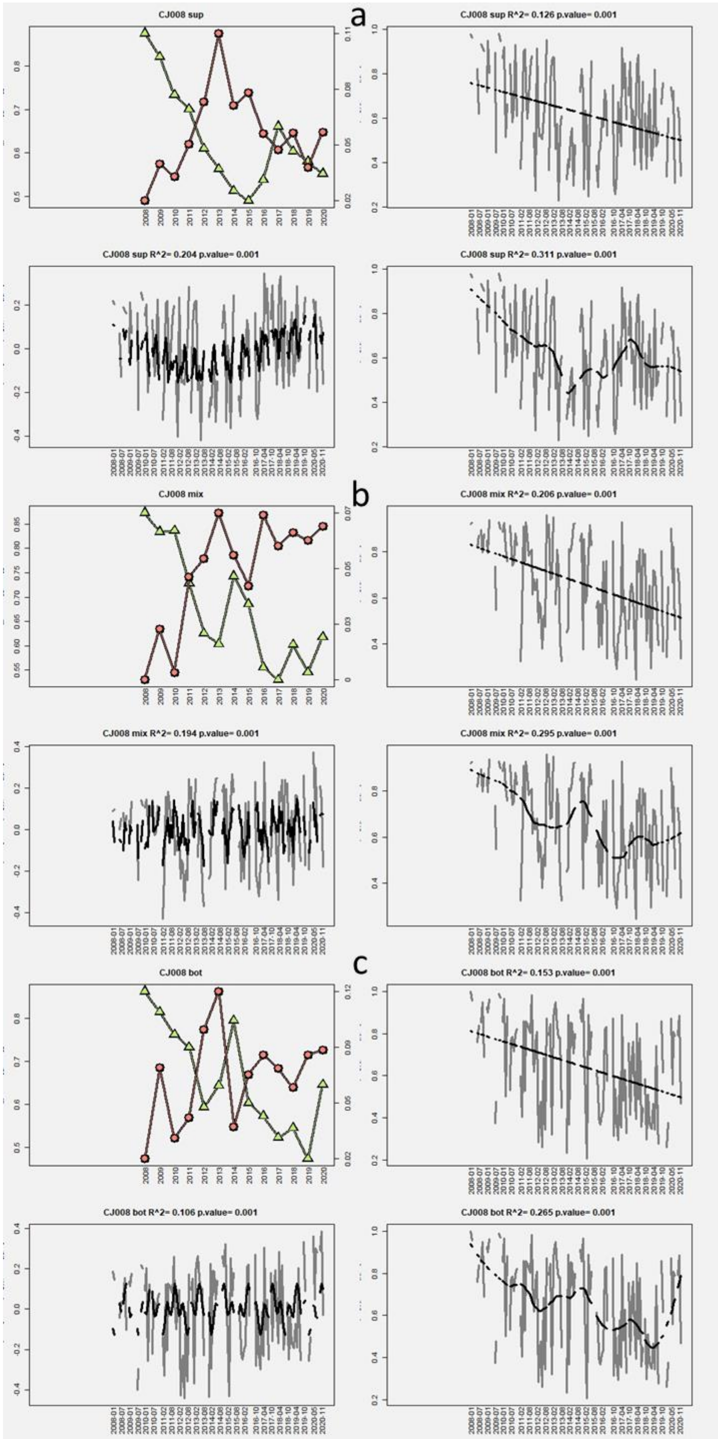

Figure S23: Berger-Parker's dominance in the (a) surface, (b) middle and (c) bottom layer of station CJ008 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

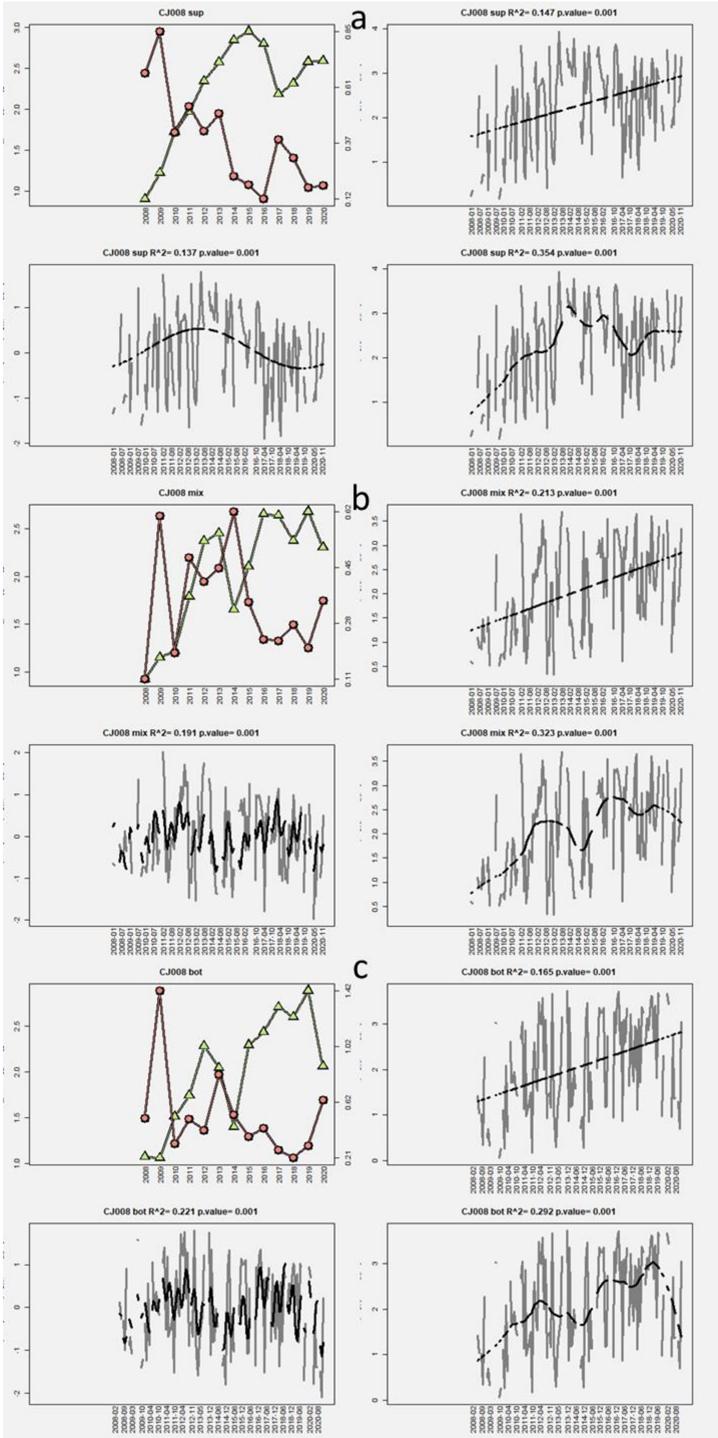

Figure S24: Rao's index in the (a) surface, (b) middle and (c) bottom layer of station CJ008 in the period 2007-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

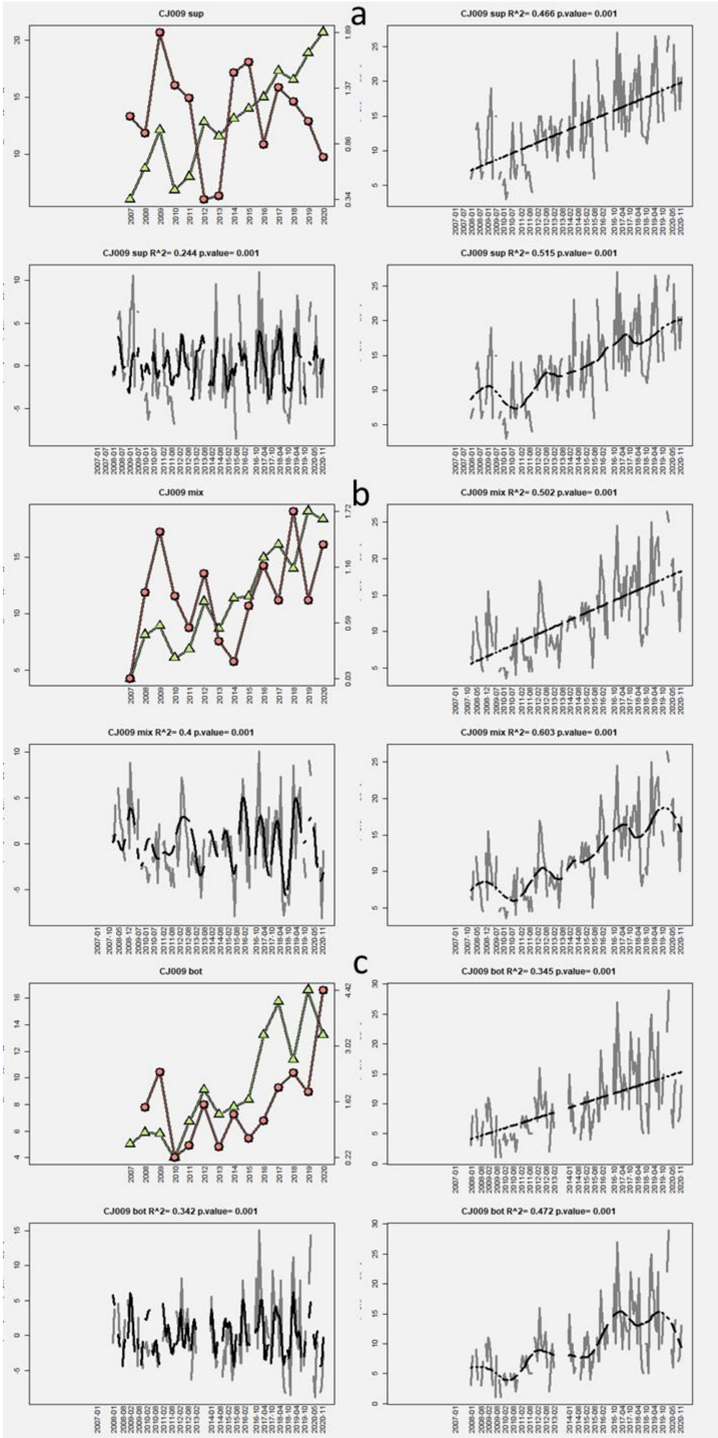

Figure S25: Genus richness in the (a) surface, (b) middle and (c) bottom layer of station CJ009 in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

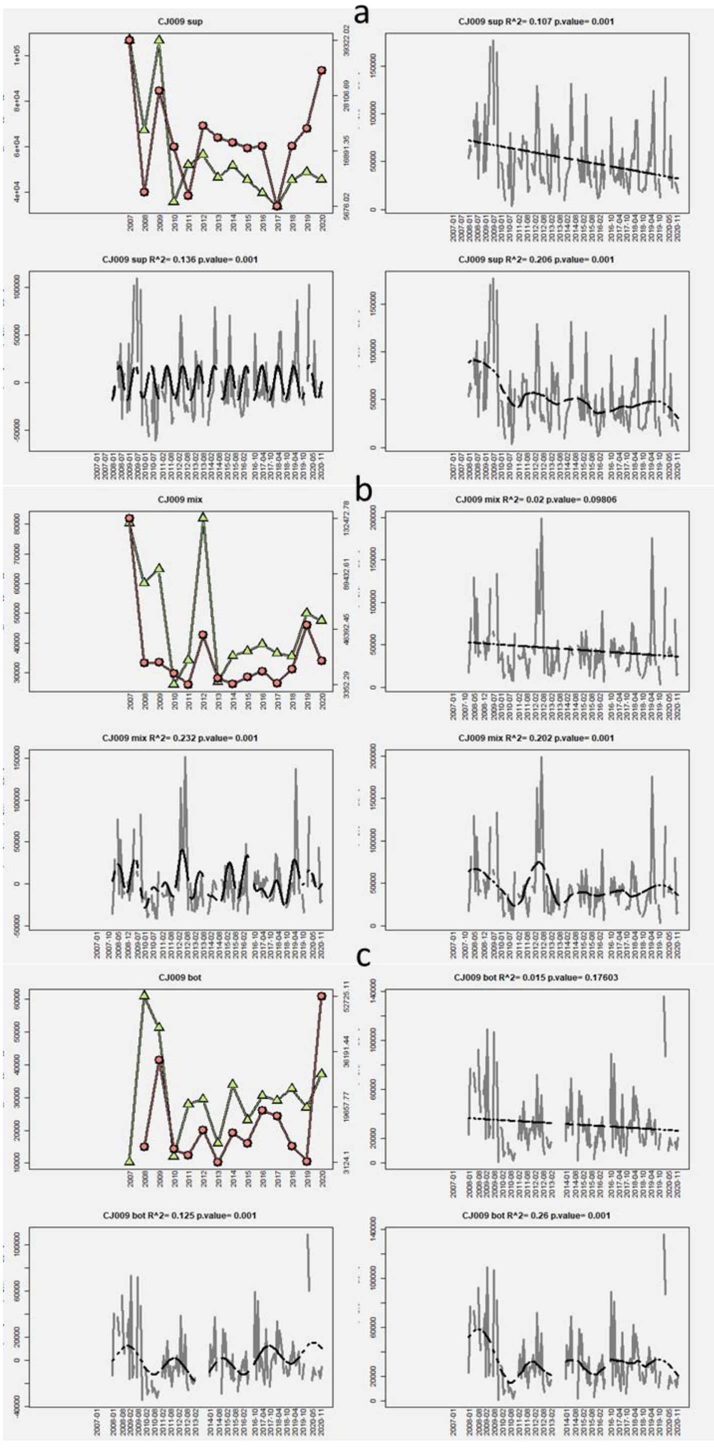

Figure S26: Phytoplankton abundance in the (a) surface, (b) middle and (c) bottom layer of station CJ009 in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

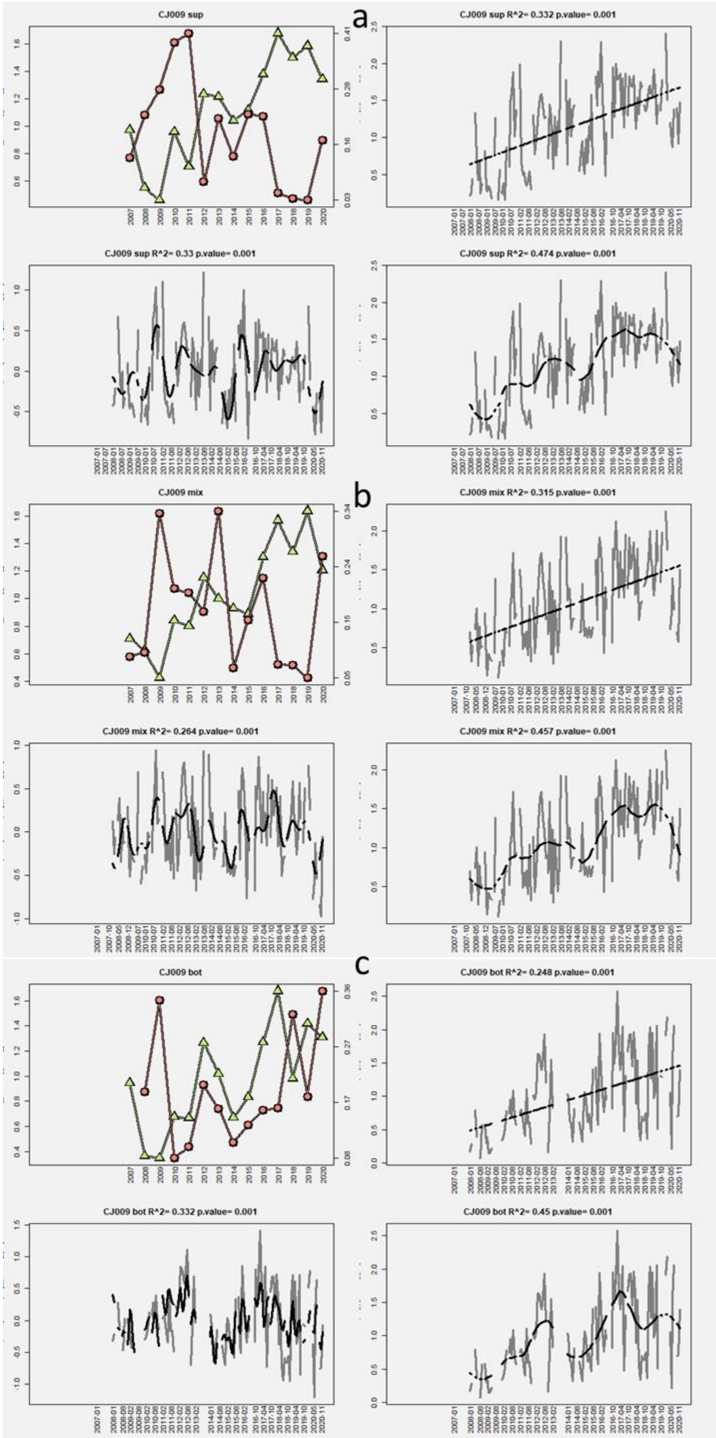

Figure S27: Shannon - Wiener's diversity index in the (a) surface, (b) middle and (c) bottom layer of station CJ009 in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

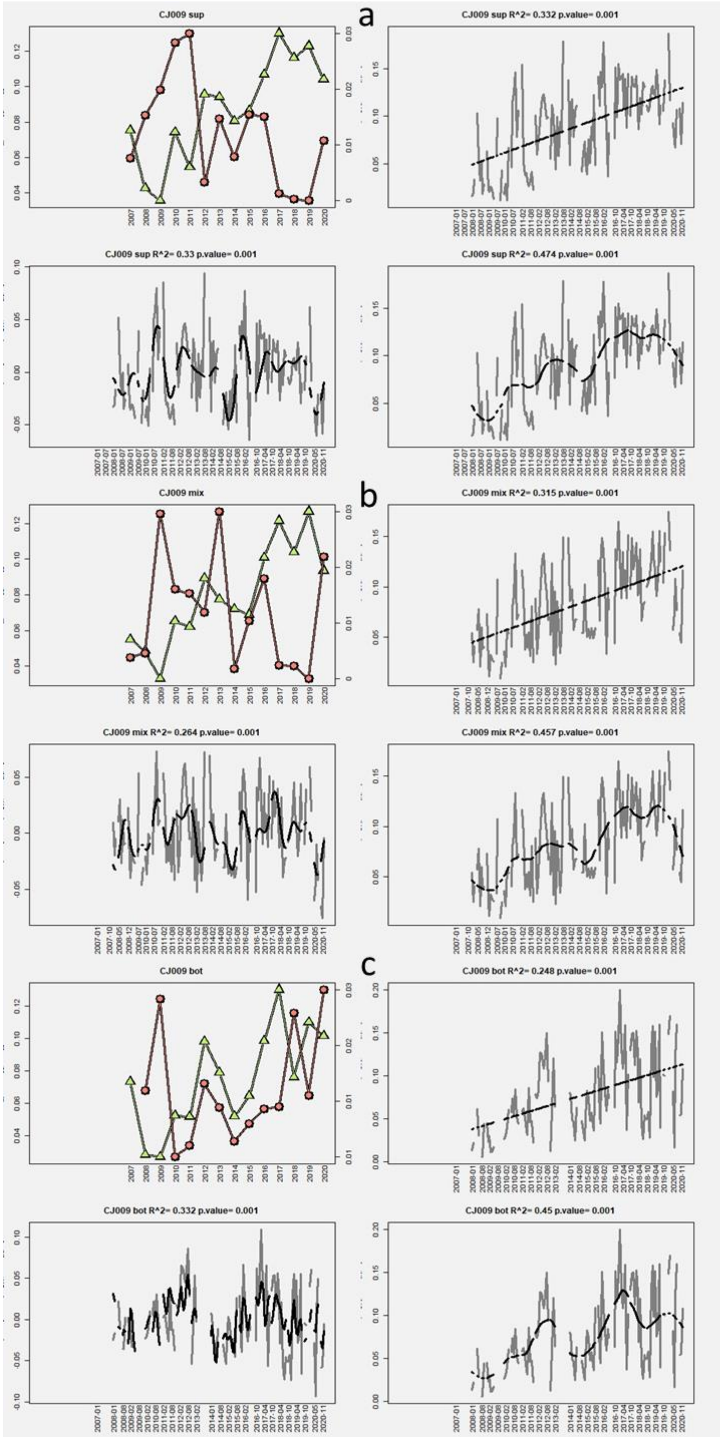

Figure 28: Pielou's evenness in the (a) surface, (b) middle and (c) bottom layer of station CJ009 in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

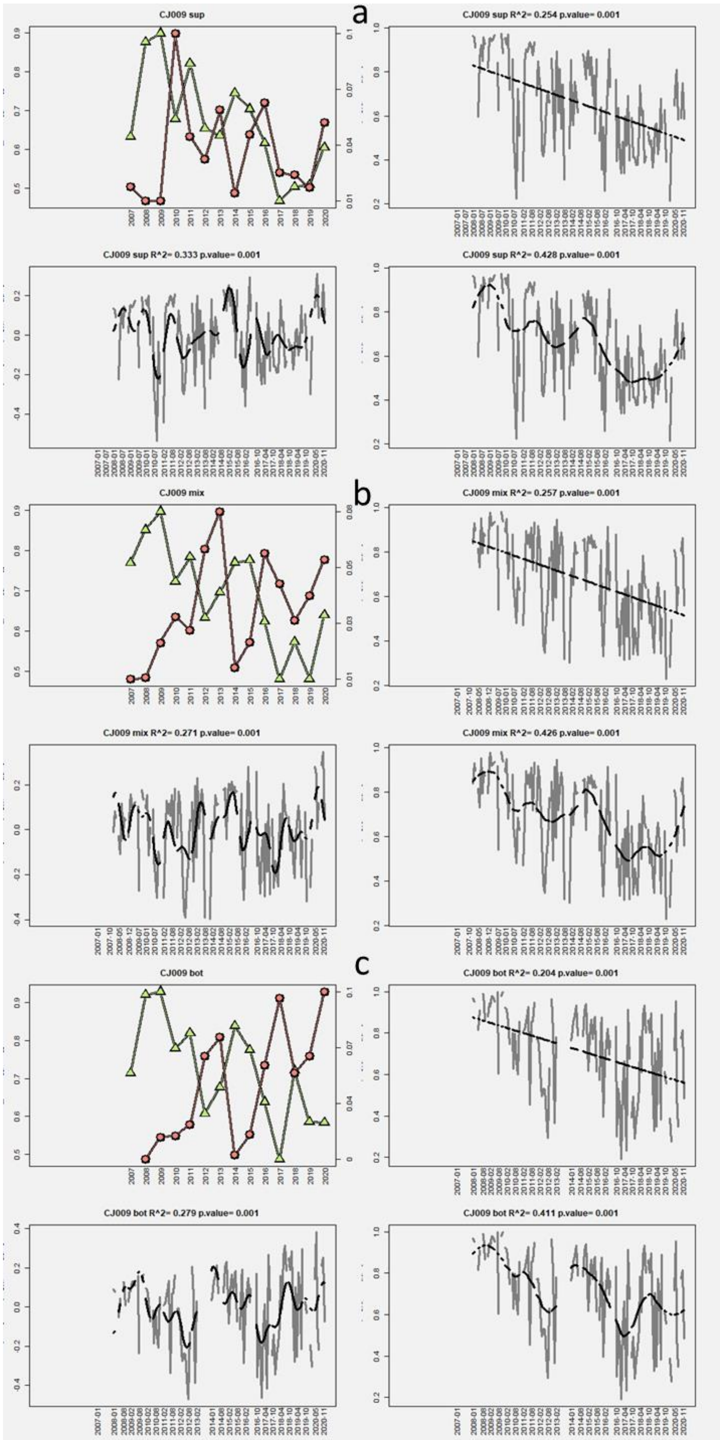

Figure S29: Berger-Parker's dominance in the (a) surface, (b) middle and (c) bottom layer of station CJ009 in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

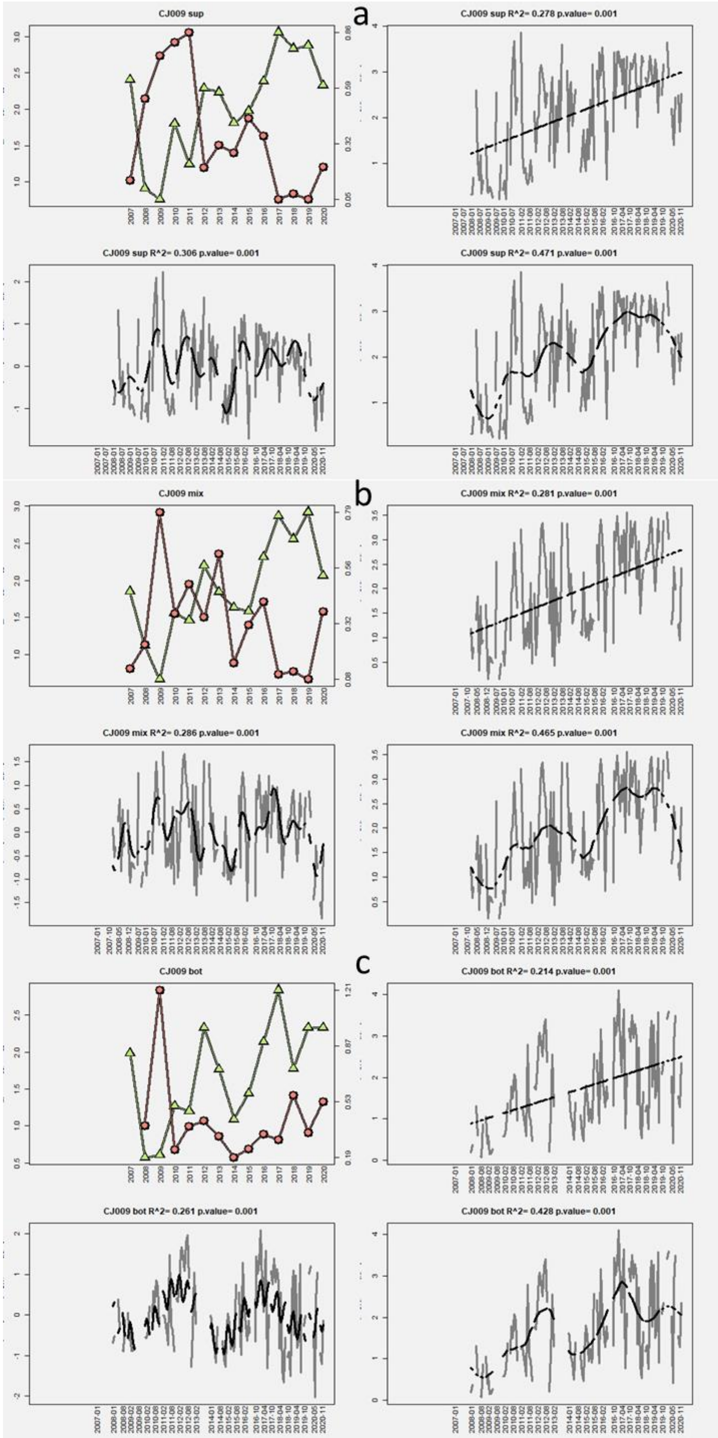

Figure S30: Rao’s index in the (a) surface, (b) middle and (c) bottom layer of station CJ009 in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

<u>Case study: Slovenia</u>

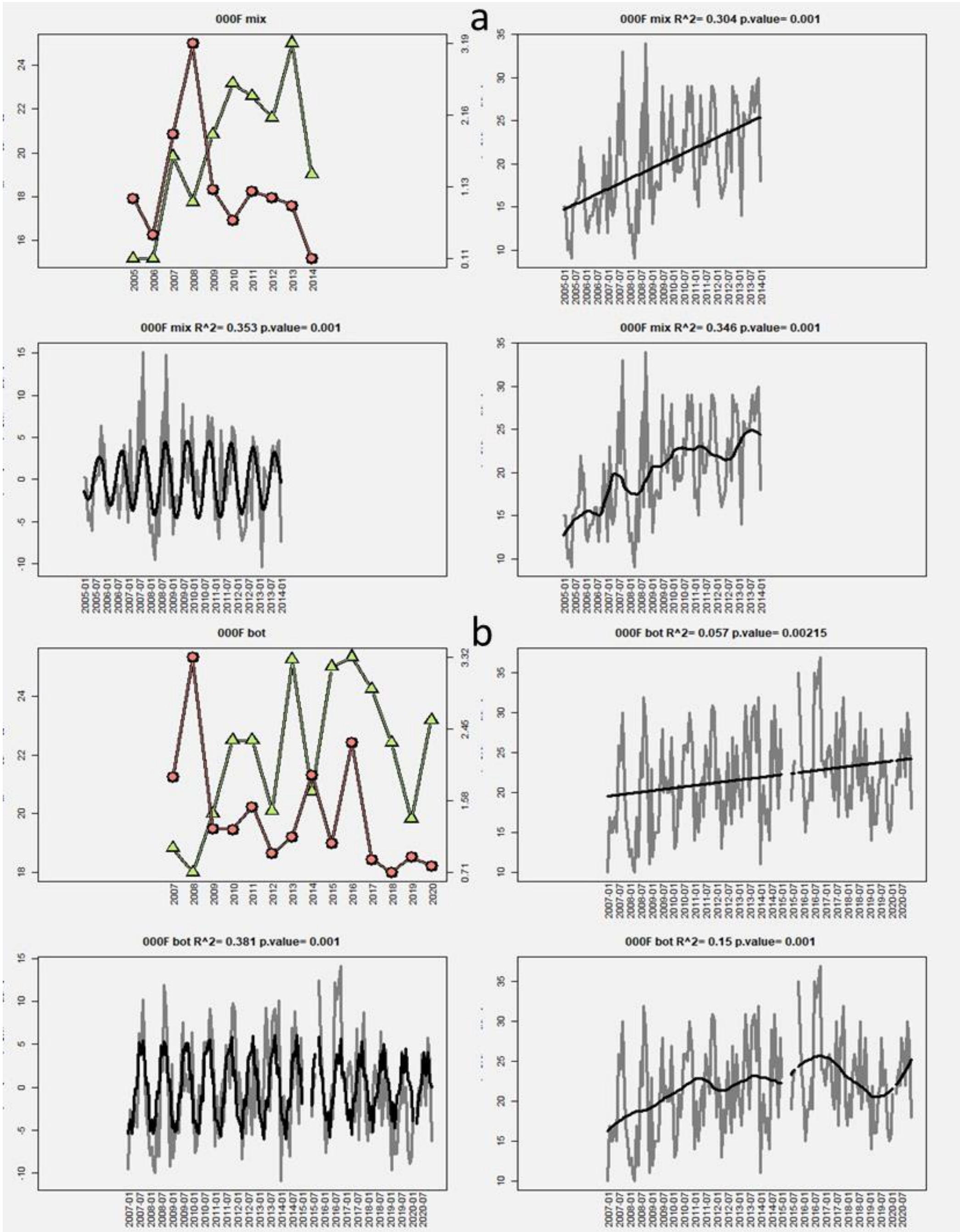

Figure S31: Genus richness in the (a) middle and (b) bottom layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

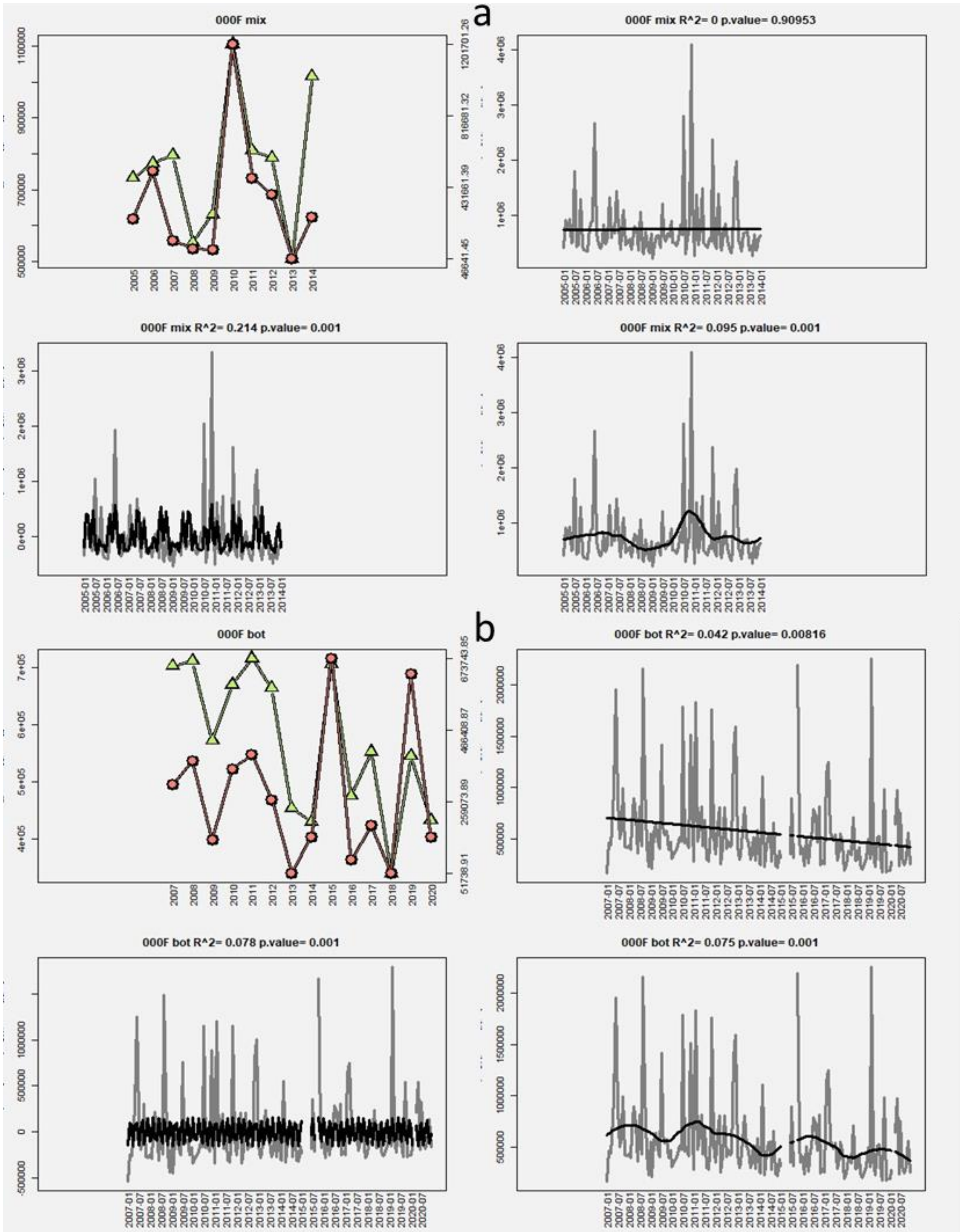

Figure S32: Phytoplankton abundance in the (a) middle and (b) bottom layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

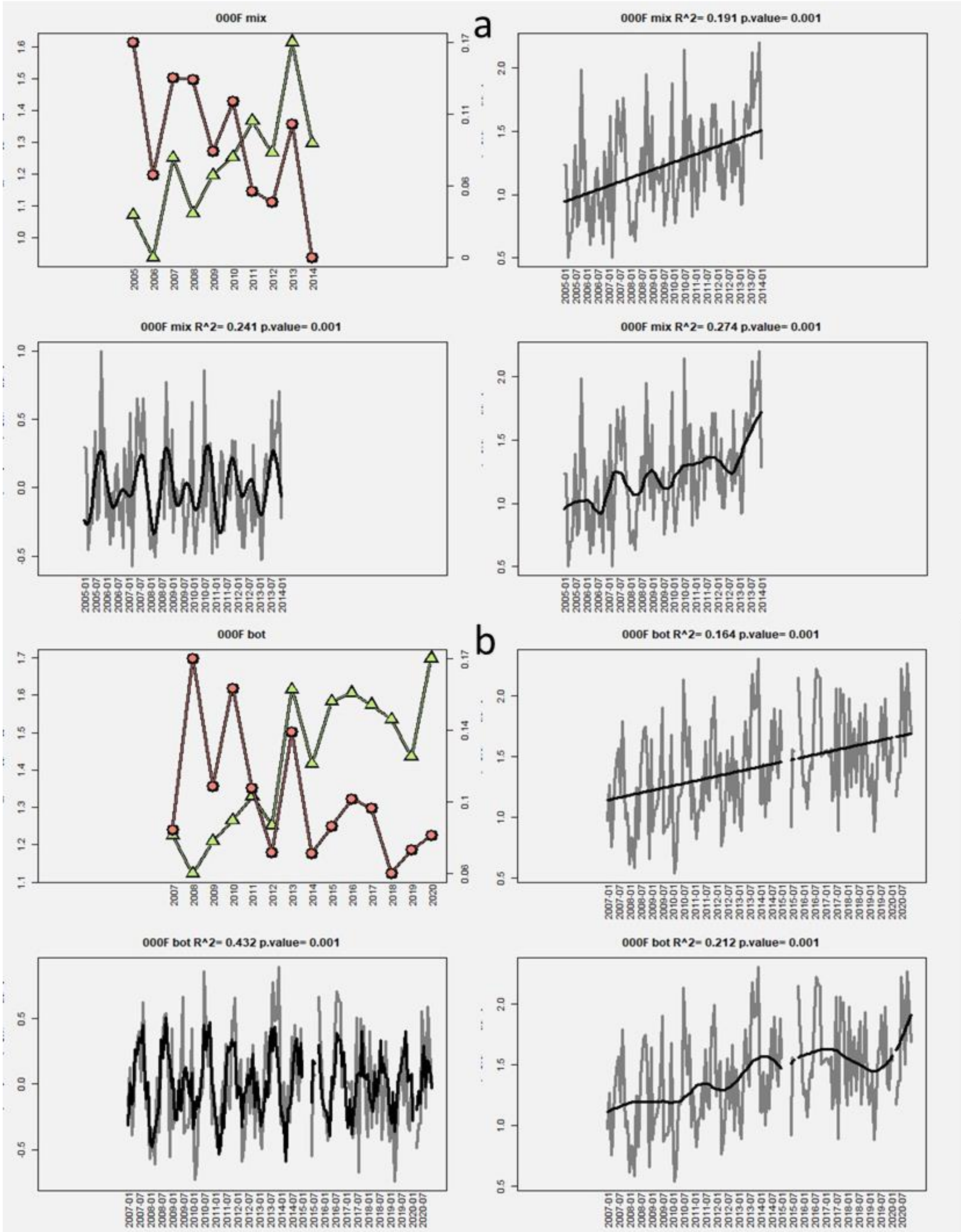

Figure S33: Shannon - Wiener's diversity index in the (a) middle and (b) bottom layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

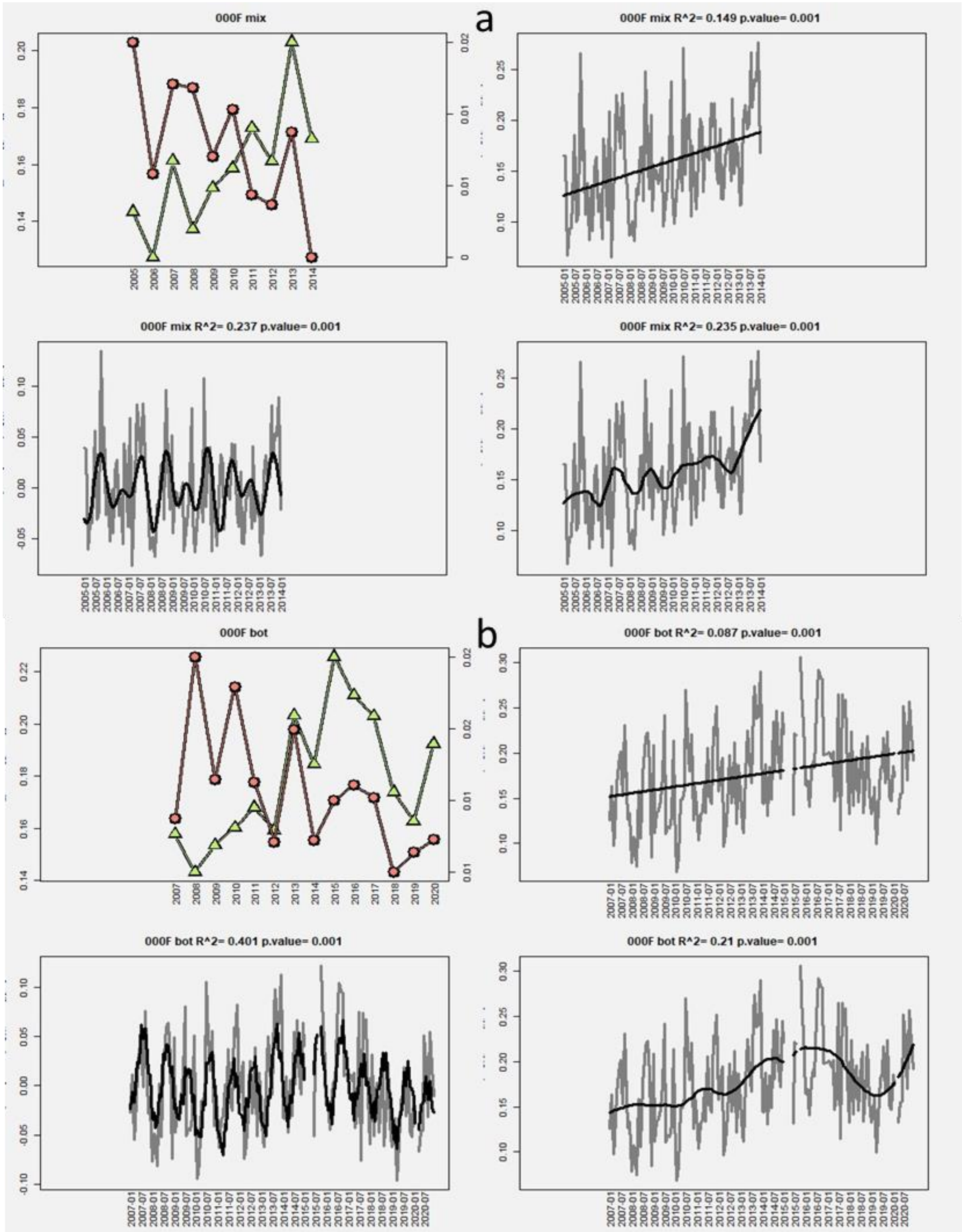

Figure S34: Pielou's evenness in the (a) middle and (b) bottom layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

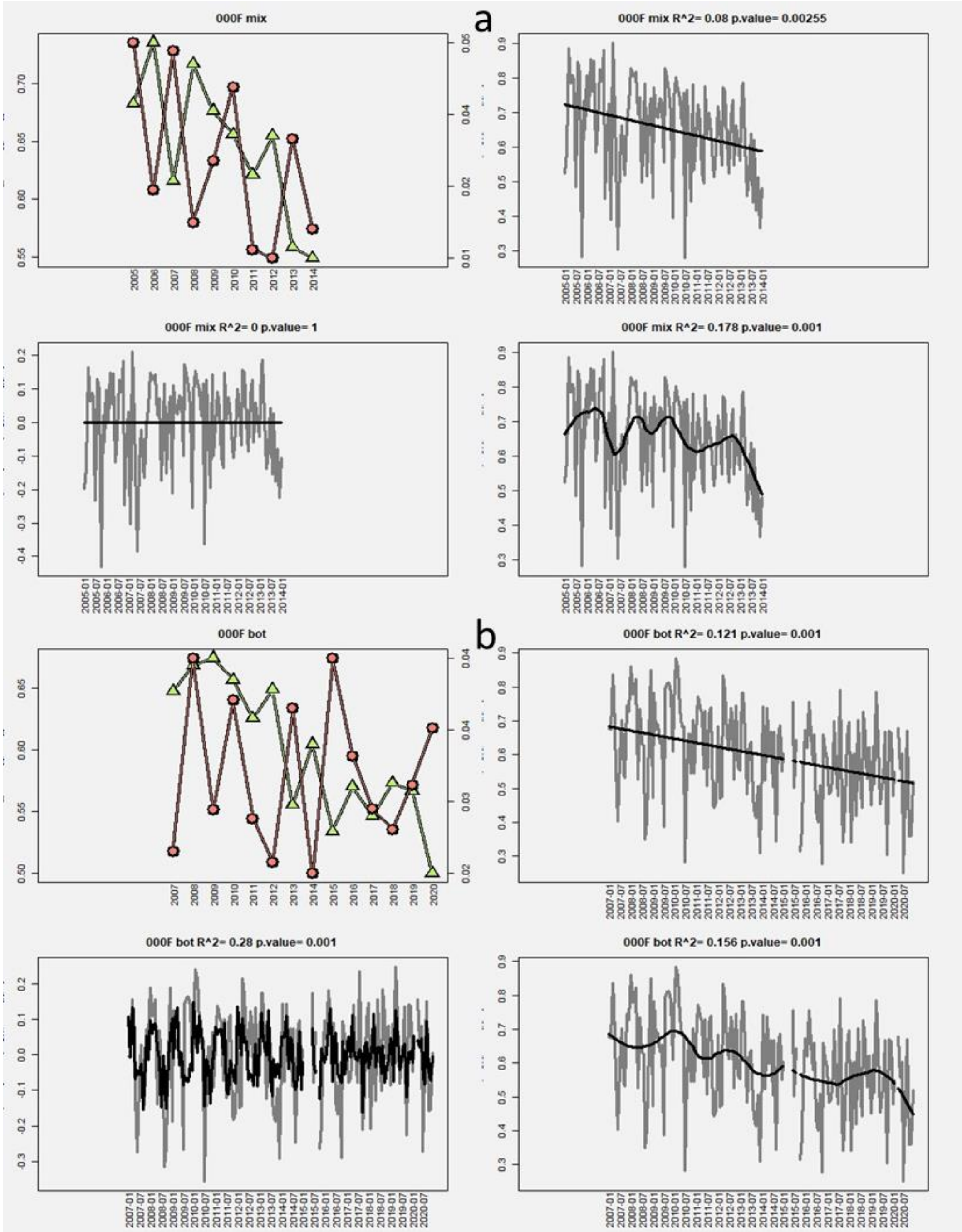

Figure S35: Berger-Parker's dominance in the (a) middle and (b) bottom layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

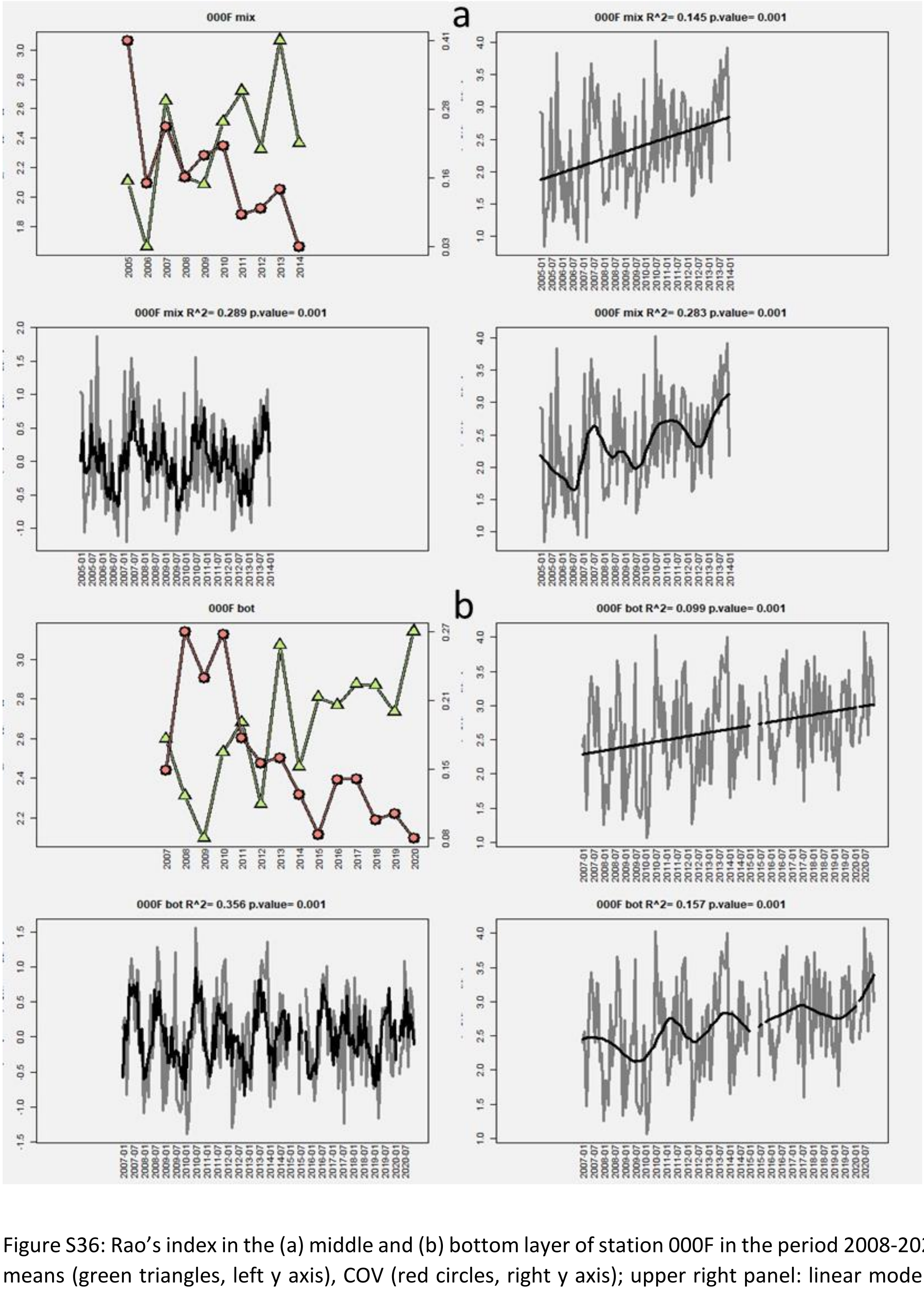

Figure S36: Rao's index in the (a) middle and (b) bottom layer of station 000F in the period 2008-2020. Upper left panel: annual means (green triangles, left y axis), COV (red circles, right y axis); upper right panel: linear model; lower left panel: periodic components; lower right panel: the LOESS (deseasonalized) model

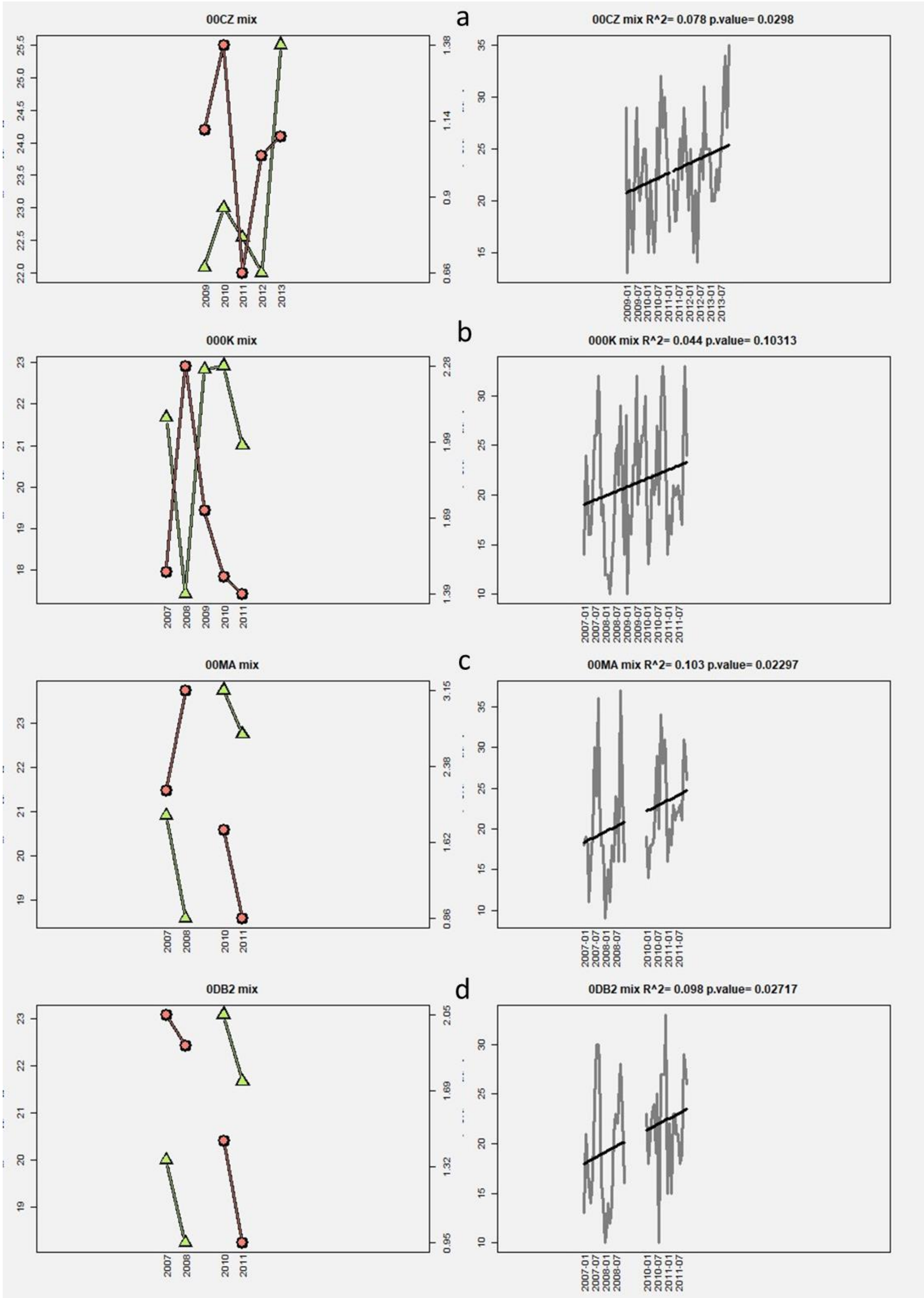

Figure S37: Genus richness in the middle layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

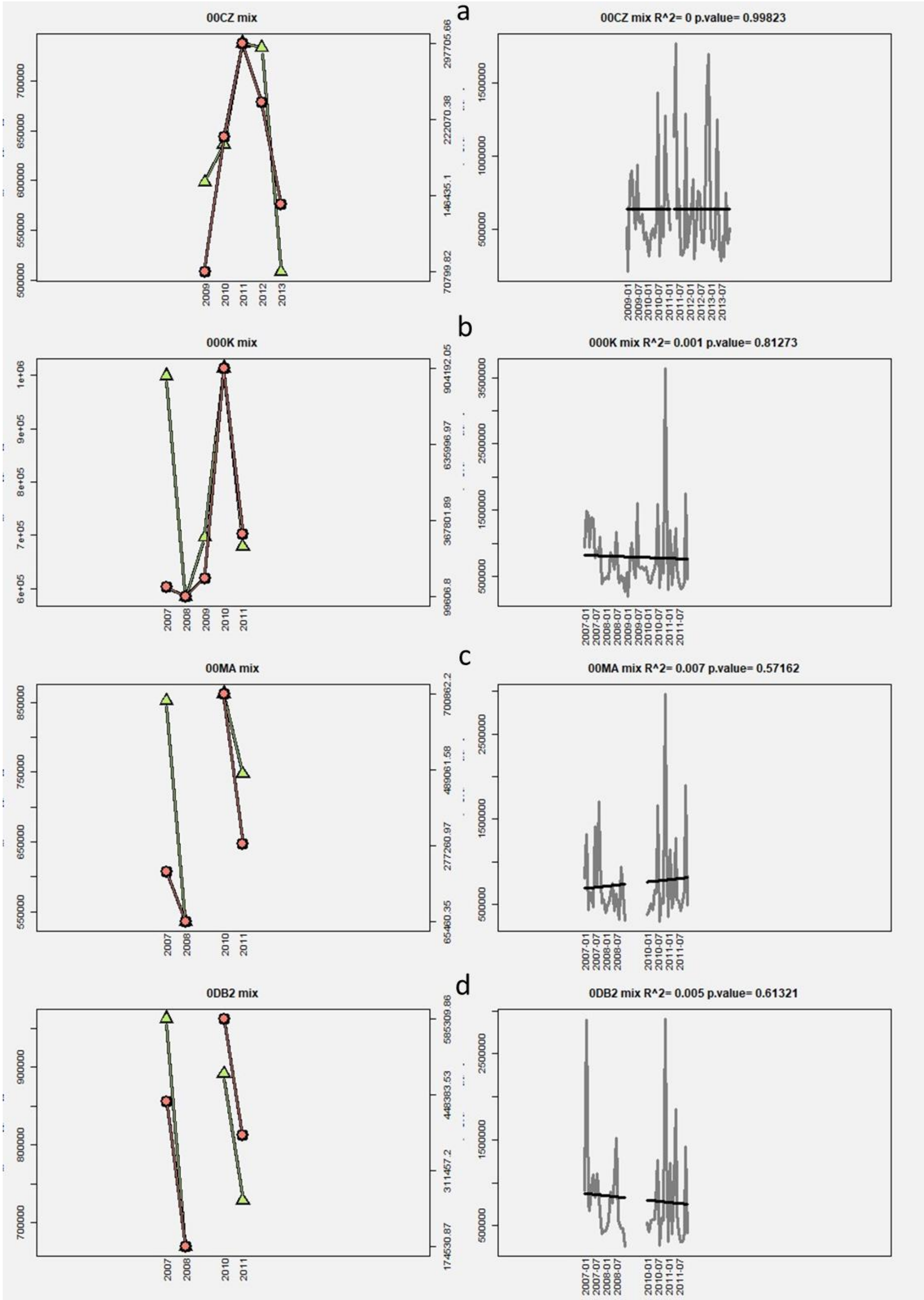

Figure S38: Phytoplankton abundance in the middle layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

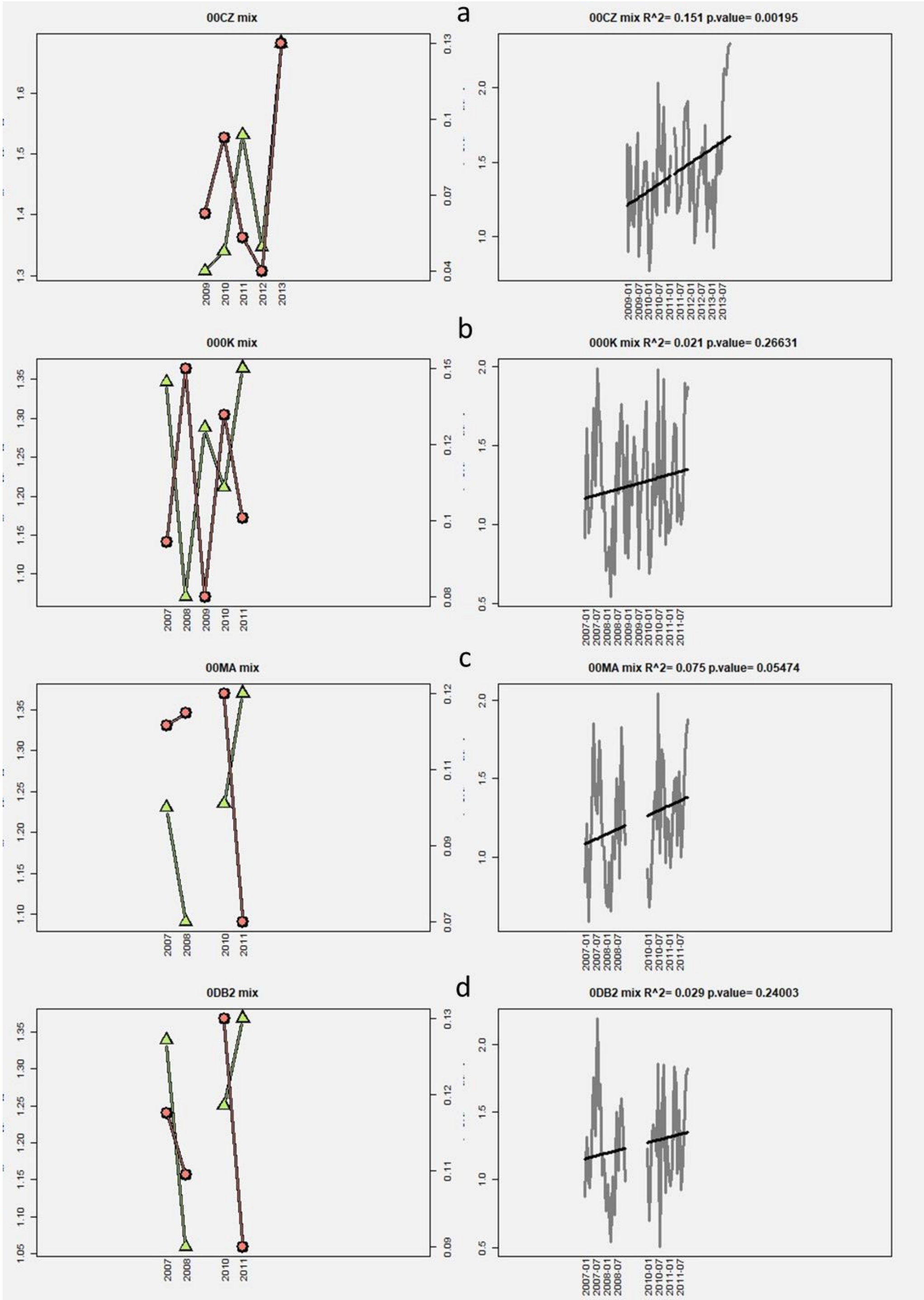

Figure S39: Shannon - Wiener's diversity index in the middle layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

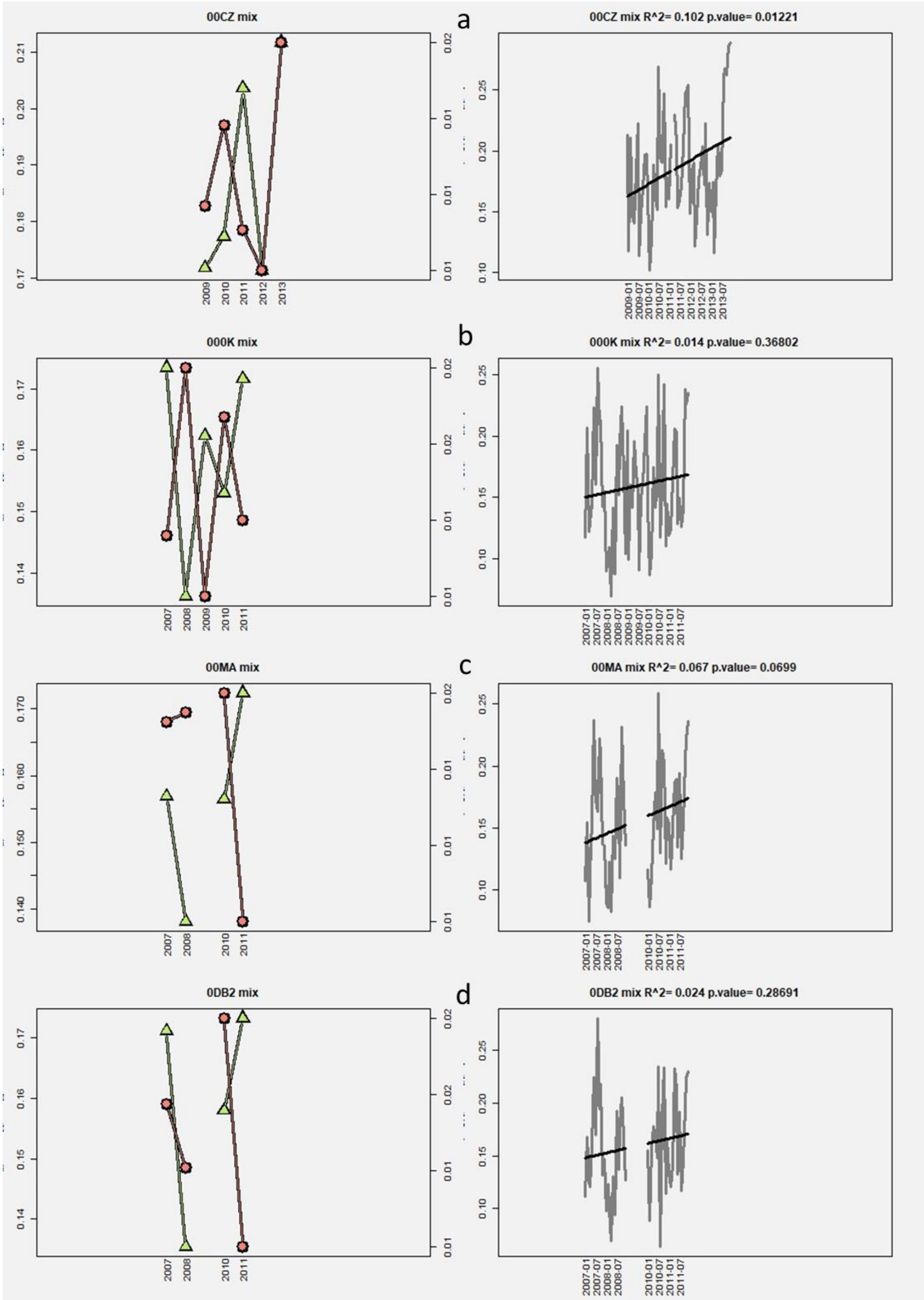

Figure S40: Pielou's evenness in the middle layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

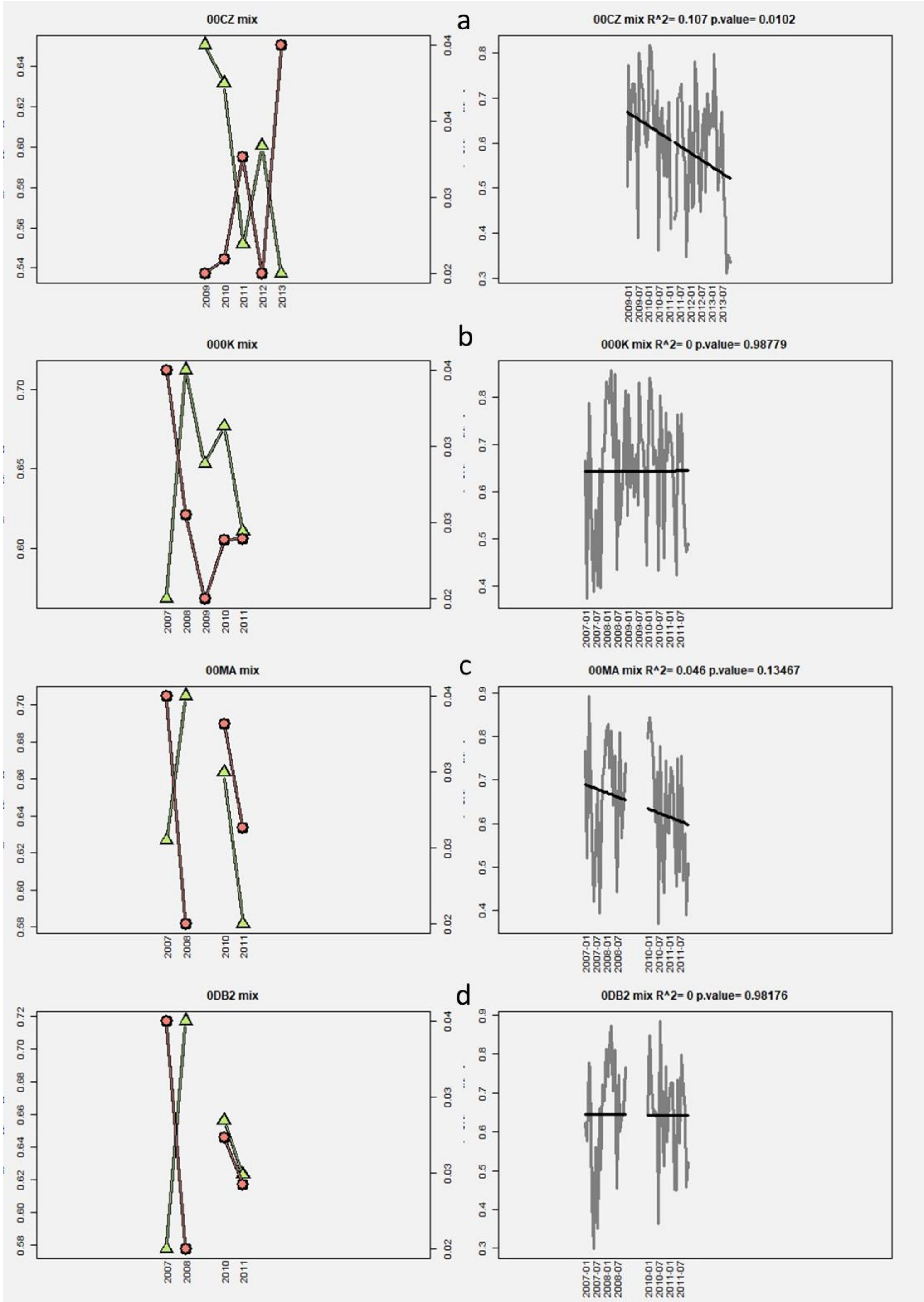

Figure S41: Berger-Parker's dominance in the middle layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

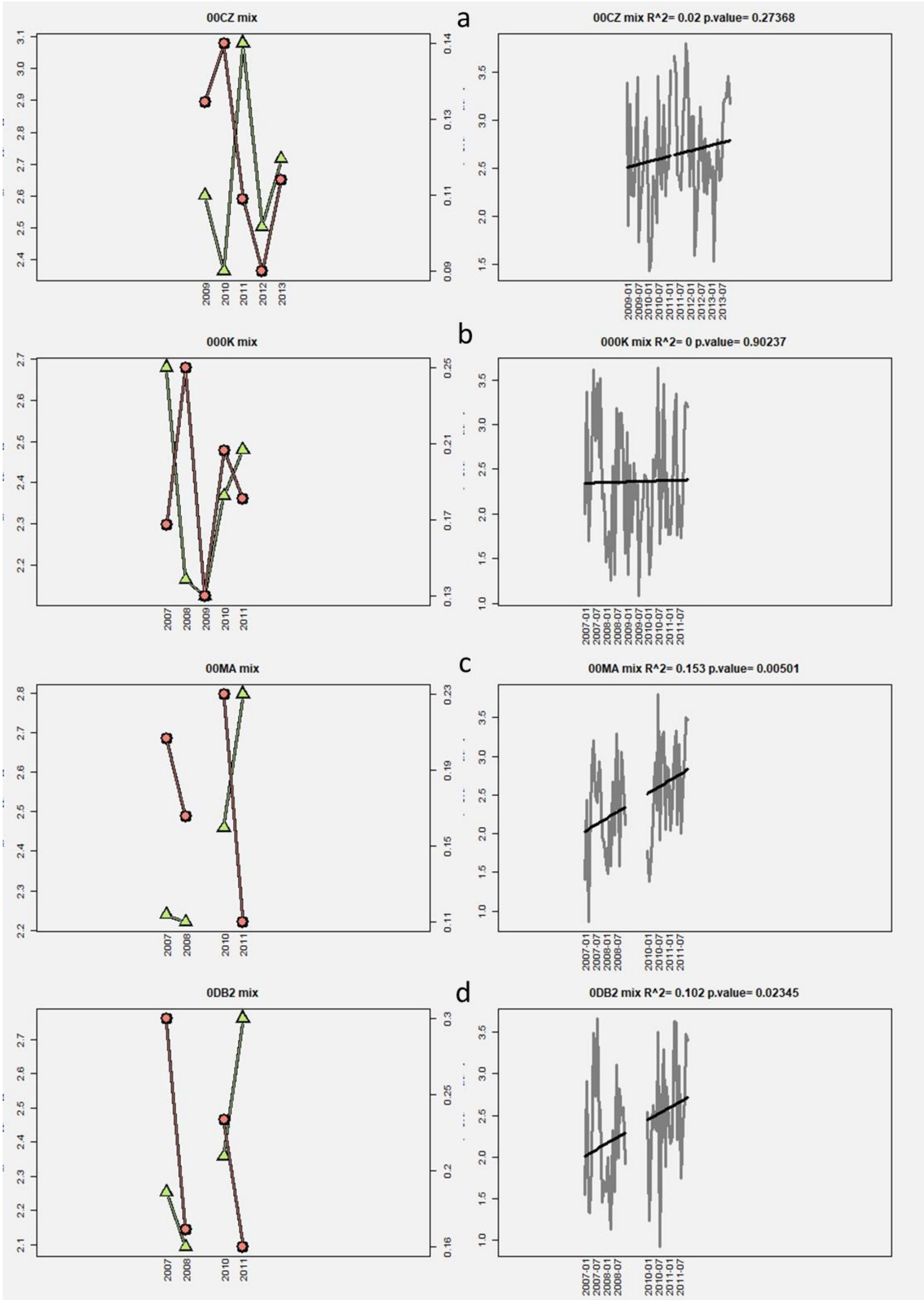

Figure S42: Rao's index in the middle layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

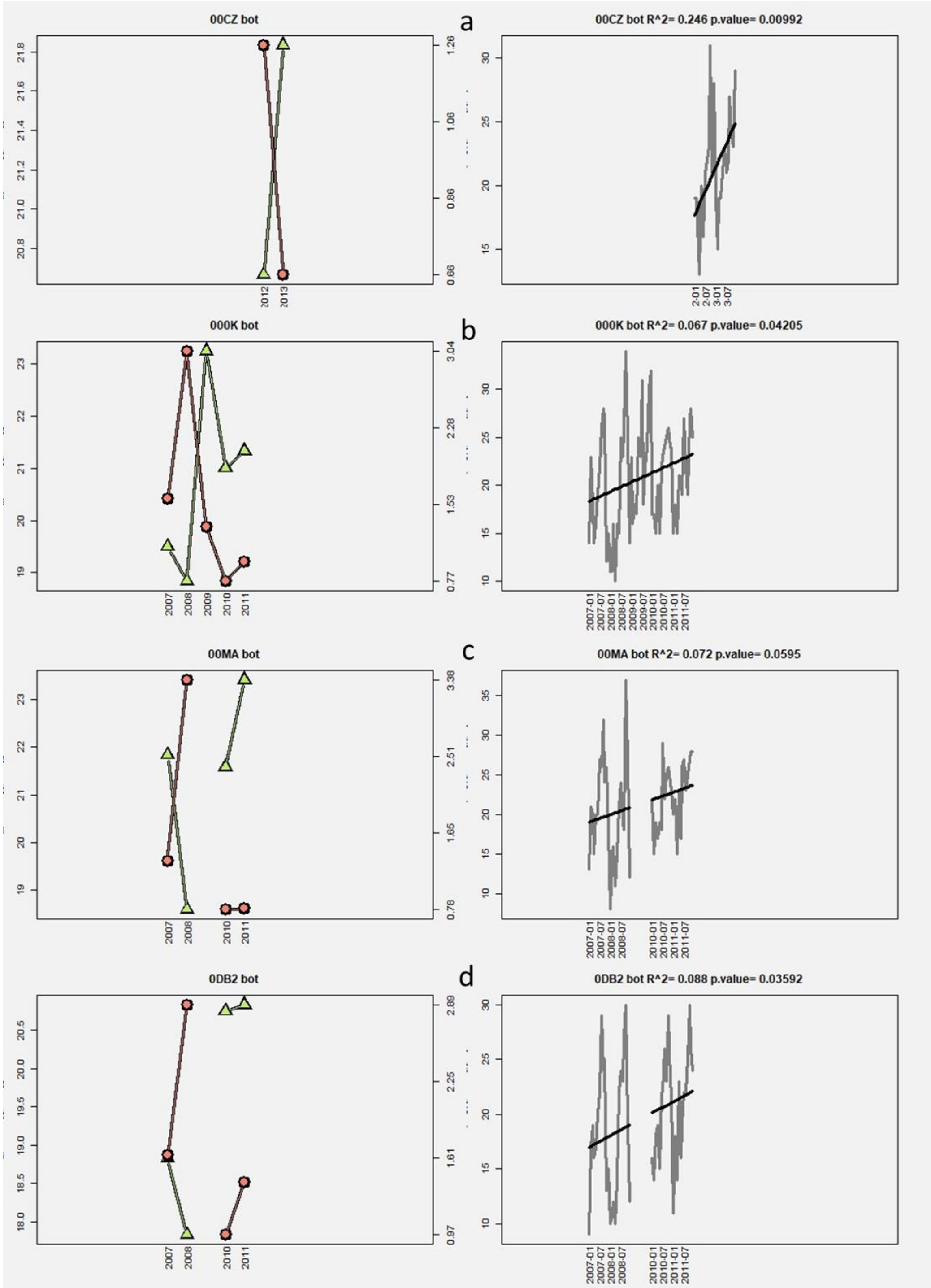

Figure S43: Genus richness in the bottom layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

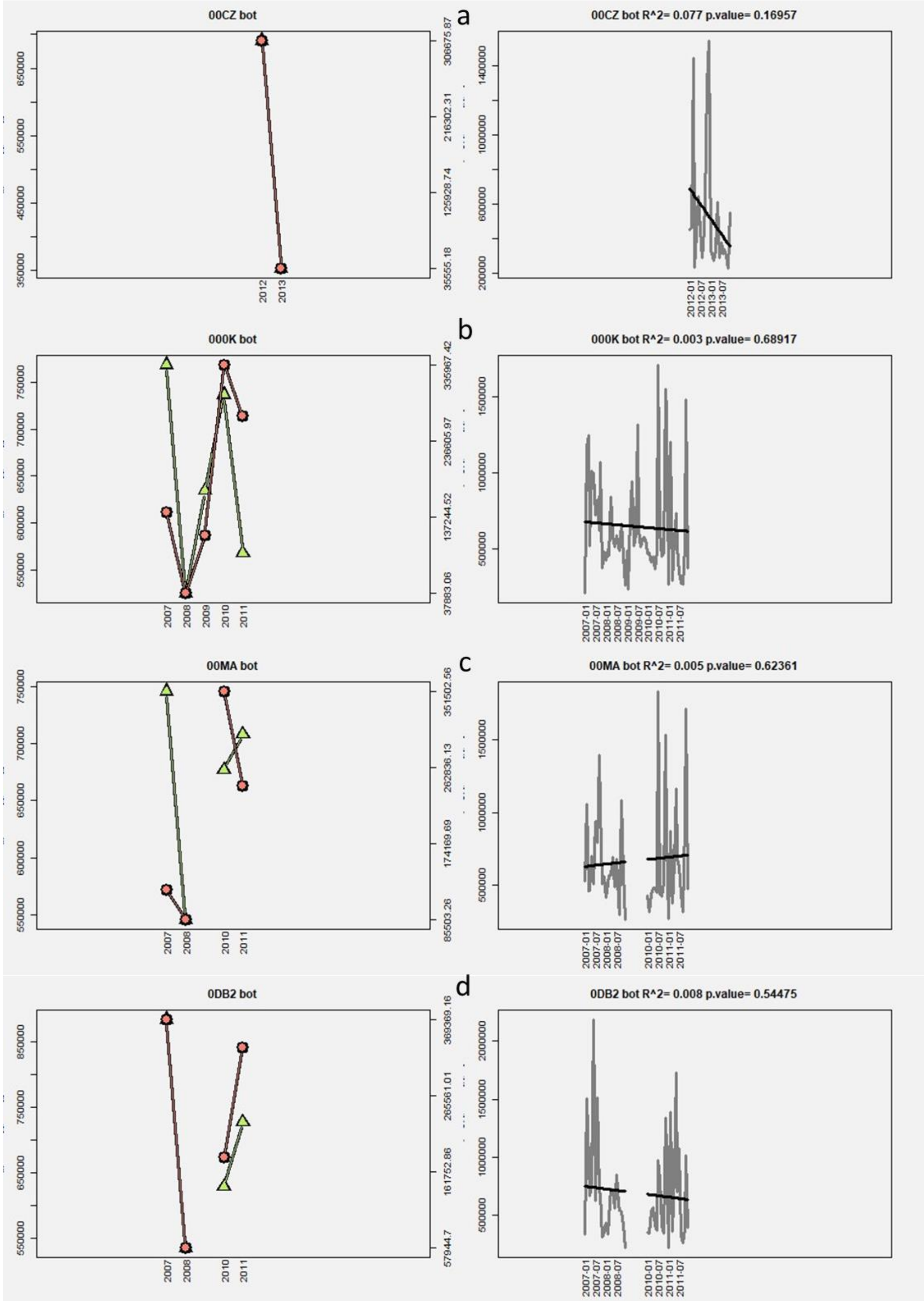

Figure S45: Phytoplankton abundance in the bottom layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

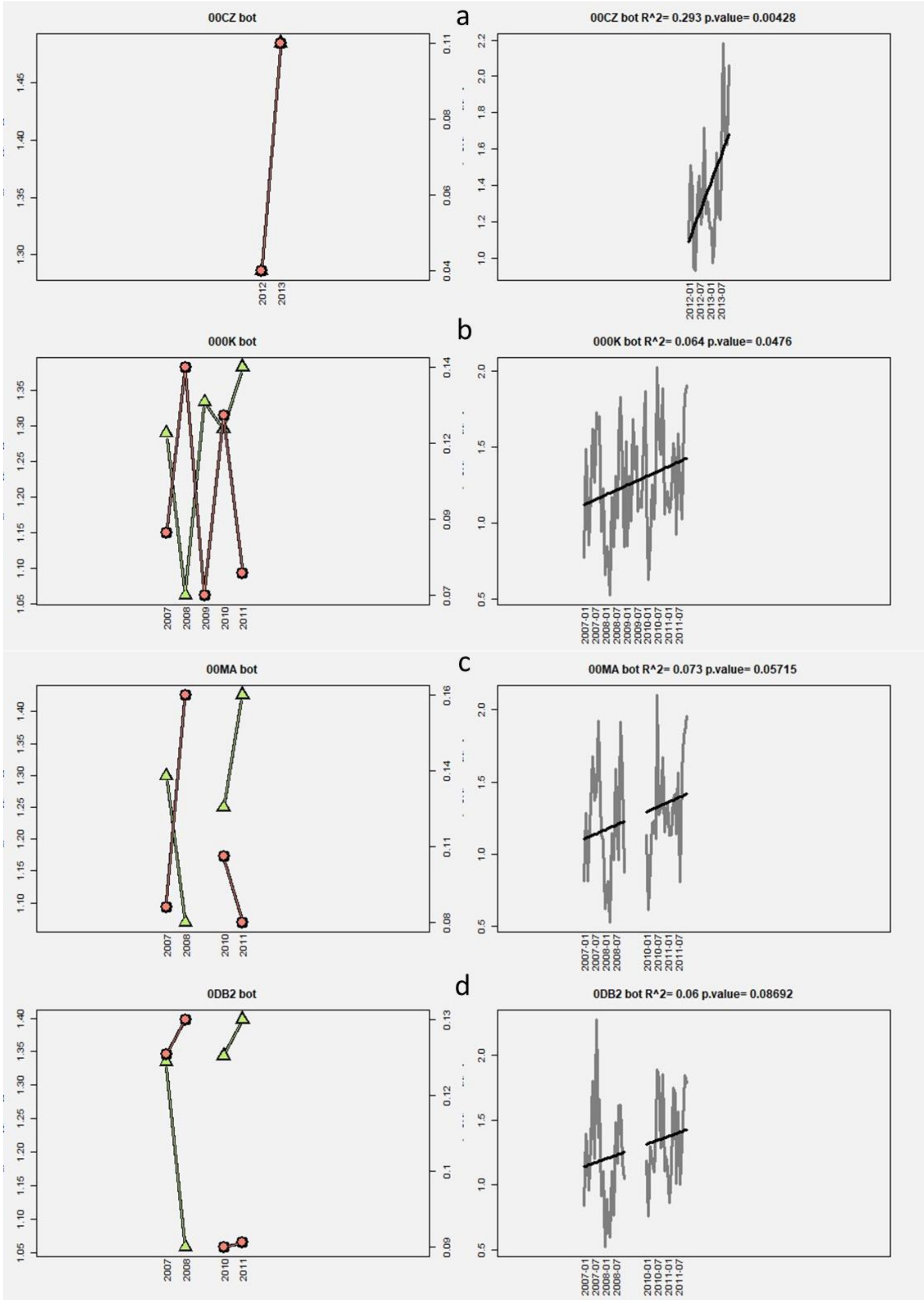

Figure S46: Shannon - Wiener's diversity index in the bottom layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

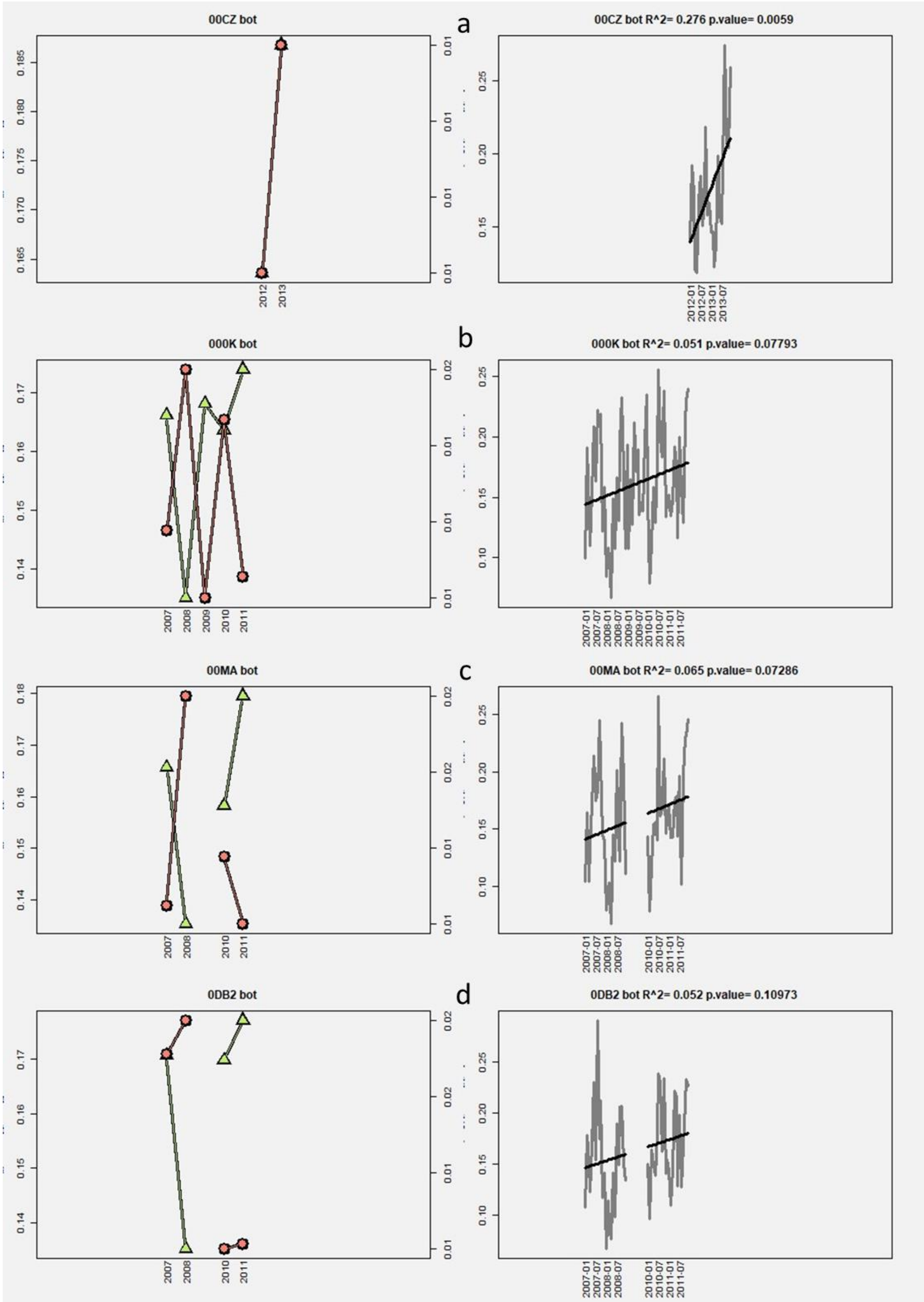

Figure S47: Pielou's evenness in the bottom layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

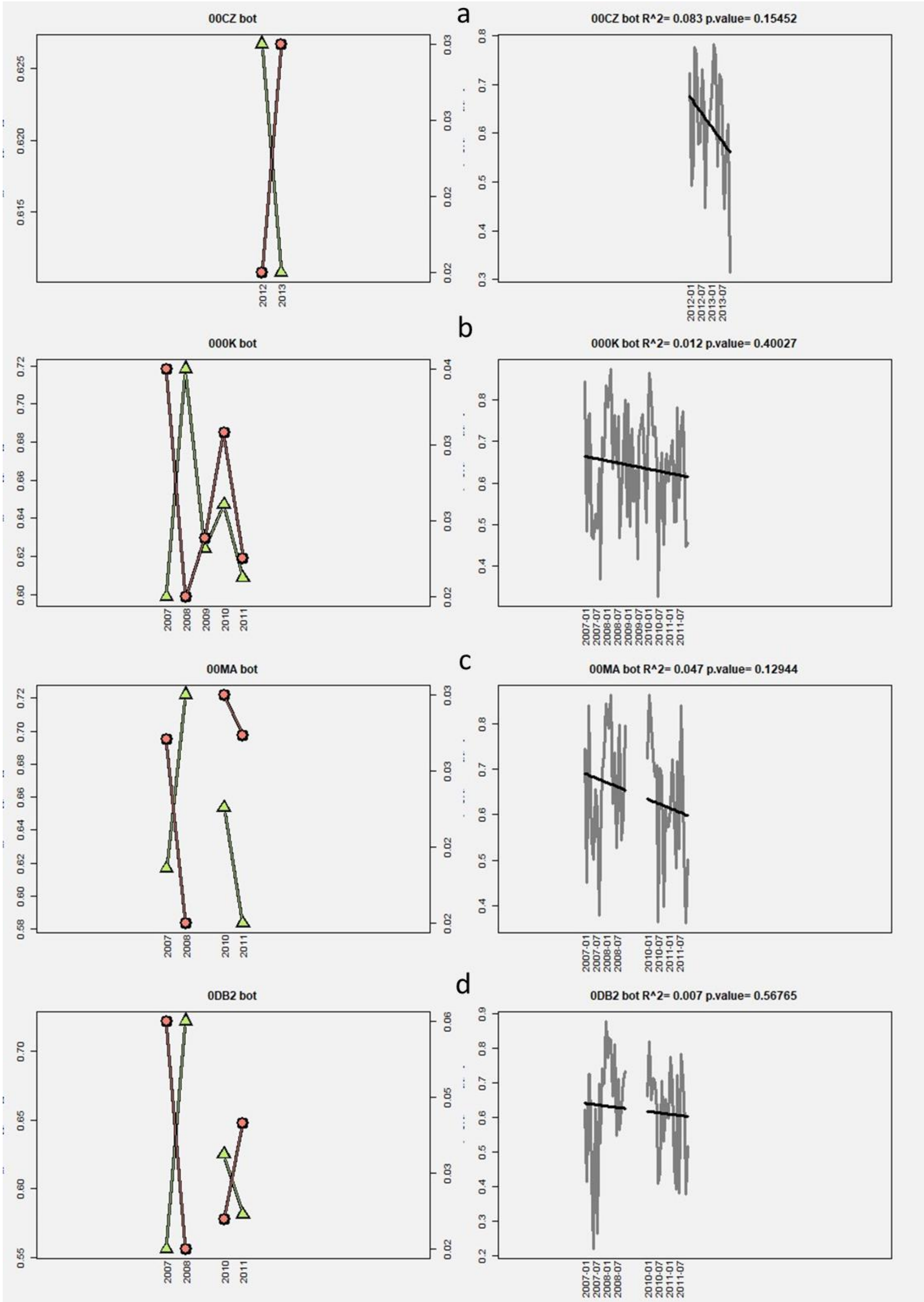

Figure S48: Berger-Parker's dominance in the bottom layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model

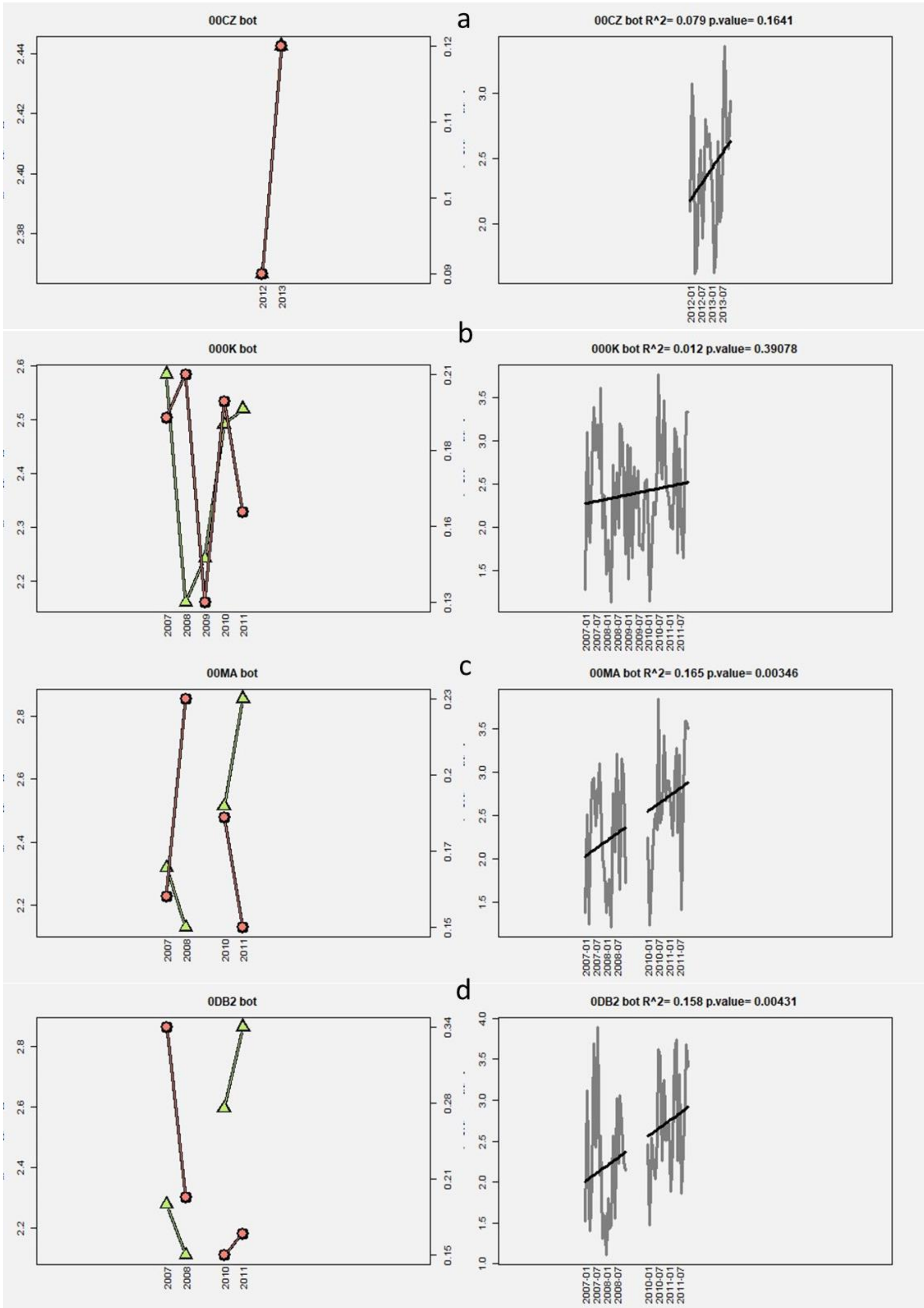

Figure S49: Rao's index in the bottom layer of stations (a) 00CZ, (b) 000K, (c) 00MA and (d) 0DB2 in the period 2007-2011. Left panels: annual means (green triangles, left y axis), COV (red circles, right y axis); Right panels: linear model www.abiommed.eu

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