The effects of hydrological extremes on denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and mineralization in a coastal lagoon

Original
The effects of hydrological extremes on denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and mineralization in a coastal lagoon / Magri, M.; Benelli, S.; Bonaglia, S.; Zilius, M.; Castaldelli, G.; Bartoli, M.. - In: SCIENCE OF THE TOTAL ENVIRONMENT. - ISSN 0048-9697. - 740:(2020), p. 140169. [10.1016/j.scitotenv.2020.140169]

Availability:
This version is available at: 11381/2881559 since: 2024-12-14T17:37:35Z

Publisher:
Elsevier B.V.

Published
DOI:10.1016/j.scitotenv.2020.140169

Elsevier Editorial System(tm) for Science of the Total Environment
Manuscript Draft

Manuscript Number: STOTEN-D-20-07243R1

Title: The effects of hydrological extremes on denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and mineralization in a coastal lagoon

Article Type: Research Paper

Keywords: coastal lagoon, hydrological extremes, benthic fluxes, nitrate respiration, nitrogen recycling

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Abstract: Hydrological extremes of unusually high or low river discharge may deeply affect the biogeochemistry of coastal lagoons, but the effects are poorly explored. In this study, microbial nitrogen processes were analyzed through intact core incubations and 15N-isotope addition at three sites in the eutrophic Sacca di Goro lagoon (Northern Adriatic Sea) both under high discharge (spring) and after prolonged low discharge (late-summer) of the main freshwater inputs.
Under high discharge/nitrate load, denitrification was the leading process and there was no internal recycling. The site located at the mouth of the main freshwater input and characterized by low salinity exhibited the highest denitrification rate (up to 1150 ± 81 µmol N m-2 h-1), mostly sustained by nitrification stimulated by burrowing macrofauna. In contrast, we recorded high internal recycling under low discharge, when denitrification dropped at all sites due to low nitrate concentrations, reduced bioturbation and nitrification. The highest recycling was measured at the sites close to the sea entrance and characterized by high salinity and particularly at the clams cultivated area (up to 1003 ± 70 µmol N m-2 h-1). At this site, internal recycling was sustained by ammonification of biodeposits, bivalve excretion and dissimilatory nitrate reduction to ammonium (DNRA), which represented 30% of nitrate reduction.
Flash floods and high nitrate loads may overwhelm the denitrification capacity of the lagoon due to the reduced residence time and to the saturation of microbial enzymatic activity, resulting in high transport of nitrate to the sea. Prolonged dry periods favor large internal recycling, due to a combination of high temperatures, low oxygen solubility and low bioturbation, which may prolong the extent of algal blooms with negative effects on lagoon biogeochemical services. We conclude that hydrological extremes, which are expected to become more frequent under climate change scenarios, strongly alter N cycling in coastal sediments.

Response to Reviewers: Dear Editorial Office of Science of Total Environment,
We are pleased to resubmit the revised version of our manuscript "The effects of hydrological extremes on denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and mineralization in a coastal lagoon". This version incorporates the suggestions provided by three anonymous reviewers, whom we would like to thank for their constructive analysis of our work. In general, we appreciate the reviewers' efforts in improving the manuscript and at the same time we are happy to read their acknowledgment of the study's merit. We have addressed their comments point-by-point and we present our answers (in Italic) below their original comments.

With best regards,
Monia Magri and co-authors

Reviewer #2:
Add the location of where the experiment was conducted (name + country) in the abstract.
Answer: We added the location of the study area in the abstract (Line 27).

The introduction would benefit from having a conceptual model showing the interlink between the different nitrogen pathways and how factors may influence each of them.
Answer: We added a conceptual model showing expected changes of benthic N cycling induced by hydrological extremes in coastal lagoons (Fig. 1). We hope this picture will help to clarify the different topics covered in the introduction.
The different pathways could also benefit from being developed further in the introductions.
Answer: We improved the description of the pathways in the conceptual model.

You should also have a section about microbes. You are mentioning investigating microbes L98 but it is a little bit out of nowhere since before you have a focus on benthic invertebrates.
Answer: we tackled our working hypotheses with a biogeochemical approach and as we stated "we analyzed microbial N transformations" (and not microbial communities) by means of 15N-based techniques, that allow to measure accurately microbially-mediated processes like nitrification, denitrification and nitrate ammonification, and by means of oxygen and inorganic N fluxes, that can be converted into rates of ammonification. Based on previous works targeting the effects of macrofauna on benthic fluxes (e.g. Welsh et al., 2015; Murphy et al., 2018), we were also able to partition some of the measured fluxes in the contribution of microbes and macrofauna, as in the clams farmed station. We are aware of increasing number of papers combining microbial community characterization via molecular tools and biogeochemical measurements; such approach may represent a follow-up of the present study.

In your methods you mention the importance of clams. Shouldn't this also be in the introduction as well as a description of their influence on the biogeochemical processes?
Answer: the importance of macrofauna activity in benthic N cycling is well known in the literature and the effects of clams on N biogeochemistry were analyzed in detail in the Sacca di Goro in different papers we cited (Nizzoli et al., 2006; Viaroli et al., 2006; Welsh et

al., 2015; Murphy et al., 2018). One of the 3 sampling stations that we investigated is cultivated with clams as this activity occurs over nearly 30% of the lagoon surface and cannot be neglected. However, clams are not central in our story, which rather targets the effects produced by hydrological extremes on benthic N cycling. We demonstrated that the seasonal shift from the dominance of removal to the dominance of recycling occurs at all sites, regardless the presence of clams or other macrofauna.

L124 Up to 10 what?
Answer: Salinity was measured by means of a YSI 556 multiple probe and values were determined from electrical conductivity and from the estimate of the ionic content, according to the practical salinity scale 1978 (PSS-78). The practical salinity, defined as the ratio of the conductivity of a sample of seawater to the conductivity of a special reference material called IAPSO Standard Seawater, has no units. The suffix PSU (practical salinity unit) is sometimes added but it is formally incorrect.

L152-153 What is the minimum distance between two sampling points?
Answer: We added in the test the distance among sampling sites, ranging from 1.5 to 4.5 Km (lines 161-162).

L153 Why do you have different sampling efforts between summer and spring
Answer: During the spring campaign we had some technical constraints, due also to the bad weather. Despite this, please note that according to the protocol that we followed, a minimum of 4 replicates is considered adequate, since “measurements of fluxes and denitrification are carried out on a minimum of 3 parallel cores” (Dalsgaard et al., 2000).

L158 Add the manufacturer etc… in brackets for the YSI
Answer: Done (Line 177).

L160 How were the core preserved until they reached the lab. A few hours is a long time when it comes to microbial activity.
Answer: We detailed in the Material and Methods section (lines 178-182) that the cores, after collection, were immediately submerged with the top open in a box filled with in situ water cooled with ice packs to slow microbial activity. Within 1-2 hours from collection they were submersed in large tanks at the Centre for Mollusc Research, Goro (Ferrara, Italy), which is <1 km from the harbor. The tanks were filled with aerated and well-mixed water from the three stations, maintained at in situ temperature and the cores were preincubated overnight. The procedure we have followed minimizes sediment disturbance, does not alter microbial activity and is standard for core incubation (Dalsgaard et al., 2000).

L161-164 Do you have any reference for this protocol? Also, how were the cores distributed in the tanks. I am pretty sure you used different tanks for different sites but maybe make it slightly clearer.
Answer: We added more details on the pre-incubation phase (lines 181-182). Once in laboratory, the cores were placed into three large tanks, one for each site, containing renewed water, at in situ O2 concentration and temperature. The reference for the protocol we used to plan the pre-incubation and the incubation phases was already reported (Dalsgaard et al., 2000) and was produced during a European project targeting the measurement of N-related microbial activities in different estuaries.

L174 Maybe add a reference to the standard protocol

Answer: For each analytical method we reported the reference. We added a reference of a collection of Standard Methods containing all the procedures that we used for the water samples analysis (APHA, 1992, line 200).

L225 What was the taxonomic resolution? How did you identify (add the key that you used, microscope and amplification)? How did you get the biomass (add the protocol)?
Answer: We added some details in the text (Lines 252-258). Organisms were retrieved from the sediments and sorted under stereomicroscope (Leica S8 APO, amplification 8x). The organisms were identified by dichotomous keys (http://species-identification.org/identify_species.php) and by scientific papers (Wagele, 1981) to the lowest possible taxonomic level and counted. The identification was strengthened by the comparison with previous studies on the macrobenthic community of the Sacca di Goro (Mistri et al., 2001; Ludovisi et al., 2013; Politi et al., 2019). For each species the dry weight was determined after drying at 80°C for 48 h. For the clams, shells were removed, and only flesh weight was measured.

Why did you not look at the microbial community? You said in your introduction that you were interested in microbial processes and yet you do nothing in that direction. What is the reason for that? I feel like your work would have benefitted at lot from assessing microbial biomass, densities and activity at a minor cost.
Answer: We agree that it would have been extremely interesting to analyze the microbial communities in the two sampling periods, but as we explained earlier, we used a biogeochemical approach to focus on microbial processes.

L246-249 It would be good to have a reference as well. It seems like a big approximation to me as nutrient loads can vary a lot over very short time periods. I wonder if modelling methods such as SWAT (or others) would not have been better to get the nutrient load rather than extrapolating data from a single sampling event for each season.
Answer: We partially agree as our group of research in Parma and Ferrara started monitoring the Sacca di Goro and the nutrient loads to this system since 1986 and produced a large body of literature including recent papers reporting the nutrient loads delivered in the last decades to the Po River Delta (Viaroli et al., 2018), loads generated during high discharge periods (Naldi et al 2010) and nutrients delivered by the Po di Volano to the Goro Lagoon (Castaldelli et al., 2013, 2020). We acknowledge that loads undergo large variations in the short-term but the hydrology of the Volano basin, which is the main nutrient source to the system is regulated as it lays below sea level and discharge is monitored and known. Loads reported in our work overlap seasonal loads reported in Castaldelli et al (2013); we added these aspects (lines 378-384)

What were the incubation temperature for all your measurements (including acclimation phase)? Was there a dark-light cycle during the incubation phase?
Answer: We detailed that overnight preincubation and incubation occurred at in situ temperatures (Table 1) and in the dark as only heterotrophic processes were measured (lines 180-187).

L279 What error measurement did you use?
Answer: density values of macrofauna were displayed as averages ± standard errors, we added the measurement in the text (Line 312).

Fig 5 is hard to read
Answer: The reviewers had different opinions about our figure (from hard to read to very clear). We have now improved it by increasing characters to the possible maximum.

Table 3 seems to have a number problem in the last significant P value reported (N2 season x site)
Answer: We are sorry, but we didn’t understand what exactly the reviewer meant in this comment. We have run again the two-way ANOVA and the number is correct.

Reviewer #4:
Highlights
The authors need to define as early as possible their definition of "N recycling", and "N regeneration" which could mean a number of things. I think in this case is the sum of the measured processes such as nitrification, mineralization, and DNRA, but for some people it could mean other things such as algal or animal uptake and excretion.
Answer: We agree with the reviewer and we clearly stated that with “recycling” we mean the fluxes of inorganic nitrogen (NH4+, NO2- and NO3-) from the benthic system to the water column. As such, they include the net result of processes as ammonification, nitrification, denitrification, DNRA and excretion by macrofauna (lines 84-87). We removed “regeneration” to avoid confusion.
Introduction
L109: It would be interesting to emphasize how "unusual' this year was, for instance, "precipitation which was 30% higher than long-term measurements" instead of "heavy precipitation" or "Low river discharge"
Answer: We agree and we added that a) May 2019 was characterized by unusually heavy rainfall with value of cumulative precipitation (mm) nearly 2.5 times higher than values measured from 2000 to 2018, b) during summer, in the Po River basin, the temperature shows a clear increasing trend from the 1970s (Brunetti et al.,2006) and c) during the summer of 2019, the average daily water temperature exceeded 30°C for 8 days, compared to what recorded from 2006 to 2018, when this period was limited to 4 days (lines 116-122).

L112: Is it salinity really the determinant factor? Or is salinity just an indicator of the influence of marine water, which is characterized by low nutrient concentrations and high S?
Answer: Both aspects are important. Salinity is recognized to influence benthic N dynamics, decreasing nitrification and denitrification processes, and favoring DNRA, mainly due to higher sulfate reduction rates that increase concentrations of sulfides in the pore-water environment, which directly impact DNRA (An and Gardner, 2002; Gardner et al., 2006; Giblin et al., 2010; Caffrey et al., 2019). But salinity directly limits coupled nitrification-denitrification due to inhibitory physiological effects on nitrifiers and denitrifiers (Rysgaard et al., 1999) and determines a decrease in nitrification rates and an increase in NH4+ effluxes due to sediment desorption (Gardner et al., 1991). At the same time NO3- concentration influences the proportion of denitrification and DNRA rates, with the latter favored at low concentration, due to a thermodynamic advantage, since reduction of nitrate to ammonium accommodates 8 electrons compared to the 5 received by denitrification (Tiedje, 1988; Nizzoli et al., 2010). We were not able to discriminate between NO3- concentration and salinity, because both these factors

varied along the seaward trajectories (the first decreased, whereas the latter increased).

L115: "loss" is mentioned twice in the third hypothesis.
Answer: we changed this term (line 126).

Methods
L139: What is "high temperature" in this site" ? range?
Answer: We agree with the reviewer, we reformulated this sentence that was not very clear. We clarified that the eastern portion of the lagoon, mainly during the summer season, is characterized by stagnant water and by temperatures usually higher than in the rest of the lagoon (Lines 153-154).

L159: 80L of water were collected
Answer: we corrected the units (line 178).

176: Define MIMS
Answer: we defined the acronym MIMS (membrane inlet mass spectrometer) in the text (lines 196-197).

L209: Do you mean effluxes from the sediment to the water column?
Answer: That’s was exactly what we meant, we added a better definition in the text (lines 235-236).

L231: Define "C"
Answer: We defined C as carbon in the text (line 264).

L231: Isotopes are "values" not "signatures", because they are not constant.
Answer: We thank the reviewer for this clarification, we corrected the term in the text (line 264).

Results
L271: It doesn't look like FA has higher NH4 concentrations in the summer in Fig. 2 as stated in the text
Answer: We reformulated the sentence to clarify the graph explanation (lines 304-309).

L274: "seasons"
Answer: we corrected the term (line 307).

L306: Comma after "spring"
Answer: Added (line 340).

Discussion
L345: What do you mean by "microphytobenthos activity", do you mean nitrogen uptake or photosynthesis, or both?
Answer: We mean both. Microphytobenthos inhibits N dissimilative pathways both through photosynthetic activity and nutrient uptake. The competition for N, mainly as NH_4^+, determines a decrease in nitrification and coupled nitrification-denitrification rates (Sundbäck et al., 2000). At the same time, the expansion of the oxic layer, due to microphytobenthos O_2 production, reduces denitrification of water column NO_3^- (Dw) due to increased diffusion pathlength to reach the anoxic sediment horizon (Bartoli et al., 2003).

L358: small amount of NO_3 being reduced

Answer: we thank the reviewer and corrected the form (line 402).

L379: Please explain here whether is in fact salinity or is it the NO3, and reductant sulphides that drive the changes in N processing.
Answer: as we explained above, both salinity and NO3- concentration may determine variations in the relative proportion of DNRA and denitrification as NO3- reduction pathways and we were not able to discriminate between these two factors, as they both varied along the seaward gradient.
In this study we did not measure sulfides concentration but results of previous studies displayed a zonation in the buffering capacity against dissolved sulfides accumulation in the Sacca di Goro lagoon. The western corner, where station Giralda is located, is characterized by a high buffer capacity that is related to the abundance of electron acceptors alternative to sulfate, as NO3-, Mn4+ and Fe3+ delivered from river, and to high bioturbation, resulting in deep penetration of O2 and NO3- within sediments (Giordani et al., 1996; Azzoni et al., 2005; Zilius et al., 2015). There are different areas of the lagoon, as the northern corner or the sheltered Valle di Gorino, where sediments are reduced and devoid of macrofauna, especially during summer macroalgal blooms that determine a high load of organic matter, and energetically favorable electron acceptors such as O2 or NO3- are rapidly exhausted. In these conditions sulfate reduction and sulfides release increase (Zilius et al., 2015). The two marine sites analyzed in our study, Gorino and Farmed Area, are located close to the sea mouth, in an area subject to tidal influence. For this reason, despite the high salinity and sulfate availability and biodeposition of faeces and preudofaeces by cultivated clams, these sites are characterized by high hydrodynamic conditions which may prevent the accumulation of organic matter and favor oxygenation at the water-sediment interface, thus partially contrasting the build-up of sulfides (Giordani et al., 1996, 1997; Azzoni et al., 2005).

L391: Not sure what do you mean by "contrasted the effects"
Answer: we reformulated the sentence (Lines 434-437).

L393: Add comma after "summer"
Answer: we added it (line 411).

L400: Explain a bit further how you got to this conclusion, my guess is that you considered terrestrial plants to be -27ppm of 13C versus marine phytoplankton, which is usually around -20 ppm
Answer: At Giralda the high organic load was derived mainly from settled particles of fluvial origin, as demonstrated by the higher C:N ratio and by the lower C and N isotopic values, within the range reported for terrestrial organic matter (~-27‰ and of 3‰ for δ13C and δ15N, respectively, Lamb et al., 2006). However, even during the high discharge period, the C:N ratio of Giralda surface sediments suggested high organic matter quality, whereas material of terrestrial origins usually displays values significantly above 12 (Yamamuro, 2000). Gorino and Farmed Area were characterized by C:N ratios closed to the Redfield one and by higher δ13C and δ15N values, closer to values reported for marine systems, suggesting a progressive increase in the proportion of organic matter from autochthonous origins (Yamamuro, 2000; Liu, 2006). The isotopic values, however, were more depleted compared to marine phytoplankton, particularly relative to δ13C values, usually ranging from -22 to -19 ‰ (Lamb et al., 2006), suggesting that sedimentary organic matter still derived from the mixture of terrestrial derived material and marine material and from the accumulation of clam biodeposits (δ13C value of

about -23.2 ‰, Mazzola and Sarà, 2001). We added the explanation in the text (Lines 443-457).

L421: Not sure what you mean by "lagoon aging", please explain.
Answer: We expected that the organic enrichment due to more than 30 years of aquaculture activity and to macroalgal blooms, have permanently affected benthic dynamics. We expected an increasing trend in O2 sediment uptake rates due to enhanced microbial activity and a decreasing trend in denitrification rates, since under highly reduced and sulfidic conditions nitrogen cycling becomes controlled by dissimilative nitrate reduction to ammonium instead of denitrification. However, long-term trends are not clearly visible, probably due to the frequent silting operations within the lagoon and the increased frequency of heavy rainfall events, which contributed to limit the organic matter accumulation.

L422: Please define IPT in the Methodology, or just write here the full name of the methodology (isotope pairing technique).
Answer: we added it (line 209).

L430: I don't think "addressed" is the best word here, maybe "associated"?
Answer: we agree with the reviewer and replaced the word (line 481).

L432: I guess it would make it more variable, not necessarily reduce it, as it would be increased in some events, and decreased in others.
Answer: we partially agree, as we demonstrated that under hydrological extremes there is a decrease in the efficiency of the lagoon to act as N filter. Particularly, under high discharge/high nitrate periods denitrification can be saturated and its efficiency decrease whereas under low discharge recycling may mobilize large amounts of ammonium that may be exported to the open sea or fuel primary production activity. We replaced the sentence "future change in climatic conditions" with "hydrological extremes", which are the focus of our study (lines 483-485).

Conclusion
L518: As it is written now, the sentence implies that denitrification will be exported to the Adriatic Sea, please rewrite.
Answer: We reformulated the sentence (Lines 567-569).

Tables
Table 3-4 could be considered for supplementary Material. Table 6 could definitely be incorporated in the text
Answer: we agree with the reviewer. We moved Table 3 and 4 to supplementary material and incorporated table 6 in the text.

Figures
The font in Figure 5 is too small. This diagrams are great, but maybe just have two one for spring and one for summer? The rest could be in supplementary material.

Answer: The reviewers had different opinions about our figure (from hard to read to very clear). We have now improved it by increasing characters to the possible maximum.

Reviewer#1
Title:

The effects of hydrological extremes on denitrification, DNRAdissimilatory nitrate reduction to ammonium, and mineralization in a coastal lagoon
Answer: we defined DNRA in the title, enclosing the acronym in brackets.

Graphical abstract:
I am surprised that there is no NH4+ during the spring time. It is true that ammonium is less mobile in soils but there are always excess NH4+. It could be much less than NO3-. Although, there is usually no positive correlation with water discharge and NH4+ like NO3-.
Answer: The reviewer is right, in the previous version we stressed only nitrate due to large seasonal variation, we have now added NH4+ and the relevance of its recycling.

What do you mean by PN? Particulate nitrogen?
Answer: we defined the term in the figure.

Change DNRA to real words in the graphic as in the title.
Answer: we defined the acronym in the title, but we think that the figure may result hard to read if we will add further text.

Highlights:
Hydrological extremes deeply alter benthic N cycling in coastal lagoonstransitional areas
Answer: we substituted the term.

Abstract:
Hydrological extremes of unusually high or low river discharge may have profound effects on biogeochemistry of coastal lagoons zones, but such effects are poorly explored. Coastal Zone is a very subject. Coastal lagoons are very small part of the subject.
Answer: we agree with the reviewer and substituted the term.

27- freshwater and two marine ones) in a eutrophic lagoon both under high discharge (spring) and after….
Your values of salinity does not indicate any freshwater. It is all brackish water. Please show some data about freshwater salinity.
Answer: we thank the reviewer for this correction, we removed from the text all references to the stations as freshwater or marine sites.

29- Under high discharge/nitrate load, denitrification was the leading process and little to no recycling was observed.Do you mean little or no INTERNAL recycling was observed? In the larger picture, the recycling is always going on in biogeochemical processes. The rates are just different

Answer: According to another comment by another reviewer we defined internal "recycling" as the sum of fluxes of inorganic nitrogen (NH4+, NO2- and NO3-) from the benthic system to the water column. As such, they include the net result of processes as ammonification, nitrification, denitrification, DNRA and excretion by macrofauna (lines 84-87). In spring DIN fluxes were negative (directed from the water column to sediments) at all three sites, so we stated that there was no internal recycling (line 29).

31 mostly sustained by nitrification stimulated by burrowing macrofauna.It might be true, but justify it in your introduction by showing some evidence or some references

Answer: Denitrification coupled to nitrification and its stimulation by burrowing macrofauna was extensively studied in the Sacca di Goro and in other coastal areas; we added appropriate references related to Corophium insidiosum and Neantes succinea (Pelegri and Blackburn, 1994; Nizzoli et al., 2007; Moraes et al., 2018).

32 recycling under low discharge,-AGAIN, DO YOU MEAN INTERNAL CYCLING?

Answer: yes, we specified it in the text (line 32).

33 reduced bioturbation and nitrification. The highest recycling was measured at the marine sites. Please use a better term that marine sites. This research is about a coastal lagoon.

Answer: we agree and reformulated the sentence that now reads: "The highest recycling was measured at the sites located close to the sea entrance and characterized by the higher salinity and particularly at the clams cultivated area" (lines 33-35).

43 under climate change scenarios, strongly alter N cycling RATES in coastal sediments lagoons. It might be true under climate change scenarios, but this is another topic. Climate change is not evident everywhere. However, there are many strong indications about it in many places. Do you have any real proof about it in your area?

Answer: In the introduction we report some of the ongoing and expected climatic changes in the area of the Po River basin (lines 110-115), including increase in average and maximum temperatures, a general decline in runoff (30-40% reduction), mainly in summer and an increase in hydrological extremes, with prolonged droughts and peaks of river discharge due to more severe and less frequent but more intense precipitation. All the papers that we cite in the introduction are relative to the Italian territory or specifically to the Po River and the North Adriatic area (Coppola and Giorgi, 2010; Tibaldi et al., 2010; Cozzi and Giani, 2011; Vezzoli et al., 2015).

38 -Flash floods and high nitrate may offset denitrification due to reduced residence time and saturation of 39-microbial enzymatic activity, (38-39 are not very clear) resulting in high transport of nitrate to the open sea.

Answer: we reformulated the sentence that now reads:
Flash floods and high nitrate load may overwhelm the denitrification capacity of the lagoon due to the reduced residence time and to the saturation of microbial enzymatic activity, resulting in high transport of nitrate to the open sea (lines 38-40).

Prolonged dry periods 40-favor large N regeneration by(in)sediments, due to combination of high temperatures, low oxygen solubility and 41 low bioturbation, which may prolong the extent of algal blooms with negative feedbacks (Please check the definition of positive and negative feedback-I think it should be a positive feedback here. It seems that you are trying to say that in worsen the situation. Is it what you are trying to say?)for the lagoon 42 biogeochemical services.

Answer: the reviewer is right, it is a positive feedback and we modified the sentence (Lines 41-42).

Keywords: nitrogen, sedimentary fluxes, estuaries, climate extremes, nitrogen loss, nitrogen recycling
-By definition of estuaries, your study site is not an estuary. There is no tide

Answer: We partially agree, our study area is a microtidal coastal lagoon. We replaced the keyword "estuary" with "coastal lagoon"

-Nitrogen is a very general word. You can use some more specific words from your paper.

Answer: We agree and we have replaced the keyword (line 45).

-Sedimentary flux also could be anything in sedimentary processes

Answer: We agree and we have replaced sedimentary fluxes with benthic fluxes, that is more appropriate keyword for studies analysing the exchange of solutes across the sediment-water interface (line 45).

Introduction:
47 Human activities, through increased fertilizer production and combustion, have more than doubled the loadingof bioavailable nitrogen (N) to coastal areas (37-66 Tg total N yr-148),that have led(or leaded. Both are accepted in English)leading to widespread eutrophication,
49 hypoxia,and anoxia (Nixon, 1995; Cloern, 2001; Diaz and Rosenberg, 2008).

Answer: we corrected the text (line 48).

49 hypoxia and anoxia (Nixon, 1995; Cloern, 2001; Diaz and Rosenberg, 2008). Management policies have
50 been more effective in regulating point than diffuse nutrient sources to aquatic ecosystems (Boesch, 2002;
51 Palmeri et al., 2005).
Management policies have been more effective in regulating point nutrient sources of aquatic ecosystems compared to diffused ones.

Answer: we corrected the text (lines 49-50).

Line 53- Recent analyses suggest that most European watersheds export to the sea the same (or 52-even higher) amount of total N than before the nitrate directive, some 30 years ago (Vybernaite-Lubiene et
53- al., 2017; Viaroli et al., 2018).

Recent analyses suggest that most European watersheds total N export to the sea has either stayed the same or even increased despite the nitrate reduction directive which was established some 30 years ago (Vybernaite-Lubiene et al., 2017; Viaroli et al., 2018). PLEASE DECIDE TO CHOOSE BETWEEN TOTAL N AND NITRATE. IN SOME PLACES NITRATE EXPORT COULD BE VERY HIGH AND IN THE OTHERS ORGANIC NITROGEN IS THE DOMINANT FORM.

Answer: We corrected as suggested by the reviewer and left "total N", which includes sites where nitrate is the dominant form, as in our study

(Naldi et al., 2005; Viaroli et al., 2006; Castaldelli et al., 2013), and sites where dissolved organic forms may dominate. (Line 51-53).

Line 54- This situation can be worsened by the effects of climate change, which affects
54 the magnitude and the seasonal pattern of precipitation and increases the frequency of high discharge, flash 55 flood periods as well as those with no precipitation and minimum river flow (Trenberth, 2005; Lehner et al.,

This situation can be worsened as a consequence of climate change, which affects the magnitude and the seasonal pattern of precipitation and the increase of the frequency of high discharge, and flashflood periods. There might also be periods with no precipitation and as a result very low river (Trenberth, 2005; Lehner et al.,
OR
This situation can be worsened by the effects of climate change, which affects the magnitude and the seasonal pattern of precipitation that increases the frequency of high discharge, flash flood periods as well as phases (or intervals) with no precipitation and minimum river flow (Trenberth, 2005; Lehner et al.,

Answer: we changed the text according to the second option, thank you (lines 53-56).

64-discharge leads to the decrease in water residence time within estuarine systems. You are not working within estuarine system.

Answer: the reviewer is correct but we are not writing specifically about the Sacca di Goro here, but about the effects of discharge on estuaries (and lagoons) residence time. We added coastal lagoon to the sentence (line 59).

Line 66-removal instead of removed….etc.

Answer: we changed the text according to this suggestion (line 69).

Line 97- The effects of climatic extremes on benthic N cycling are therefore multifaceted, site-specific and thus 98 difficult to forecast (Najjar et al., 2010; Statham, 2012).
I agree with this statement. However, you have made generalized conclusions even though, your research is site-specific. Please adjust your text accordingly.

Answer: we partially agree, as this sentence belongs to the introduction and not to the conclusion section. With that statement and the citations reported we stress that the pathways of nutrients under climatic extremes are potentially multiple and difficult to predict. In our conclusion we state that in the analysed coastal lagoon hydrological extremes lead to higher inorganic N export to the sea and increased internal recycling in the summer.

2 Material and methods
124- which can be up to 10. Unite?
Answer: Salinity was measured by means of a YSI 556 multiple probe and values were determined from electrical conductivity and from the estimate of the ionic content, according to the practical salinity scale 1978

(PSS-78). The practical salinity, defined as the ratio of the conductivity of a sample of seawater to the conductivity of a special reference material called IAPSO Standard Seawater, has no units. The suffix PSU (practical salinity unit) is sometimes added but it is formally incorrect.

129- uptakein the lagoon (Bartoli et al., 2001; Nizzoli et al., 2006; Viaroli et al., 2006). During specific meteorological130- conditions (high temperature, low wind),macroalgal blooms might be followed by dystrophic events.
During high temperature and low wind macroalgal blooms might be followed by dystrophic…..
Answer: we changed the text according to these suggestions (lines 142-143).

High water temperature or high air temperature? Please specify.
Answer: both of them. The Sacca di Goro is a shallow lagoon and responds to variation in air temperature faster than the open sea.

131 -causing massive damages to the ecosystem and to the local economy (Viaroli et al., 2006).
What type of damage to the ecosystem? Some meteorological events might damage in longer term, it actually could be beneficial.
Answer: we were not referring to meteorological events, but to macroalgal blooms and their collapse, which are favoured under specific meteorological conditions, particularly during periods of high temperature and calm wind. The collapse of macroalgal production leads to dystrophy, anoxia, sulfide accumulation and loss of biodiversity. We reformulated the sentence to clarify this concept.

which hosts most of the 140-licensed areas for clams farming, is continuously flushed by marine seawater, which prevents organic matter 141 accumulation and forms sandy deposits.
If flashed continuously then it must be well oxygenated all the time. Although, the way that you describe the site, the circulation and mixing should be very slow except may be for spring flooding.Please check the definition of tidal prism and its effect on the circulation of the water in the lagoons.
As you mention it, tides are weak and wind circulation is not very strong and except for spring discharge, there is very little forcing for mixing and maybe salty water intrusion from the Adriatic Sea causes some slow circulation. This is also evident by the formation of the sandy spit in the area. The possible circulation and exchange of water with Adriatic Sea can possibly explained by slow river mixing and the continuity equation. The water that leaves the lagoon cannot be more than the river discharge (neglecting the evaporation). That is why you get different salinity values across the lagoon.
Answer: Clams are cultivated in the proximity of the lagoon-sea mouth, which is the area that is relatively more flushed in the entire lagoon system. The lagoon-sea mouth is the section through which nearly 30% of the lagoon water is daily exchanged with the sea. So, this system is microtidal, but it allows the cultivation of nearly 15,000 tons of clams per year, something impossible in a non-tidal system. Specific meteorological conditions (e.g. Scirocco winds) may contrast tidal forcing and increase stagnation also in the lagoon-sea mouth, with anoxic risk for clams. We better clarified these points (lines 154-158), added appropriate references on water circulation and hydrodynamic models for

the Lagoon (Marinov et al., 2006, 2008; Arpae-Emilia Romagna, Bologna University, CNR-Ismar, 2019).

143- 48' N 12°19' E) at the edge of the Valle di Gorino, and a site near the sea mouth within thefarmed areaFarmed Area(Unless, this is propr name.)
Answer: we changed the text according to these suggestions (line 161).

Line 153- 154. When exactly? Spring and summer are just season. Please indicate the exact dates.
Answer: we added sampling dates (Line 170).

149- to 2019). Precipitations related to the closing section?of the Po basin at Pontelagoscuro were also retrieved 150 for the period 2000-2019 from ARPAE.
Answer: we changed the text to clarify the meaning of the sentence (lines 167-168).

159 each site, 80 l of water was collected for cores maintenance, pre-incubation and incubation periods.
80 L? Line 171- 100 µl to 100 µL. Although, both are supposedly correct but L is the most accepted standard.
Answer: we thank the reviewer and corrected the units (line 178-line 193).

Line 181- to 183- Please check the units, it seems that they are wrong when metric dimensional analysis is carried out.
Answer: the units are correct. Concentration values are expressed in µmol L-1 or mmol L-1, the volume in L, the sediment surface in m2 and the incubation time in h.

Line 197- anammox contribution seems to be always insignificant in these situation.
Answer: We agree with the reviewer, it was an expected result since the highest contribution of anammox to NO2- reduction are typically found at deep sites characterized by low organic content (Thamdrup, 2012).

Study area well explained.
2.3 Measurement of denitrification and DNRA rates very well explained. Please check the grammar as well

Answer: Done

2.5 Rivers discharge and reactive N loadings
Since there is no real data are available. Please justify your choices more.
For example, the latter was calculated from monthly data of May and 249- September. THIS IS NOT VERY CLEAR AND NOT VERY REPLICABLE for readers.
Answer: We previously answered to reviewer #2 that the Universities of Parma and Ferrara monitor the Sacca di Goro lagoon (including loads from the Volano watershed and benthic processes) since 1986. As the Po di Volano watershed lays below the sea level and waters are pumped to avoid flooding, discharge is well known as well as loads (see Castaldelli et al., 2013, 2020). We therefore compared benthic processes (either denitrification or internal recycling) to real data of loads, from real measurements of discharge and concentration from the main tributaries during the two sampling periods.

Specifically, data of river discharge for the Po di Volano, Collettore Giralda, Canal Bianco and Canale Bonello were provided by the local water management authority (Consorzio di Bonifica Pianura di Ferrara). This authority continuously monitors the water discharge and provides daily or weekly average values.

The water released from the locks connecting the Po di Goro with the lagoon (30 m3 s-1), was calculated from a Hydrodynamic Model (Final Report of the Hydrodynamic Modelling System of the Sacca di Goro lagoon, Arpae-Emilia Romagna, Bologna University, CNR-Ismar, 2019). During the samplings carried out in May and September, water samples were collected in triplicates at each tributary for NH4+, NO2- and NO3- (DIN) determination. We calculated the daily load of dissolved inorganic N delivered to the lagoon from each tributary by multiplying the concentration measured by the mean water discharge.

Results:

Please explain more about the effect(s) of bioturbation.

Answer: We detailed the effects of amphipods and clam bioturbation in lines 393-409.

Tables:

Table 1. Unit of salinity? NH4+ is significant in spring time but it does not show in your graphical abstract.

Answer: we already answered to these questions above.

Table 2.,C/N ratios of your data actually indicates that organic matter is mostly local. C/N ratio higher than 20 is usually allochthone that will cause low or insignificant nitrification. Your values of C/N ratios are low and the nitrification is high the amount of which is a first rate kinetic reaction, thus depending on the nitrate concentration. Please be more careful in using C/N ratio indicator. It is much more into it. So, explain better about it in your results and use some references.

Answer: we have reformulated this section, also according to the comments of reviewer #4 (Lines 443-457).

Table 3. I am not so sure if such a detailed table is necessary. Same thing for table 4.

Answer: we moved tables 3 and 4 in Supplementary Material (Tables S1 and S2).

Table 6. Where are the outputs?

Answer: Outputs are not reported as they were not measured and as the aim of this table was to compare the amount of N potentially removed via sedimentary denitrification and the amount of N potentially recycled with respect to N inputs to the lagoon system.

Figures:

If possible show the results in NH4-N. So, they are consistent with other results that you have shown. Are there any data for NO3-?

Answer: we thank the reviewer. The results were already displayed as NH4+-N (µM), but we specified that in the units, like in the other figures. For the pore water we did not measure the NO3- concentration.

Figure 3. NH4-N and NO2-N, NO3-N, and N2-N.

Answer: we modified the units.

Figure 6- Very interesting figure. Very artistic and well presented. If I were you I would have used it for graphical abstract.

Answer: thank you!

Figure 7 a. This graph does not show any freshwater salinity. Units?

Answer: we already answered in some of the questions above.

PLEASE ADD THE MONTHLY-AVERAGED discharge of freshwater (rivers) into the lagoon. And explain the circulation based on salt water intrusion, wind, and the fact that water is very shallow. A moderate wind can create wave large enough to affect this very shallow lagoon.

Answer: We have detailed (lines 116-122; lines 504-511; lines 537-541) why the sampling times represent hot periods for the Sacca di Goro lagoon based on historical data. In particular, we detailed how the spring phase was characterized by unusual freshwater discharge and how the summer phase was characterized by low discharge and elevated water temperatures. We have also provided appropriate references supporting the evidence that climatic anomalies and hydrological extremes will be more and more frequent in the Po River Plain (lines 143-148). We believe that what we reported is enough to support the relevance of our findings, which derive from a biogeochemical and not from a hydrological approach. The core of our results is the experimental analysis of benthic processes via 15N stable isotopes, which is the most accurate technique at present available to measure denitrification and nitrate ammonification. Our main results show that under high discharge/high nitrate periods denitrification can be saturated and its efficiency decrease whereas under low discharge recycling may mobilize large amounts of ammonium. We then conclude that if expected anomalies will increase in frequency the benthic system will likely react as we described.

At the end, just for reading, I send you the following. I am not sure from where I took it. So, I cannot send you the reference. Just I had it in my notes.
"Although there is a consensus among reputable scientists that global warming is underway, it has become a major policy, political,and economic issue that engenders heated discussion. It is a complex topic made even more so by the natural variations in climate that occur over decades of time.
Recent trends in global temperature that have been measured with particular accuracy since the late 1900s using satellite instrumentation tend to support the idea that global warming is taking place. According to studies performed by theU.S. Goddard Institute for Space Studies, during the time period from 1880 to the present the 10 warmest years recorded have occurred since 1997. The near record warmth of 2007 is all the more remarkable because the year was at a minimum ofsolar irradiance and the natural El Niño–La Niña cycle of the equatorial PacificOcean was in its cool phase. Although the coolest year since 2000, the year 2008 stillranks seventh to tenth of these record warm years."

Answer: thank you. We are aware that, also from Italian Alpine Lakes long-term series of temperature data, inland aquatic ecosystems, including coastal lagoon, are accumulating heat. We believe that results of our study should be useful to stimulate further research linking climate change to coastal ecosystem functioning.

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Dear Editor,

We submit our manuscript “The effects of hydrological extremes on denitrification, DNRA and mineralization in a coastal lagoon” to Science of the Total Environment on the study of nitrogen (N) dynamics during two hydrological extremes in a eutrophic coastal lagoon. In our study, we analyzed inorganic N fluxes, denitrification, and DNRA rates, both during a period characterized by heavy rainfall and high freshwater river discharge (spring) and after a period characterized by prolonged low river discharge and high temperature (late summer). Results from this study suggest a sharp seasonal transition among dominant microbial processes driving the benthic N dynamics and a reduction in the effectiveness of the lagoon to act as a nutrient filter during both the extreme scenarios. During the high discharge period, in fact, removal processes dominated over recycling, but the high load of imported N, increased by rainfall and runoff, is partially exported to the open sea due to reduced residence time within the lagoon. During the summer drought, the high temperature and the low O_2 concentration, determine a large increase in internal recycling processes, which largely exceed the amount of N delivered from the watershed. Our study shows how hydrological and thermal extremes, whose frequency is expected to increase in the next decades, affect N benthic dynamics in transitional areas, increasing the amount of nutrients exported to the open sea or acting as a new possible driver of algal blooms.

We hope our paper is of interest for you and the readers of Science of the Total Environment.

With best regards,
Monia Magri and co-authors

The effects of hydrological extremes on denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and mineralization in a coastal lagoon

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***Responses to Reviewers Comments**

Dear Editorial Office of Science of Total Environment,

We are pleased to resubmit the revised version of our manuscript "The effects of hydrological extremes on denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and mineralization in a coastal lagoon". This version incorporates the suggestions provided by three anonymous reviewers, whom we would like to thank for their constructive analysis of our work. In general, we appreciate the reviewers' efforts in improving the manuscript and at the same time we are happy to read their acknowledgment of the study's merit. We have addressed their comments point-by-point and we present our answers (in Italic) below their original comments.

With best regards,

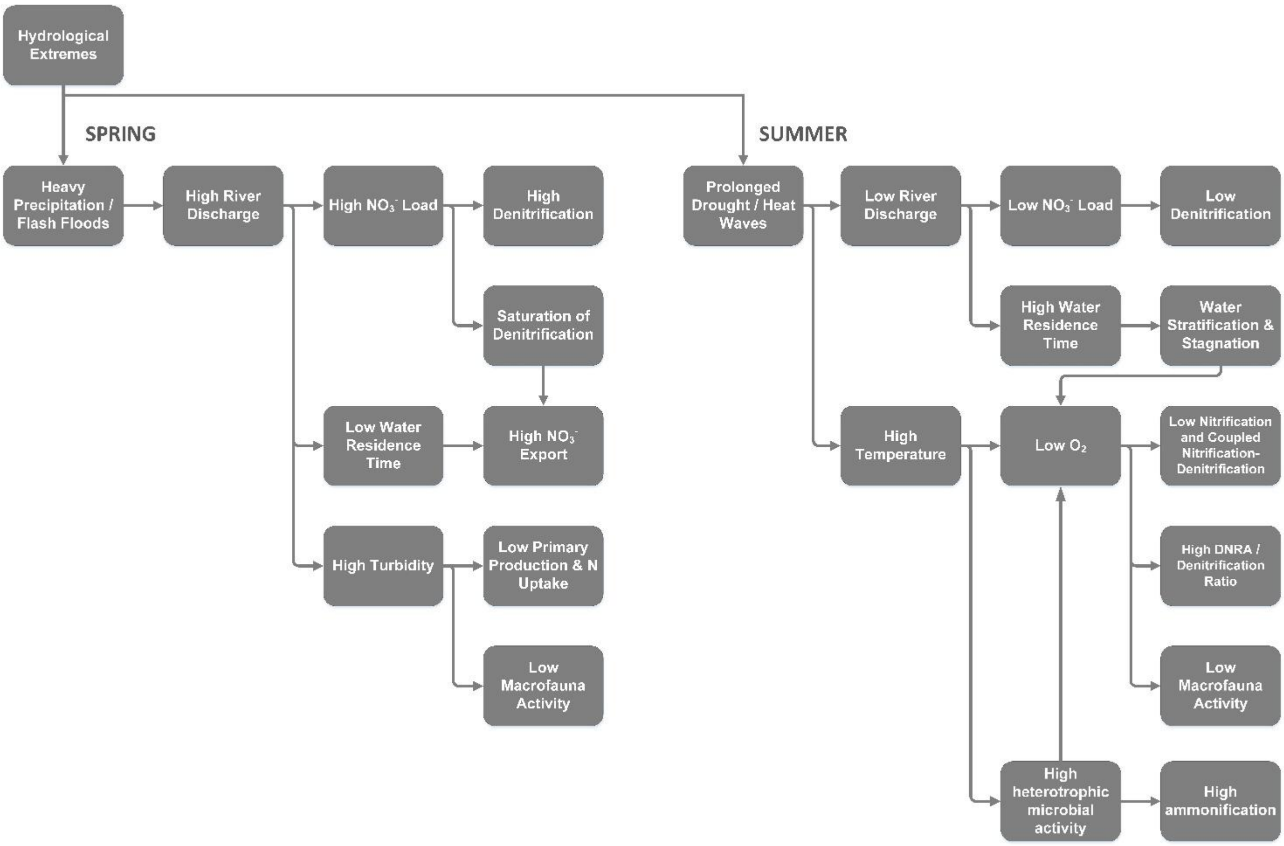
Monia Magri and co-authors

Reviewer #2:
Add the location of where the experiment was conducted (name + country) in the abstract.

Answer: We added the location of the study area in the abstract (Line 27).

The introduction would benefit from having a conceptual model showing the interlink between the different nitrogen pathways and how factors may influence each of them.

Answer: We added a conceptual model showing expected changes of benthic N cycling induced by hydrological extremes in coastal lagoons (Fig. 1). We hope this picture will help to clarify the different topics covered in the introduction.



The different pathways could also benefit from being developed further in the introductions.

Answer: We improved the description of the pathways in the conceptual model.

You should also have a section about microbes. You are mentioning investigating microbes L98 but it is a little bit out of nowhere since before you have a focus on benthic invertebrates.

Answer: we tackled our working hypotheses with a biogeochemical approach and as we stated "we analyzed microbial N transformations" (and not microbial communities) by means of ^{15}N-based techniques, that allow to measure accurately microbially-mediated processes like nitrification, denitrification and nitrate ammonification, and by means of oxygen and inorganic N fluxes, that can be converted into rates of ammonification. Based on previous works targeting the effects of macrofauna on benthic fluxes (e.g. Welsh et al., 2015; Murphy et al., 2018), we were also able to partition some of the measured fluxes in the contribution of microbes and macrofauna, as in the clams farmed station. We are aware of increasing number of papers combining microbial community characterization via molecular tools and biogeochemical measurements; such approach may represent a follow-up of the present study.

In your methods you mention the importance of clams. Shouldn't this also be in the introduction as well as a description of their influence on the biogeochemical processes?

Answer: the importance of macrofauna activity in benthic N cycling is well known in the literature and the effects of clams on N biogeochemistry were analyzed in detail in the Sacca di Goro in different papers we cited (Nizzoli et al., 2006; Viaroli et al., 2006; Welsh et al., 2015; Murphy et al., 2018). One of the 3 sampling stations that we investigated is cultivated with clams as this activity occurs over nearly 30% of the lagoon surface and cannot be neglected. However, clams are not central in our story, which rather targets the effects produced by hydrological extremes on benthic N cycling. We demonstrated that the seasonal shift from the dominance of removal to the dominance of recycling occurs at all sites, regardless the presence of clams or other macrofauna.

L124 Up to 10 what?

Answer: Salinity was measured by means of a YSI 556 multiple probe and values were determined from electrical conductivity and from the estimate of the ionic content, according to the practical salinity scale 1978 (PSS-78). The practical salinity, defined as the ratio of the conductivity of a sample of seawater to the conductivity of a special reference material called IAPSO Standard Seawater, has no units. The suffix PSU (practical salinity unit) is sometimes added but it is formally incorrect.

L152-153 What is the minimum distance between two sampling points?

Answer: We added in the test the distance among sampling sites, ranging from 1.5 to 4.5 Km (lines 161-162).

L153 Why do you have different sampling efforts between summer and spring

Answer: During the spring campaign we had some technical constraints, due also to the bad weather. Despite this, please note that according to the protocol that we followed, a minimum of 4 replicates is considered adequate, since "measurements of fluxes and denitrification are carried out on a minimum of 3 parallel cores" (Dalsgaard et al., 2000).

L158 Add the manufacturer etc… in brackets for the YSI

Answer: Done (Line 177).

L160 How were the core preserved until they reached the lab. A few hours is a long time when it comes to microbial activity.

Answer: We detailed in the Material and Methods section (lines 178-182) that the cores, after collection, were immediately submerged with the top open in a box filled with in situ *water cooled with ice packs to slow microbial activity. Within 1-2 hours from collection they were submersed in large tanks at the Centre for Mollusc Research, Goro (Ferrara, Italy), which is <1 km from the harbor. The tanks were filled with aerated and well-mixed water from the three stations, maintained at in situ temperature and the cores were preincubated overnight. The procedure we have followed minimizes sediment disturbance, does not alter microbial activity and is standard for core incubation (Dalsgaard et al., 2000).*

L161-164 Do you have any reference for this protocol? Also, how were the cores distributed in the tanks. I am pretty sure you used different tanks for different sites but maybe make it slightly clearer.

Answer: We added more details on the pre-incubation phase (lines 181-182). Once in laboratory, the cores were placed into three large tanks, one for each site, containing renewed water, at in situ *O_2 concentration and temperature. The reference for the protocol we used to plan the pre-incubation and the incubation phases was already reported (Dalsgaard et al., 2000) and was produced during a European project targeting the measurement of N-related microbial activities in different estuaries.*

L174 Maybe add a reference to the standard protocol

Answer: For each analytical method we reported the reference. We added a reference of a collection of Standard Methods containing all the procedures that we used for the water samples analysis (APHA, 1992, line 200).

L225 What was the taxonomic resolution? How did you identify (add the key that you used, microscope and amplification)? How did you get the biomass (add the protocol)?

Answer: We added some details in the text (Lines 252-258). Organisms were retrieved from the sediments and sorted under stereomicroscope (Leica S8 APO, amplification 8x). The organisms were identified by dichotomous keys (http://species-identification.org/identify_species.php) and by scientific papers (Wagele, 1981) to the lowest possible taxonomic level and counted. The identification was strengthened by the comparison with previous studies on the macrobenthic community of the Sacca di Goro (Mistri et al., 2001; Ludovisi et al., 2013; Politi et al., 2019). For each species the dry weight was determined after drying at 80°C for 48 h. For the clams, shells were removed, and only flesh weight was measured.

Why did you not look at the microbial community? You said in your introduction that you were interested in microbial processes and yet you do nothing in that direction. What is the reason for that? I feel like your work would have benefitted at lot from assessing microbial biomass, densities and activity at a minor cost.

Answer: We agree that it would have been extremely interesting to analyze the microbial communities in the two sampling periods, but as we explained earlier, we used a biogeochemical approach to focus on microbial processes.

L246-249 It would be good to have a reference as well. It seems like a big approximation to me as nutrient loads can vary a lot over very short time periods. I wonder if modelling methods such as SWAT (or others) would not have been better to get the nutrient load rather than extrapolating data from a single sampling event for each season.

Answer: We partially agree as our group of research in Parma and Ferrara started monitoring the Sacca di Goro and the nutrient loads to this system since 1986 and produced a large body of literature including *recent papers reporting the nutrient loads delivered in the last decades to the Po River Delta (Viaroli et al., 2018), loads generated during high discharge periods (Naldi et al 2010) and nutrients delivered by the Po di Volano to the Goro Lagoon (Castaldelli et al., 2013, 2020). We acknowledge that loads undergo large variations in the short-term but the hydrology of the Volano basin, which is the main nutrient source to the system is regulated as it lays below sea level and discharge is monitored and known. Loads reported in our work overlap seasonal loads reported in Castaldelli et al (2013); we added these aspects (lines 378-384)*

What were the incubation temperature for all your measurements (including acclimation phase)? Was there a dark-light cycle during the incubation phase?

Answer: We detailed that overnight preincubation and incubation occurred at in situ temperatures (Table 1) and in the dark as only heterotrophic processes were measured (lines 180-187).

L279 What error measurement did you use?

A*nswer: density values of macrofauna were displayed as averages ± standard errors, we added the measurement in the text (Line 312).*

Fig 5 is hard to read

Answer: The reviewers had different opinions about our figure (from hard to read to very clear). We have now improved it by increasing characters to the possible maximum.

Table 3 seems to have a number problem in the last significant P value reported (N2 season x site)

Answer: We are sorry, but we didn't understand what exactly the reviewer meant in this comment. We have run again the two-way ANOVA and the number is correct.

Reviewer #4:

Highlights
The authors need to define as early as possible their definition of "N recycling", and "N regeneration" which could mean a number of things. I think in this case is the sum of the measured processes such as nitrification, mineralization, and DNRA, but for some people it could mean other things such as algal or animal uptake and excretion.

Answer: We agree with the reviewer and we clearly stated that with "recycling" we mean the fluxes of inorganic nitrogen (NH4+, NO2- and NO3-) from the benthic system to the water column. As such, they include the net result of processes as ammonification, nitrification, denitrification, DNRA and excretion by macrofauna (lines 84-87). We removed "regeneration" to avoid confusion.

Introduction
L109: It would be interesting to emphasize how "unusual' this year was, for instance, "precipitation which was 30% higher than long-term measurements" instead of "heavy precipitation" or "Low river discharge"

Answer: We agree and we added that a) May 2019 was characterized by unusually heavy rainfall with value of cumulative precipitation (mm) nearly 2.5 times higher than values measured from 2000 to 2018, b) during summer, in the Po River basin, the temperature shows a clear increasing trend from the 1970s (Brunetti et al.,2006) and c) during the summer of 2019, the average daily water temperature exceeded 30°C for 8 days, compared to what recorded from 2006 to 2018, when this period was limited to 4 days (lines 116-122).

L112: Is it salinity really the determinant factor? Or is salinity just an indicator of the influence of marine water, which is characterized by low nutrient concentrations and high S?

Answer: Both aspects are important. Salinity is recognized to influence benthic N dynamics, decreasing nitrification and denitrification processes, and favoring DNRA, mainly due to higher sulfate reduction rates that increase concentrations of sulfides in the pore-water environment, which directly impact DNRA (An and Gardner, 2002; Gardner et al., 2006; Giblin et al., 2010; Caffrey et al., 2019). But salinity directly limits coupled nitrification-denitrification due to inhibitory physiological effects on nitrifiers and denitrifiers (Rysgaard et al., 1999) and determines a decrease in nitrification rates and an increase in NH_4^+ *effluxes due to sediment desorption (Gardner et al., 1991). At the same time* NO_3^- *concentration influences the proportion of denitrification and DNRA rates, with the latter favored at low concentration, due to a thermodynamic advantage, since reduction of nitrate to ammonium accommodates 8 electrons compared to the 5 received by denitrification (Tiedje, 1988; Nizzoli et al., 2010). We were not able to discriminate between* NO_3^- *concentration and salinity, because both these factors varied along the seaward trajectories (the first decreased, whereas the latter increased).*

L115: "loss" is mentioned twice in the third hypothesis.

Answer: we changed this term (line 126).

Methods

L139: What is "high temperature" in this site" ? range?

Answer: We agree with the reviewer, we reformulated this sentence that was not very clear. We clarified that the eastern portion of the lagoon, mainly during the summer season, is characterized by stagnant water and by temperatures usually higher than in the rest of the lagoon (Lines 153-154).

L159: 80L of water were collected

Answer: we corrected the units (line 178).

176: Define MIMS

Answer: we defined the acronym MIMS (membrane inlet mass spectrometer) in the text (lines 196-197).

L209: Do you mean effluxes from the sediment to the water column?

Answer: That's was exactly what we meant, we added a better definition in the text (lines 235-236).

L231: Define "C"

Answer: We defined C as carbon in the text (line 264).

L231: Isotopes are "values" not "signatures", because they are not constant.

Answer: We thank the reviewer for this clarification, we corrected the term in the text (line 264).

Results

L271: It doesn't look like FA has higher NH4 concentrations in the summer in Fig. 2 as stated in the text

Answer: We reformulated the sentence to clarify the graph explanation (lines 304-309).

L274: "seasons"

Answer: we corrected the term (line 307).

L306: Comma after "spring"

Answer: Added (line 340).

Discussion

L345: What do you mean by "microphytobenthos activity", do you mean nitrogen uptake or photosynthesis, or both?

Answer: We mean both. Microphytobenthos inhibits N dissimilative pathways both through photosynthetic activity and nutrient uptake. The competition for N, mainly as NH_4^+*, determines a decrease in nitrification and coupled nitrification-denitrification rates (Sundbäck et al., 2000). At the same time, the expansion of the oxic layer, due to microphytobenthos* O_2 *production, reduces denitrification of water column* NO_3^- *(Dw) due to increased diffusion pathlength to reach the anoxic sediment horizon (Bartoli et al., 2003).*

L358: small amount of NO3 being reduced

Answer: we thank the reviewer and corrected the form (line 402).

L379: Please explain here whether is in fact salinity or is it the NO3, and reductant sulphides that drive the changes in N processing.

Answer: as we explained above, both salinity and NO_3^- *concentration may determine variations in the relative proportion of DNRA and denitrification as* NO_3^- *reduction pathways and we were not able to discriminate between these two factors, as they both varied along the seaward gradient.*

In this study we did not measure sulfides concentration but results of previous studies displayed a zonation in the buffering capacity against dissolved sulfides accumulation in the Sacca di Goro lagoon. The western corner, where station Giralda is located, is characterized by a high buffer capacity that is related to the abundance of electron acceptors alternative to sulfate, as NO_3^-*,* Mn^{4+} *and* Fe^{3+} *delivered from river, and to high bioturbation, resulting in deep penetration of* O_2 *and* NO_3^- *within sediments (Giordani et al., 1996; Azzoni et al., 2005; Zilius et al., 2015). There are different areas of the lagoon, as the northern corner or the sheltered Valle di Gorino, where sediments are reduced and devoid of macrofauna, especially during summer macroalgal blooms that determine a high load of organic matter, and energetically favorable electron acceptors such as* O_2 *or* NO_3^- *are rapidly exhausted. In these conditions sulfate reduction and sulfides release increase (Zilius et al., 2015). The two marine sites analyzed in our study, Gorino and Farmed Area, are located close to the sea mouth, in an area subject to tidal influence. For this reason, despite the high salinity and sulfate availability and biodeposition of faeces and preudofaeces by cultivated clams, these sites are characterized by high hydrodynamic conditions which may prevent the accumulation of organic matter and favor oxygenation at the water-sediment interface, thus partially contrasting the build-up of sulfides (Giordani et al., 1996, 1997; Azzoni et al., 2005).*

L391: Not sure what do you mean by "contrasted the effects"

Answer: we reformulated the sentence (Lines 434-437).

L393: Add comma after "summer"

Answer: we added it (line 411).

L400: Explain a bit further how you got to this conclusion, my guess is that you considered terrestrial plants to be -27ppm of 13C versus marine phytoplankton, which is usually around -20 ppm

Answer: At Giralda the high organic load was derived mainly from settled particles of fluvial origin, as demonstrated by the higher C:N ratio and by the lower C and N isotopic values, within the range reported for terrestrial organic matter (~-27‰ and of 3‰ for $\delta^{13}C$ *and* $\delta^{15}N$*, respectively, Lamb et al., 2006). However, even during the high discharge period, the C:N ratio of Giralda surface sediments suggested high organic matter quality, whereas material of terrestrial origins usually displays values significantly above 12 (Yamamuro, 2000). Gorino and Farmed Area were characterized by C:N ratios closed to the Redfield one and by higher* $\delta^{13}C$ *and* $\delta^{15}N$ *values, closer to values reported for marine systems, suggesting a progressive increase in the proportion of organic matter from autochthonous origins (Yamamuro, 2000; Liu, 2006). The isotopic values, however, were more depleted compared to marine phytoplankton, particularly relative to* $\delta^{13}C$ *values, usually ranging from -22 to -19 ‰ (Lamb et al., 2006), suggesting that sedimentary organic matter still derived from the mixture of terrestrial derived material and marine material and from the accumulation of clam biodeposits (*$\delta^{13}C$ *value of about -23.2 ‰, Mazzola and Sarà, 2001). We added the explanation in the text (Lines 443-457).*

L421: Not sure what you mean by "lagoon aging", please explain.

Answer: We expected that the organic enrichment due to more than 30 years of aquaculture activity and to macroalgal blooms, have permanently affected benthic dynamics. We expected an increasing trend in O_2 *sediment uptake rates due to enhanced microbial activity and a decreasing trend in denitrification rates, since under highly reduced and sulfidic conditions nitrogen cycling becomes controlled by dissimilative nitrate reduction to ammonium instead of denitrification. However, long-term trends are not clearly visible, probably due to the frequent silting operations within the lagoon and the increased frequency of heavy rainfall events, which contributed to limit the organic matter accumulation.*

L422: Please define IPT in the Methodology, or just write here the full name of the methodology (isotope pairing technique).
Answer: we added it (line 209).

L430: I don't think "addressed" is the best word here, maybe "associated"?
Answer: we agree with the reviewer and replaced the word (line 481).

L432: I guess it would make it more variable, not necessarily reduce it, as it would be increased in some events, and decreased in others.

Answer: we partially agree, as we demonstrated that under hydrological extremes there is a decrease in the efficiency of the lagoon to act as N filter. Particularly, under high discharge/high nitrate periods denitrification can be saturated and its efficiency decrease whereas under low discharge recycling may mobilize large amounts of ammonium that may be exported to the open sea or fuel primary production activity. We replaced the sentence "future change in climatic conditions" with "hydrological extremes", which are the focus of our study (lines 483-485).

Conclusion

L518: As it is written now, the sentence implies that denitrification will be exported to the Adriatic Sea, please rewrite.

Answer: We reformulated the sentence (Lines 567-569).

Tables

Table 3-4 could be considered for supplementary Material. Table 6 could definitely be incorporated in the text
Answer: we agree with the reviewer. We moved Table 3 and 4 to supplementary material and incorporated table 6 in the text.

Figures

The font in Figure 5 is too small. This diagrams are great, but maybe just have two one for spring and one for summer? The rest could be in supplementary material.

Answer: The reviewers had different opinions about our figure (from hard to read to very clear). We have now improved it by increasing characters to the possible maximum.

Reviewer#1

Title:

The effects of hydrological extremes on denitrification, ~~DNRA~~dissimilatory nitrate reduction to ammonium, and mineralization in a coastal lagoon

Answer: we defined DNRA in the title, enclosing the acronym in brackets.

Graphical abstract:

I am surprised that there is no NH4+ during the spring time. It is true that ammonium is less mobile in soils but there are always excess NH4+. It could be much less than NO3-. Although, there is usually no positive correlation with water discharge and NH4+ like NO3-.

Answer: The reviewer is right, in the previous version we stressed only nitrate due to large seasonal variation, we have now added NH_4^+ *and the relevance of its recycling.*

What do you mean by PN? Particulate nitrogen?

Answer: we defined the term in the figure.

Change DNRA to real words in the graphic as in the title.

Answer: we defined the acronym in the title, but we think that the figure may result hard to read if we will add further text.

Highlights:

Hydrological extremes deeply alter benthic N cycling in coastal lagoons~~transitional areas~~

Answer: we substituted the term.

Abstract:

Hydrological extremes of unusually high or low river discharge may have profound effects on biogeochemistry of coastal lagoons ~~zones,~~ but such effects are poorly explored. Coastal Zone is a very subject. Coastal lagoons are very small part of the subject.

Answer: we agree with the reviewer and substituted the term.

27- freshwater and two marine ones) in a eutrophic lagoon both under high discharge (spring) and after….

Your values of salinity does not indicate any freshwater. It is all brackish water. Please show some data about freshwater salinity.

Answer: we thank the reviewer for this correction, we removed from the text all references to the stations as freshwater or marine sites.

29- Under high discharge/nitrate load, denitrification was the leading process and little to no recycling was observed.Do you mean little or no INTERNAL recycling was observed? In the larger picture, the recycling is always going on in biogeochemical processes. The rates are just different

Answer: According to another comment by another reviewer we defined internal "recycling" as the sum of fluxes of inorganic nitrogen (NH4+, NO2- and NO3-) from the benthic system to the water column. As such, they include the net result of processes as ammonification, nitrification, denitrification, DNRA and excretion by macrofauna (lines 84-87). In spring DIN fluxes were negative (directed from the water column to sediments) at all three sites, so we stated that there was no internal recycling (line 29).

31 mostly sustained by nitrification stimulated by burrowing macrofauna.It might be true, but justify it in your introduction by showing some evidence or some references

Answer: Denitrification coupled to nitrification and its stimulation by burrowing macrofauna was extensively studied in the Sacca di Goro and in other coastal areas; we added appropriate references related to Corophium insidiosum *and* Neantes succinea *(Pelegri and Blackburn, 1994; Nizzoli et al., 2007; Moraes et al., 2018).*

32 recycling under low discharge,-AGAIN, DO YOU MEAN INTERNAL CYCLING?

Answer: yes, we specified it in the text (line 32).

33 reduced bioturbation and nitrification. The highest recycling was measured at the marine sites. Please use a better term that marine sites. This research is about a coastal lagoon.

Answer: we agree and reformulated the sentence that now reads: "The highest recycling was measured at the sites located close to the sea entrance and characterized by the higher salinity and particularly at the clams cultivated area" (lines 33-35).

43 ~~under climate change scenarios~~, strongly alter N cycling RATES in coastal ~~sediments~~ lagoons. It might be true under climate change scenarios, but this is another topic. Climate change is not evident everywhere. However, there are many strong indications about it in many places. Do you have any real proof about it in your area?

Answer: In the introduction we report some of the ongoing and expected climatic changes in the area of the Po River basin (lines 110-115), including increase in average and maximum temperatures, a general decline in runoff (30-40% reduction), mainly in summer and an increase in hydrological extremes, with prolonged droughts and peaks of river discharge due to more severe and less frequent but more intense precipitation. All the papers that we cite in the introduction are relative to the Italian territory or specifically to the Po River and the North Adriatic area (Coppola and Giorgi, 2010; Tibaldi et al., 2010; Cozzi and Giani, 2011; Vezzoli et al., 2015).

38 -Flash floods and high nitrate may offset denitrification due to reduced residence time and saturation of 39-microbial enzymatic activity, (38-39 are not very clear) resulting in high transport of nitrate to the open sea.

Answer: we reformulated the sentence that now reads:
Flash floods and high nitrate load may overwhelm the denitrification capacity of the lagoon due to the reduced residence time and to the saturation of microbial enzymatic activity, resulting in high transport of nitrate to the open sea (lines 38-40).

Prolonged dry periods 40-favor large N regeneration by(in)sediments, due to combination of high temperatures, low oxygen solubility and 41 low bioturbation, which may prolong the extent of algal blooms with negative feedbacks (Please check the definition of positive and negative feedback-I think it should be a positive feedback here. It seems that you are trying to say that in worsen the situation. Is it what you are trying to say?)for the lagoon 42 biogeochemical services.

Answer: the reviewer is right, it is a positive feedback and we modified the sentence (Lines 41-42).

Keywords: nitrogen, sedimentary fluxes, estuaries, climate extremes, nitrogen loss, nitrogen recycling
-By definition of estuaries, your study site is not an estuary. There is no tide

Answer: We partially agree, our study area is a microtidal coastal lagoon. We replaced the keyword "estuary" with "coastal lagoon"

-Nitrogen is a very general word. You can use some more specific words from your paper.

Answer: We agree and we have replaced the keyword (line 45).

-Sedimentary flux also could be anything in sedimentary processes

Answer: We agree and we have replaced sedimentary fluxes with benthic fluxes, that is more appropriate keyword for studies analysing the exchange of solutes across the sediment-water interface (line 45).

Introduction:
47 Human activities, through increased fertilizer production and combustion, have more than doubled the loadingof bioavailable nitrogen (N) to coastal areas (37-66 Tg total N yr-148),that have led(or leaded. Both are accepted in English)~~leading~~ to widespread eutrophication,
49 hypoxia,and anoxia (Nixon, 1995; Cloern, 2001; Diaz and Rosenberg, 2008).

Answer: we corrected the text (line 48).

49 hypoxia and anoxia (Nixon, 1995; Cloern, 2001; Diaz and Rosenberg, 2008). Management policies have 50 been more effective in regulating point than diffuse nutrient sources to aquatic ecosystems (Boesch, 2002; 51 Palmeri et al., 2005).
Management policies have been more effective in regulating point nutrient sources of aquatic ecosystems compared to diffused ones.

Answer: we corrected the text (lines 49-50).

Line 53- Recent analyses suggest that most European watersheds export to the sea the same (or 52-even higher) amount of total N than before the nitrate directive, some 30 years ago (Vybernaite-Lubiene et 53- al., 2017; Viaroli et al., 2018).

Recent analyses suggest that most European watersheds total N export to the sea has either stayed the same or even increased despite the nitrate reduction directive which was established some 30 years ago (Vybernaite-Lubiene et al., 2017; Viaroli et al., 2018). PLEASE DECIDE TO CHOOSE BETWEEN TOTAL N AND NITRATE. IN SOME PLACES NITRATE EXPORT COULD BE VERY HIGH AND IN THE OTHERS ORGANIC NITROGEN IS THE DOMINANT FORM.

Answer: We corrected as suggested by the reviewer and left "total N", which includes sites where nitrate is the dominant form, as in our study (Naldi et al., 2005; Viaroli et al., 2006; Castaldelli et al., 2013), and sites where dissolved organic forms may dominate. (Line 51-53).

Line 54- This situation can be worsened by the effects of climate change, which affects
54 the magnitude and the seasonal pattern of precipitation and increases the frequency of high discharge, flash 55 flood periods as well as those with no precipitation and minimum river flow (Trenberth, 2005; Lehner et al.,

This situation can be worsened as a consequence of climate change, which affects the magnitude and the seasonal pattern of precipitation and the increase of the frequency of high discharge, and flashflood periods. There might also be periods with no precipitation and as a result very low river (Trenberth, 2005; Lehner et al.,

OR

This situation can be worsened by the effects of climate change, which affects the magnitude and the seasonal pattern of precipitation that increases the frequency of high discharge, flash flood periods as well as phases (or intervals) with no precipitation and minimum river flow (Trenberth, 2005; Lehner et al.,

Answer: we changed the text according to the second option, thank you (lines 53-56).

64-discharge leads to the decrease in water residence time within estuarine systems. You are not working within estuarine system.

Answer: the reviewer is correct but we are not writing specifically about the Sacca di Goro here, but about the effects of discharge on estuaries (and lagoons) residence time. We added coastal lagoon to the sentence (line 59).

Line 66-removal instead of removed….etc.

Answer: we changed the text according to this suggestion (line 69).

Line 97- The effects of climatic extremes on benthic N cycling are therefore multifaceted, site-specific and thus 98 difficult to forecast (Najjar et al., 2010; Statham, 2012).
I agree with this statement. However, you have made generalized conclusions even though, your research is site-specific. Please adjust your text accordingly.

Answer: we partially agree, as this sentence belongs to the introduction and not to the conclusion section. With that statement and the citations reported we stress that the pathways of nutrients under climatic extremes are potentially multiple and difficult to predict. In our conclusion we state that in the analysed coastal lagoon hydrological extremes lead to higher inorganic N export to the sea and increased internal recycling in the summer.

2 Material and methods

124- which can be up to 10. Unite?
Answer: Salinity was measured by means of a YSI 556 multiple probe and values were determined from electrical conductivity and from the estimate of the ionic content, according to the practical salinity scale 1978 (PSS-78). The practical salinity, defined as the ratio of the conductivity of a sample of seawater to the conductivity of a special reference material called IAPSO Standard Seawater, has no units. The suffix PSU (practical salinity unit) is sometimes added but it is formally incorrect.

129- uptakein the lagoon (Bartoli et al., 2001; Nizzoli et al., 2006; Viaroli et al., 2006). ~~During specific meteorological130- conditions~~ (high temperature, low wind),macroalgal blooms might be followed by dystrophic events.
During high temperature and low wind macroalgal blooms might be followed by dystrophic…..
Answer: we changed the text according to these suggestions (lines 142-143).

High water temperature or high air temperature? Please specify.
Answer: both of them. The Sacca di Goro is a shallow lagoon and responds to variation in air temperature faster than the open sea.

131 -causing massive damages to the ecosystem and to the local economy (Viaroli et al., 2006).
What type of damage to the ecosystem? Some meteorological events might damage in longer term, it actually could be beneficial.

Answer: we were not referring to meteorological events, but to macroalgal blooms and their collapse, which are favoured under specific meteorological conditions, particularly during periods of high temperature and calm wind. The collapse of macroalgal production leads to dystrophy, anoxia, sulfide accumulation and loss of biodiversity. We reformulated the sentence to clarify this concept.

which hosts most of the 140-licensed areas for clams farming, is continuously flushed by marine seawater, which prevents organic matter 141 accumulation and forms sandy deposits.
If flashed continuously then it must be well oxygenated all the time. Although, the way that you describe the site, the circulation and mixing should be very slow except may be for spring flooding.Please check the definition of tidal prism and its effect on the circulation of the water in the lagoons.
As you mention it, tides are weak and wind circulation is not very strong and except for spring discharge, there is very little forcing for mixing and maybe salty water intrusion from the Adriatic Sea causes some slow circulation. This is also evident by the formation of the sandy spit in the area. The possible circulation and exchange of water with Adriatic Sea can possibly explained by slow river mixing and the continuity equation. The water that leaves the lagoon cannot be more than the river discharge (neglecting the evaporation). That is why you get different salinity values across the lagoon.
Answer: Clams are cultivated in the proximity of the lagoon-sea mouth, which is the area that is relatively more flushed in the entire lagoon system. The lagoon-sea mouth is the section through which nearly 30% of the lagoon water is daily exchanged with the sea. So, this system is microtidal, but it allows the cultivation of nearly 15,000 tons of clams per year, something impossible in a non-tidal system. Specific meteorological conditions (e.g. Scirocco winds) may contrast tidal forcing and increase stagnation also in the lagoon-sea mouth, with anoxic risk for clams. We better clarified these points (lines 154-158), added appropriate references on water circulation and hydrodynamic models for the Lagoon (Marinov et al., 2006, 2008; Arpae-Emilia Romagna, Bologna University, CNR-Ismar, 2019).

143- 48' N 12°19' E) at the edge of the Valle di Gorino, and a site near the sea mouth within thefarmed area~~Farmed Area(Unless, this is propr name.)~~
Answer: we changed the text according to these suggestions (line 161).

Line 153- 154. When exactly? Spring and summer are just season. Please indicate the exact dates.
Answer: we added sampling dates (Line 170).

149- to 2019). Precipitations related to the closing section?of the Po basin at Pontelagoscuro were also retrieved 150 for the period 2000-2019 from ARPAE.
Answer: we changed the text to clarify the meaning of the sentence (lines 167-168).

159 each site, 80 l of water was collected for cores maintenance, pre-incubation and incubation periods.
80 L? Line 171- 100 µl to 100 µL. Although, both are supposedly correct but L is the most accepted standard.
Answer: we thank the reviewer and corrected the units (line 178-line 193).

Line 181- to 183- Please check the units, it seems that they are wrong when metric dimensional analysis is carried out.
Answer: the units are correct. Concentration values are expressed in µmol L^{-1} or mmol L^{-1}, the volume in L, the sediment surface in m^2 and the incubation time in h.

Line 197- anammox contribution seems to be always insignificant in these situation.
Answer: We agree with the reviewer, it was an expected result since the highest contribution of anammox to NO_2^- reduction are typically found at deep sites characterized by low organic content (Thamdrup, 2012).

Study area well explained.
2.3 Measurement of denitrification and DNRA rates very well explained.
Please check the grammar as well

Answer: Done

2.5 Rivers discharge and reactive N loadings

Since there is no real data are available. Please justify your choices more.
For example, the latter was calculated from monthly data of May and 249- September. THIS IS NOT VERY CLEAR AND NOT VERY REPLICABLE for readers.

Answer: We previously answered to reviewer #2 that the Universities of Parma and Ferrara monitor the Sacca di Goro lagoon (including loads from the Volano watershed and benthic processes) since 1986. As the Po di Volano watershed lays below the sea level and waters are pumped to avoid flooding, discharge is well known as well as loads (see Castaldelli et al., 2013, 2020). We therefore compared benthic processes (either denitrification or internal recycling) to real data of loads, from real measurements of discharge and concentration from the main tributaries during the two sampling periods.
Specifically, data of river discharge for the Po di Volano, Collettore Giralda, Canal Bianco and Canale Bonello were provided by the local water management authority (Consorzio di Bonifica Pianura di Ferrara). This authority continuously monitors the water discharge and provides daily or weekly average values.
The water released from the locks connecting the Po di Goro with the lagoon (30 m^3 s^{-1}), was calculated from a Hydrodynamic Model (Final Report of the Hydrodynamic Modelling System of the Sacca di Goro lagoon, Arpae-Emilia Romagna, Bologna University, CNR-Ismar, 2019). During the samplings carried out in May and September, water samples were collected in triplicates at each tributary for NH_4^+, NO_2^- and NO_3^- (DIN) determination. We calculated the daily load of dissolved inorganic N delivered to the lagoon from each tributary by multiplying the concentration measured by the mean water discharge.

Results:
Please explain more about the effect(s) of bioturbation.

Answer: We detailed the effects of amphipods and clam bioturbation in lines 393-409.

Tables:
Table 1. Unit of salinity? **NH4+** is significant in spring time but it does not show in your graphical abstract.

Answer: we already answered to these questions above.

Table 2.,C/N ratios of your data actually indicates that organic matter is mostly local. C/N ratio higher than 20 is usually allochthone that will cause low or insignificant nitrification. Your values of C/N ratios are low and the nitrification is high the amount of which is a first rate kinetic reaction, thus depending on the nitrate concentration. Please be more careful in using C/N ratio indicator. It is much more into it. So, explain better about it in your results and use some references.
Answer: we have reformulated this section, also according to the comments of reviewer #4 (Lines 443-457).

Table 3. I am not so sure if such a detailed table is necessary. Same thing for table 4.

Answer: we moved tables 3 and 4 in Supplementary Material (Tables S1 and S2).

Table 6. Where are the outputs?

Answer: Outputs are not reported as they were not measured and as the aim of this table was to compare the amount of N potentially removed via sedimentary denitrification and the amount of N potentially recycled with respect to N inputs to the lagoon system.

Figures:
If possible show the results in NH4-N. So, they are consistent with other results that you have shown. Are there any data for NO3-?

Answer: we thank the reviewer. The results were already displayed as NH_4^+-N (μM), but we specified that in the units, like in the other figures. For the pore water we did not measure the NO_3^- concentration.

Figure 3. NH4-N and NO2-N, NO3-N, and N2-N.

Answer: we modified the units.

Figure 6- Very interesting figure. Very artistic and well presented. If I were you I would have used it for graphical abstract.

Answer: thank you!

Figure 7 a. This graph does not show any freshwater salinity. Units?

Answer: we already answered in some of the questions above.

PLEASE ADD THE MONTHLY-AVERAGED discharge of freshwater (rivers) into the lagoon. And explain the circulation based on salt water intrusion, wind, and the fact that water is very shallow. A moderate wind can create wave large enough to affect this very shallow lagoon.

Answer: We have detailed (lines 116-122; lines 504-511; lines 537-541) why the sampling times represent hot periods for the Sacca di Goro lagoon based on historical data. In particular, we detailed how the spring phase was characterized by unusual freshwater discharge and how the summer phase was characterized by low discharge and elevated water temperatures. We have also provided appropriate references supporting the evidence that climatic anomalies and hydrological extremes will be more and more frequent in the Po River Plain (lines 143-148). We believe that what we reported is enough to support the relevance of our findings, which derive from a biogeochemical and not from a hydrological approach. The core of our results is the experimental analysis of benthic processes via ^{15}N stable isotopes, which is the most accurate technique at present available to measure denitrification and nitrate ammonification. Our main results show that under high discharge/high nitrate periods denitrification can be saturated and its efficiency decrease whereas under low discharge recycling may mobilize large amounts of ammonium. We then conclude that if expected anomalies will increase in frequency the benthic system will likely react as we described.

At the end, just for reading, I send you the following. I am not sure from where I took it. So, I cannot send you the reference. Just I had it in my notes.
“Although there is a consensus among reputable scientists that global warming is underway, it has become a major policy, political,and economic issue that engenders heated discussion. It is a complex topic made even more so by the natural variations in climate that occur over decades of time.
Recent trends in global temperature that have been measured with particular accuracy since the late 1900s using satellite instrumentation tend to support the idea that global warming is taking place. According to studies performed by theU.S. Goddard Institute for Space Studies, during the time period from 1880 to the present the 10 warmest years recorded have occurred since 1997. The near record warmth of 2007 is all the more remarkable because the year was at a minimum ofsolar irradiance and the natural El Niño–La Niña cycle of the equatorial PacificOcean was in its cool phase. Although the coolest year since 2000, the year 2008 stillranks seventh to tenth of these record warm years.”

Answer: thank you. We are aware that, also from Italian Alpine Lakes long-term series of temperature data, inland aquatic ecosystems, including coastal lagoon, are accumulating heat. We believe that results of our study should be useful to stimulate further research linking climate change to coastal ecosystem functioning.

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The effects of hydrological extremes on denitrification, ~~DNRA~~ dissimilatory nitrate reduction to ammonium (DNRA) and mineralization in a coastal lagoon

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Abstract

Hydrological extremes of unusually high or low river discharge may ~~have profound effects on~~deeply affect the biogeochemistry of coastal ~~zones~~lagoons, but the effects are poorly explored. In this study, microbial nitrogen processes were analyzed ~~by means of~~through intact core incubations and~~ the~~ ^{15}N-isotope addition at three sites ~~(a freshwater and two marine onesthe first located at the … of the main river input and the other two)~~ in the~~a~~ eutrophic ~~lagoon~~Sacca di Goro lagoon (Northern Adriatic Sea) both under high discharge (spring) and after prolonged low discharge (late-summer) of the main freshwater inputs.

Under high discharge/nitrate load, denitrification was the leading process and there was ~~little to~~ no internal recycling~~ was observed~~. The ~~freshwater~~ site located at the mouth of the main freshwater input and characterized by low salinity exhibited the highest denitrification rate (up to 1150 ± 81 µmol N m^{-2} h^{-1}), mostly sustained by nitrification stimulated by burrowing macrofauna. In contrast, we recorded high internal recycling under low discharge, when denitrification dropped at all sites due to low nitrate concentrations, reduced bioturbation and nitrification. The highest recycling was measured at the ~~marine~~ sites close to the sea entrance and characterized by high salinity and particularly at the clams cultivated area (up to 1003 ± 70 µmol N m^{-2} h^{-1}). At this site, internal recycling was sustained by ammonification of biodeposits, bivalve excretion and dissimilatory nitrate reduction to ammonium (DNRA), which~~ became an important path of N recycling, representing~~ represented~~on average~~ 30% of nitrate reduction.

Flash floods and high nitrate loads may overwhelm the denitrification capacity of the lagoon due to the reduced residence time and to the saturation of microbial enzymatic activity, resulting in high transport of nitrate to the sea~~Flash floods determine an enhancement of N loads may offset denitrification due to reduced residence time and saturation of microbial enzymatic activity, and a reduction of the residence time, decreasing the denitrification capacity of the lagoon and resulting in high transport of nitrate to the open sea~~. Prolonged dry periods favor large internal ~~regeneration~~recycling, due to a combination of high temperatures, low oxygen solubility and low bioturbation, which may prolong the extent of algal blooms with negative ~~feedbacks~~ effects on lagoon biogeochemical services. We conclude that ~~extreme~~ hydrological ~~events~~extremes, which are expected to become more frequent under climate change scenarios, strongly alter N cycling in coastal sediments.

Keywords: coastal lagoon, hydrological extremes, benthic fluxes, ~~nitrogen, sedimentary fluxes, estuaries, climate hydrological extremesnitrogen l~~nitrate respiration, ~~nitrogen~~ nitrogen recycling ~~benthic fluxes~~

1 Introduction

Human activities, through increased fertilizer production and combustion, have more than doubled the load of bioavailable nitrogen (N) to coastal areas (37-66 Tg total N yr^{-1}), that have ~~leading~~ led to widespread eutrophication, hypoxia, and anoxia (Nixon, 1995; Cloern, 2001; Diaz and Rosenberg, 2008). Management policies have been more effective in regulating point nutrient sources of aquatic ecosystems compared to~~than diffuse nutrient sources to aquatic ecosystems~~ diffuse ones (Boesch, 2002; Palmeri et al., 2005). Recent analyses suggest that in most European watersheds the total N exported to the sea has either stayed the same or even increased, despite the nitrate reduction directive which was established some 30 years ago (Vybernaite-Lubiene et al., 2017; Viaroli et al., 2018). This situation can be worsened by ~~the effects of~~ climate change, which affects the magnitude and the seasonal pattern of precipitation, increasing the frequency of high discharge, flash flood periods as well as phases with no precipitation and minimum river flow, with negative impacts on the ecosystem functioning (Trenberth, 2005; Lehner et al., 2006; Zhang et al., 2019). Different studies reported that the consequences of these sharp fluctuations of water supply, combined with increasing temperature and changes in the pattern of salinity due to sea level rise, may be amplified in transitional systems, such as estuaries and coastal lagoons (Anthony et al., 2009; Ferrarin et al., 2014). Understanding the net effect of such changes in these systems is particularly important, as they play a crucial role in the retention and transformations of nutrients by removing approximately 25% of the total reactive N delivered from the watershed (Nixon, 1981; Asmala et al., 2017; Sharples et al., 2017).

Extreme rainfall events are predicted to increase the amount and affect the composition of nutrients exported from the watershed to transitional systems (Fig. 1) (Howarth et al., 2006; Chen et al., 2018). The enhanced load of nutrients may stimulate some processes, such as N removal through denitrification, whose rates increase with increasing nitrate (NO_3^-) in the water column, until saturating concentrations (Ogilvie et al., 1997; Dong et al., 2000). Following these events, however, the high river discharge leads to the decrease in water residence time~~within estuarine~~, shortening the processing time during which N can be repeatedly cycled through uptake by primary producers, sedimentation of organic matter and mineralization or ~~removed~~ removal ~~as inert gas~~ through coupled nitrification-denitrification (Nixon et al., 1996; Dettmann, 2001; Seitzinger et al., 2006). High runoff increases water column turbidity and reduces light penetration, affecting benthic primary producers activity (Pratt et al., 2014) and depressing their ability to regulate nutrient fluxes at the water-sediment interface (Risgaard- Petersen et al., 1994; Sundbäck et al., 2000). Enhanced transport of fluvial material may also alter significantly the structure and the functioning of the macrobenthic community, decreasing the biodiversity and the total biomass and favoring the establishment of opportunistic species (Ellis et al., 2002; Cardoso et al., 2008). The loss of specific functional groups strongly influences the ecosystem biogeochemistry because macrofauna, through bioturbation, feeding activity, excretion and biodeposition of labile organic matter, significantly alter N dynamics (Laverock et al., 2011; Stief, 2013). Burrowing benthic animals have contrasting effects on benthic processes stimulating N removal via nitrification and denitrification (Rysgaard et al., 1995; Moraes et al., 2018), or enhancing processes leading to ammonium (NH_4^+) recycling, as recently demonstrated for deep burrowing alien worms (Bonaglia et al., 2013; Benelli et al., 2019).

At the opposite situation, low freshwater discharge after prolonged drought~~ characterized by low freshwater discharge~~ seasonally decreases the amount of nutrients delivered to coastal areas and may decline the relative importance of external inputs compared to internal recycling~~ processes, as the main source of nutrients for primary producers in estuaries and coastal lagoon systems~~ (Fig.1) ~~to external inputs~~ (Howarth et al., 2000; Feyen and Dankers, 2009). Internal recycling is here defined as the sum of dissolved inorganic nitrogen fluxes (NH_4^+, nitrite and NO_3^-) directed from the benthic system to the water column and is the net result of different processes as ammonification, nitrification, denitrification, DNRA and excretion by macrofauna. Higher temperatures combined with low freshwater inflow, which characterize low rainfall periods, ~~will~~ lead to the increase in water residence time and contribute to water stratification~~ in estuarine systems~~, which in turn increases the extent of hypoxia or anoxia (Statham, 2012; Du et al., 2018; Hallett et al., 2018). The decrease in oxygen (O_2) concentration in the bottom water negatively affects biological communities and biogeochemical processes (Diaz and Rosenberg, 1995, 2008). Shift in redox conditions, which takes place under O_2 shortage, determines an increase in mineralization rates and release of NH_4^+ from the sediment (Nunnally et al., 2012; Roberts et al., 2012). Reduction of oxic layer stimulates denitrification by shortening the physical distance that nitrate (NO_3^-) must go through to reach the anoxic layer (Hietanen and Lukkari, 2007), but at the same time decreases the occurrence of nitrification and coupled nitrification-denitrification (Kemp et al., 2005; Conley et al., 2007; Roberts et al., 2012). Oxygen shortage may favor DNRA, a NO_3^-

reduction pathway alternative to denitrification (McCarthy et al., 2008; Jäntti and Hietanen, 2012). DNRA is a process that does not remove N from the system but instead recycles it to the water column in a bioavailable form (Burgin and Hamilton, 2007). This process is also favored by increased salinity and sulphate reduction, which occur as a result of long periods of low river discharge (Rysgaard et al., 1999; An and Gardner, 2002; Gardner et al., 2006; Giblin et al., 2010). The increment of water temperature and NH_4^+ concentration due to the combined effect of DNRA, increase in mineralization rates and decrease in nitrification, may favor primary producers activity offsetting efforts to contrast eutrophication and extending the duration of blooms (Conley et al., 2007).

The effects of climatic extremes on benthic N cycling are therefore multifaceted, site-specific and thus difficult to forecast (Najjar et al., 2010; Statham, 2012). In this study, we analyzed microbial N transformations in the Sacca di Goro, a eutrophic coastal lagoon connected to the Adriatic Sea (Northern Mediterranean Sea). The lagoon is located downstream of the Po River watershed, one of the most impacted areas in Europe (Viaroli et al., 2018) and in the past years it was affected by macroalgal blooms, followed by anoxic events ~~with consequent damages to the local economy~~ (Viaroli et al., 2006). Combined retrospective analysis and model simulations allow to predict for the Po River basin an increase in average and maximum temperatures (Coppola and Giorgi, 2010; Tibaldi et al., 2010) and a general decline in runoff (30-40% reduction), mainly in summer (June-August) (Coppola and Giorgi, 2010; Cozzi and Giani, 2011). Simultaneously, it is expected that the frequency and the intensity of extreme events will increase, with prolonged droughts and peaks of river discharge due to more severe and less frequent precipitation (Vezzoli et al., 2015). In this study, investigations were conducted to evaluate variations in benthic N dynamics in response to the recent climatic anomalies of 2019. A spring sampling was conducted in May, which was characterized by unusually heavy rainfall with values of cumulative precipitation nearly 2.5 times higher compared to the past 20 years. A late-summer campaign was conducted at the beginning of September, following a period characterized by high temperature and low river discharge. Summer temperatures in the Po River basin show a clear increasing trend from the 1970s (Brunetti et al.,2006). In the Sacca di Goro, during summer 2019, water temperatures exceeded 30°C for 8 days, whereas during the 2006 - 2018 period such threshold was exceeded for 3.5 days. We hypothesized that: (1) low salinity and high NO_3^- availability, together with high densities of burrowing macrofauna lead to high denitrification efficiency and low N recycling during spring; (2) high salinities and low NO_3^- availability, together with low bioturbation lead to decreased denitrification efficiency and high N recycling during late-summer; (3) hydrological extremes lead to the loss of ecosystem services such as N ~~loss~~removal.

2 Material and methods

2.1 Study area

The Sacca di Goro is a shallow (average depth 1.5 m) microtidal lagoon (27 km^2) located in the southern part of the Po River Delta (NE Italy) (Fig. 2~~1~~). The lagoon is connected to the Adriatic Sea through a 3 km wide mouth and receives freshwater inputs from the Po di Volano and Po di Goro, and from three minor artificial channels (Collettore Giralda, Canal Bianco, Canale Bonello). The salinity is highly variable due to fluctuations in freshwater and marine inflows, with the widest daily variations in the area near the sea mouth, which can be up to 10. The lagoon is intensively exploited for clam farming (*Ruditapes philippinarum*) at present covering 41% of the bottom surface with densities higher than 500 ind. m^{-2} (Bartoli et al., 2016). Heavy loads of NO_3^- generated in the Po River basin have been considered the main cause for seaweeds blooms (*Ulva* sp., *Gracilaria* sp. and *Cladophora* sp.) that characterize the lagoon since the 1980s (Viaroli et al., 2006). Besides external loads, different studies stressed the importance of clams activity on internal recycling. High densities of filter feeders determine the delivery of high amounts of organic matter on the sediment surfaces, as faeces and pseudofaeces. This labile substratum fuels microbial activity, increasing benthic O_2 uptake and nutrient recycling, which in turn sustain primary producers activity (Bartoli et al., 2001; Nizzoli et al., 2006; Viaroli et al., 2006; Naldi et al., 2020). ~~During specific meteorological conditions (high temperature, low wind)~~During periods characterized by high temperature and low wind, macroalgal blooms might be followed by ~~dystrophic events~~a sudden collapse of their production. The decomposition of macroalgal mats leads to~~, … of organic matter started to decompose causing~~ anoxia and determines the onset of anaerobic processes and the release of sulfides to the water column. ~~causing massive damages~~These phenomena, in the last 30 years, caused massive damage to the ecosystem and the local economy (Viaroli et al., 2006).

The lagoon is generally divided into three areas based on sedimentary and hydrological characteristics (Marinov et al., 2006). The western portion is located at the mouth of the Po di Volano and is characterized by the highest nutrients concentration and the lowest salinity; the sediments are muddy-clayish, with a high organic matter content and are highly bioturbated by surface and deep burrowers, such as *Corophium insidiosum* and *Alitta succinea* (Bartoli et al., 2012; Politi et al., 2019). The eastern part, called Valle di Gorino, is shallow (average depth 0.6 m), it is characterized by muddy-sandy sediments and it receives freshwater inputs from different locks connecting the lagoon with the Po di Goro. This sheltered area~~,~~ ~~shallow and sheltered~~ is characterized by slow water exchange and generally by higher temperature compared to the rest of the lagoon. The central portion, which hosts most of the licensed areas for clams farming, is affected by tidal exchanges that determine more intense water circulation and prevent the~~is continuously flushed by marine seawater, which prevents~~ organic matter accumulation~~ and forms sandy deposits~~. Specific meteorological conditions, for example prevailing Scirocco winds, may contrast tidal forcing and increase stagnation also in this area, with anoxic risk for clams.

In the present study, sampling was carried out at three sites located within these representative areas: Giralda ("GI", 44° 49' N 12°16' E) in the western area, Gorino ("GO", 44° 48' N 12°19' E) at the edge of the Valle di Gorino, and a site near the sea mouth within the ~~Farmed Area~~farmed area ("FA", 44° 48' N 12°18' E) (Fig. 2~~1~~). The distance between stations was between 1.5 and 4.5 Km.

To understand ongoing changes in the Sacca di Goro lagoon in the context of climate anomalies, historical monthly averages of water temperature and salinity were retrieved for Gorino from samplings carried out by Parma University (1987 to 1991; Bencivelli et al., 1991, 1993) and from the Regional Agency of Environmental Protection of the Emilia Romagna Region – ARPAE (https://simc.arpae.it/dext3r/) (2006 to 2019). Precipitation data related to the area of ~~the closing section of the Po basin at Pontelagoscuro~~the Po River Delta were also retrieved for the period 2000-2019 from ARPAE.

2.2 Sediments sampling ~~ample collection~~ and benthic flux measurements

Samplings were carried out on May 27th (spring campaign) and on September 2nd (summer campaign) 2019. At each site intact sediment cores (Plexiglass liners, i.d. 8.4 cm, length 30 cm) were randomly collected by hand for benthic fluxes (8 cores at Farmed Area and 4 cores per site at Giralda and Gorino in spring; 8 cores per site in summer) and denitrification and DNRA measurements (8 cores per site at Giralda and Farmed Area and 4 cores at Gorino in spring; 8 cores per site in summer). Concurrently, 6 intact sediment cores

(Plexiglass liners, i.d. 4.6 cm, length 20 cm) were collected at each site for the sediment characterization and the determination of pore water NH_4^+ concentration. Water column temperature, pH, salinity and O_2 concentration were measured at the three sites by means of a ~~n YSI 556~~ multiple probe (YSI Instruments, Mod 556). In addition, from each site, 80 L of water was collected for cores maintenance, pre-incubation, and incubation periods. The intact cores were immediately submerged with the top open in a box filled with *in situ* water, cooled with ice packs to slow microbial activity and transferred to the laboratory within a couple of hours. Once in the laboratory, the cores were placed into three large tanks, one for each site, filled with unfiltered water, maintained at *in situ* temperature and they were left to settle overnight ~~containing in situ unfiltered~~~~renewed~~ ~~water~~, ~~at *in situ* O_2 concentration and temperature~~ (Dalsgaard et al., 2000). The water in the tanks was continuously aerated by aquarium pumps. Each core was equipped with a Teflon-coated magnet rotating at 40 rpm driven by a central magnet. Each magnet was suspended about 6 cm above the sediment surface ~~in order~~ to mix the water column, avoiding resuspension.

After overnight pre-incubations, the water within the tanks was replaced and the larger cores were incubated in the dark (Dalsgaard et al., 2000). Incubations for aerobic respiration and net N_2 and nutrient fluxes lasted 2-3 hours in order to keep O_2 concentration within 20% of initial values and started when gas-tight lids were positioned on the top of the cores (Dalsgaard et al., 2000). Dissolved O_2 concentration was measured with a microelectrode (OX-50, Unisense A/S, DK), whereas water samples were collected from each tank (4 replicates) at the beginning of incubation and from the water phase of each core at the end of incubation. In both cases, an aliquot of water was transferred and flushed to 12-ml exetainers (Exetainer®, Labco Limited, UK), and fixed with 100 μL~~l~~ of 7 M $ZnCl_2$ to stop microbial activity for N_2 determination. Another aliquot of 20 ml was filtered (Whatman GF/F glass fiber filters) and transferred to scintillation vials to analyze dissolved inorganic N compounds via standard spectrophotometric techniques. Samples for N_2 were analyzed to determine changes in N_2:Ar ratios via a membrane inlet mass spectrometer (MIMS) equipped with a copper reduction column maintained at 600 °C (~~MIMS,~~ Bay instrument, MD, USA) (Kana et al., 1994). Ammonium was determined using salicylate and hypochlorite in the presence of sodium nitroprussiate (Bower and Holm-Hansen, 1980). Nitrate was determined after reduction to nitrite (NO_2^-) in the presence of cadmium, and NO_2^- was determined using sulphanilamide and N-(1-

naphthyl)ethylenediamine (APHA, 1992; Golterman et al., 1978). Gas and nutrient fluxes at the sediment-water interface were calculated according to the equation below:

$$F_x = \frac{(C_f - C_i) \times V}{A \times t}$$

where F_x is the flux of the chemical species x expressed in µmol or mmol m^{-2} h^{-1}, C_i and C_f (µM or mM) are concentration values of the chemical species x at the beginning and at the end of incubation, respectively, V is the water column volume (L), A (m^2) is the sediment surface and t (h) is the incubation time.

2.3 Measurement of denitrification and DNRA rates

After the first incubation, the water in the tanks was renewed and the open cores were left submerged for 2 hours in *in situ* and well-mixed water. Thereafter, a second incubation was performed to quantify the denitrification rates with the isotope pairing technique (IPT, Nielsen, 1992). The water in the tanks was lowered just below the top of the cores and $^{15}NO_3^-$ from a stock solution of 20 mM $^{15}NO_3^-$ ($Na^{15}NO_3^-$, Sigma Aldrich) was added to the water phase of each core. When 8 cores were collected, labelled NO_3^- was added ~~in order~~ to have a final ^{15}N atom % of 50% (4 cores) and 100% (4 cores); when 4 cores were collected a 50% labelling was carried out. A water sample was collected from each core before and after the $^{15}NO_3^-$ addition to determine the ^{15}N-enrichment of the NO_3^- pools. Thereafter, the cores were capped and incubated for 2-3 hours in dark conditions as described for nutrient flux measurements. At the end of the incubation, the whole sediment column was mixed with the water column and homogenized. An aliquot of the slurry was transferred to 12-ml exetainers, allowing abundant overflow and fixed with 200 µL~~l~~ of 7 M $ZnCl_2$ to stop the microbial activity. The abundance of $^{29}N_2$ and $^{30}N_2$ was determined via MIMS. As the genuine $^{28}N_2$ production was independent from the $^{15}NO_3^-$ level we assumed that anammox contribution to N_2 production was negligible, as reported in previous denitrification measurements in the Sacca di Goro sediments (Moraes et al., 2018). Denitrification rates were calculated from the production of $^{29}N_2$ (p29) and $^{30}N_2$ (p30) as follows:

$$D_{15} = p29 + 2p30$$

$$D_{14} = D_{15} \times (p29/2p30)$$

where D_{15} is the denitrification rate of the $^{15}NO_3^-$, whereas D_{14} is the denitrification rate of $^{14}NO_3^-$. From the total denitrification rate, the denitrification of nitrate diffusing to the anoxic layer from the water column (D_w) and the denitrification of nitrate produced within the sediments due to nitrification (D_n) were calculated as described by Nielsen (1992):

$$D_w = (^{14}NO_3^- / ^{15}NO_3^-) \times D_{15}$$

$$D_n = D_{14} - D_w$$

where $^{14}NO_3^-$ is the ambient nitrate concentration (µM) and $^{15}NO_3^-$ is the concentration of labelled nitrate added to the cores.

Denitrification efficiency (DE), defined as the percentage of total processed inorganic N released as N_2, was calculated according to Eyre and Ferguson (2009) as:

$$DE = \frac{D_{tot}}{DIN + D_{tot}}$$

where D_{tot} is total denitrification (D_w+D_n) and DIN represents the sum of dissolved inorganic N fluxes (NH_4^++NO_2^-+NO_3^-) directed from the sediment to the water column (effluxes)~~ of inorganic N compounds (NH_4^++NO_2^-+NO_3^-)~~.

Within the same denitrification experiment, an additional aliquot of the slurred sediment (30 ml) was collected to determine the rates of DNRA. The samples were transferred to 50-ml falcon tubes and treated with KCl (2 M) for the determination of the exchangeable ammonium pool and the $^{15}NH_4^+$ fraction. Briefly, tubes were shaken for 1 h, then centrifuged (1800 rpm for 15 min) and the supernatant was filtered (GF/F glass fiber filters) into 20-ml scintillation vials for later analyses. These samples were purged with helium for 10 minutes, ~~in order~~ to eliminate $^{29}N_2$ and $^{30}N_2$ pools produced during the incubations. Samples were then transferred to exetainers and treated with alkaline hypobromite solution, to oxidize NH_4^+ to N_2 (Warembourg, 1993). The abundance of $^{29}N_2$ and $^{30}N_2$ was determined via MIMS. Assuming that DNRA occurs in the same sediment horizon as denitrification, total DNRA rates were calculated from the production of $^{15}NH_4^+$ ($p^{15}NH_4^+$), according to the equation reported in Risgaard-Petersen and Rysgaard (1995):

$$DNRA \;\; = p^{15}NH_4^+ \times (D_{14}/D_{15})$$

Total DNRA rates were divided into direct DNRA of NO_3^- from the water column ($DNRA_w$) and coupled DNRA ($DNRA_n$) and were calculated as follows:

$$DNRA_w \; = \; (^{14}NO_3^- / ^{15}NO_3^-) \times \; p^{15}NH_4^+$$

$$DNRA_n \; = \; DNRA \;\; - \; DNRA_w$$

At the end of the incubation, sediments from all cores were sieved (0.5 mm mesh size) ~~in order~~ to ~~determine the abundance and the biomass of~~retrieve the macrofauna. Organisms were sorted under a stereomicroscope (Leica S8 APO, amplification 8x), identified by dichotomous keys (http://species-identification.org/identify_species.php) and by scientific papers (Wägele et al., 1981) to the lowest possible taxonomic level and counted. The identification was strengthened by the comparison with previous studies on the macrobenthic community of the Sacca di Goro (Mistri et al., 2001, Ludovisi et al., 2013, Politi et al., 2019). For each species, the dry weight was determined after drying at 80°C for 48 h. For the clams, shells were removed, and only flesh weight was measured.

2.4 Sediment and pore water characterization

The six additional sediment cores were extruded and sliced in five layers: 0-1, 1-2, 2-3, 3-5 and 5-10 cm for physical and chemical sediment characterization. Briefly, in half of the cores, the slices were rapidly homogenized, and subsamples of 5 ml were collected using cut-off syringes, to determine physical properties. Sediment porosity was determined from the loss of wet weight after 48 h at 70 °C. Later, sediments were analyzed for carbon (C) and N content and their isotopic ~~signatures~~ composition in the upper 0-2 cm sediment layer with a mass spectrometer (Thermo Scientific Delta V) coupled with element analyzer (FlashEA 1112, Thermo Electron Corporation) at the Center for Physical Sciences and Technology (Lithuania). Before measurements samples were grinded and acidified with 1 N HCl in order to remove carbonates. The last three cores were sliced ~~in order~~ to analyze the vertical distribution of pore water NH_4^+ concentration. Water was extracted by centrifugation of wet sediment (1800 rpm for 15 min), the supernatant was then filtered (Whatman GF/F glass fiber filters) and analyzed to determine NH_4^+ concentration as described in the section 2.2.

2.5 Rivers discharge and reactive N loadings

The Consorzio di Bonifica Pianura di Ferrara provided data on Po di Volano, Collettore Giralda, Canal Bianco, and Canale Bonello discharges. This authority continuously monitors the water discharge and provides daily or weekly average values. River discharges for Po di Goro were not available, then mean annual data derived from the Final Report of the Hydrodynamic Modelling System of the Sacca di Goro lagoon (Arpae-Emilia Romagna, Bologna University, CNR-Ismar, 2019) were used. It was assumed that other diffuse sources were negligible. At each tributary, water samples were collected in triplicates in May and September and immediately filtered into 20-ml vials for NH_4^+, NO_2^- and NO_3^- determination as described in the section 2.2. Sampling stations were located at a certain distance from the mouth of the canals to minimize the variability due to marine water intrusion. The daily load of dissolved inorganic N was obtained by multiplying the concentration measured at each sampling date by the mean daily discharge. The latter was calculated from monthly data of May and September.

2.6 Statistical analysis

Two-way analysis of variance (ANOVA) was used to assess the significance of sites and seasons in explaining differences among benthic net fluxes, denitrification and DNRA rates. The normality and the homogeneity of variance were checked using the Shapiro-Wilk test and the Levene median test, respectively. If significant heteroscedasticity was found, data were log-transformed. Pairwise multiple comparison of means was carried out using the Tukey's test for all the significant factors. Statistical significance was set at p level lower than 0.05. All statistical analyses were performed with R software v. 3.5.1 (R Core Team, 2018). Graphs were made with Sigma Plot 11.0.

3 Results

3.1 General features of water column, sediments and macrofauna

The concentration of dissolved inorganic N, temperature and salinity displayed strong spatial and temporal variability influenced by different hydrological regimes. During spring the high freshwater discharge associated ~~to~~with unusually heavy rainfall, resulted in low salinity, low temperatures and high NO_3^- concentrations (Table 1). During summer drought, water temperatures increased by 3-6 °C as compared to spring, O_2 saturation decreased by 20% and NO_3^- concentrations decreased at all sites by a factor of 4.

Salinities reflected limited riverine discharge with values close to marine~~marine values~~ measured at Gorino and Farmed Area (Table 1).

Sediment properties revealed sharp differences between Giralda and the other two sites, mainly due to the riverine influence~~the freshwater and the marine sites~~. This site was characterized by muddy-clayish sediments with higher porosity and higher C and N content, which decreased in summer. Particulate matter displayed more depleted δ^{13}C and δ^{15}N signatures and higher C:N compared to ~~marine sandy sites~~sites located closer to the sea entrance (Table 2).

At Giralda~~, and to a minor extent at the marine sites~~, vertical profiles of pore water NH_4^+ revealed an increasing trend, with the highest values observed in summer, when concentration peaked at ~600 µM at the 5-10 cm layer (Fig. 3~~2~~). At the ~~marine~~ other sites, NH_4^+ concentration was less variable along the depth profiles, and increased from spring to summer at Gorino, whereas it showed similar seasonal values at Farmed Area. In both seasons, pore water NH_4^+ concentration exceeded that in the bottom water, suggesting upwards diffusive fluxes, generally increasing from spring to summer and with gradients peaking in the warmest season at the sediment-water interface (Fig. 3~~2~~).

The ~~A~~abundance of dominating macrofaunal taxonomic groups differed among sites and seasons. In spring at Giralda the sediments appeared heavily bioturbated, particularly by *C. insidiosum* and *A. succinea*, with densities of 7,071 ± 260 and 2,226 ± 69 ind. m^{-2} (averages ± standard errors), respectively, which accounted on average for 80% of the total biomass. In summer the densities of these organisms drastically dropped to 105 ± 10 and 270 ± 17 ind. m^{-2} for *C. insidiosum* and *A. succinea*, respectively. At Gorino the biodiversity and the abundance of the macrobenthic community were relatively low and mainly dominated by *A. succinea* (361 ± 20 ind. m^{-2} in spring, 135 ± 15 ind. m^{-2} in summer) and by the isopod *Cyathura carinata* (180 ± 30 ind. m^{-2} in spring and 1,865 ± 81 ind. m^{-2} in summer). Within Farmed Area *R. philippinarum* constituted more than 95% of the total macrofauna biomass, with densities of 768 ± 56 and 407 ± 10 ind. m^{-2} in spring and summer, respectively.

3.2 Inorganic N fluxes at the sediment-water interface

Inorganic N fluxes at the sediment-water interface varied among sites depending on seasons ($p < 0.001$, Table S1 in Supplementary Material). In all three sites and ~~in~~ both seasons, sediments were net NH_4^+

sources, with Giralda as exception during spring (Fig. 4~~3~~a). At all sites NH_4^+ fluxes significantly increased (*p* < 0.001, Table S1) from spring (average rate 146 ± 59 µmol m^{-2} h^{-1}) to summer (average rate 726 ± 73 µmol m^{-2} h^{-1}), and Farmed Area displayed the highest NH_4^+ recycling in both seasons.

Nitrite and NO_3^- were more erratic without clear patterns among sites and seasons (Table S1~~3~~). In spring, high water column NO_3^- concentrations resulted in large uptake (average rate -730 ± 150 µmol m^{-2} h^{-1}) peaking at Giralda (Tukey pairwise comparison, *p* < 0.001; Fig. 4~~3~~b). Giralda and Farmed Area in summer turned into net NO_3^- sources, with fluxes of 445 ± 135 and 168 ± 55 µmol m^{-2} h^{-1}, respectively, while Gorino displayed values comparable to the spring season. Fluxes of NO_2^- were always nearly one order of magnitude lower than those of NO_3^-. In both seasons sediments from the three sites released NO_2^- to the overlying bottom water, with Farmed Area as exception in spring (Fig. 4~~3~~c).

Measured net N_2 fluxes were largely positive suggesting the dominance of denitrification over N_2-fixation (Fig. ~~3~~4d). In spring sediment at Giralda displayed the highest N_2 effluxes (1,150 ± 81 µmol N m^{-2} h^{-1}), exceeding by a factor of 5 rates measured at Gorino and Farmed Area. In summer there was a general decline in net N_2 production, in particular at Giralda, which showed significant differences between seasons (Tukey pairwise comparison, *p* < 0.001).

3.3 Aerobic respiration, denitrification and DNRA rates

Benthic O_2 uptake ranged from -1.74 to -8.77 mmol m^{-2} h^{-1} and significantly varied among the three sites in the two seasons (Fig. 5~~4~~a, *p* < 0.001, Table S2 in Supplementary Material). In spring, Giralda displayed the highest O_2 uptake (-6.78 ± 0.32 mmol m^{-2} h^{-1}), which~~that~~ almost halved in summer despite the increase in temperature. Gorino and Farmed Area were characterized by an opposite seasonal trend, with higher fluxes measured in summer, and peaking at Farmed Area (-8.77 ± 0.87 mmol m^{-2} h^{-1}; Tukey pairwise comparison, *p* < 0.001).

Total denitrification rates ($D_{tot} = D_w + D_n$) were more elevated in spring at all sites, with the highest rates measured at Giralda (Tukey pairwise comparison, *p* < 0.001, Fig. 5~~4~~b). At this site denitrification was supported mainly by coupled nitrification-denitrification (625 ± 50 µmol N m^{-2} h^{-1}) and to a lesser extent by NO_3^- diffusing from the water column (442 ± 64 µmol N m^{-2} h^{-1}). Spring rates of D_n and D_w were ~5 times lower at Gorino and Farmed Area as compared to Giralda (Tukey pairwise comparison, *p* < 0.001). Despite the peak of denitrification matched with the peak of NO_3^- concentration in the water column, the D_n prevailed over the D_w in all the investigated sites, contributing nearly 60% of total denitrification (Table 3~~5~~). In summer at all three sites total denitrification rates decreased compared to spring (Tukey pairwise comparison, $p < 0.001$ for Giralda and Farmed Area). The greater change occurred at Giralda, where D_w and D_n rates dropped to 85 ± 18 and 132 ± 43 µmol N m^{-2} h^{-1}, respectively. The share of denitrification supported by nitrification was more variable in summer, ranging from 53 to 73% at Gorino and Farmed Area, respectively (Table 3~~5~~). In spring denitrification efficiency was generally high at all sites (>92%), whereas it substantially dropped in summer (Table 3~~5~~).

The highest rates of DNRA were found at Giralda both in spring and summer (Fig. 5~~4~~c). At all three sites values tended to increase in summer, but only at Gorino seasonal differences were significant (Tukey pairwise comparison, $p < 0.001$). In spring at Farmed Area DNRA represented 10% of total NO_3^- reduction pathways, whereas at Giralda and Gorino it represented a minor portion. During summer the share of DNRA to NO_3^- reduction increased at all sites and reached nearly 33% at ~~the two more marine sites~~Gorino and Farmed Area (Table 3~~5~~).

3.4 External loads versus internal removal and recycling

To compare the magnitude of external loads and internal processes, total DIN delivered from the ~~river~~lagoon watershed were normalized by the lagoon total surface, whereas removal and recycling rates were calculated by averaging denitrification rates and DIN effluxes measured at each sampling site. During spring the load of DIN ~~delivered to the Sacca di Goro from the watershed~~ was 27.25 ± 1.30 mmol m^{-2} d^{-1} (average ± standard error~~s~~). About 40% of the imported N ~~delivered to the Sacca di Goro from the watershed~~ was removed via denitrification (11.86 ± 1.02 mmol m^{-2} d^{-1}), whereas inorganic N recycling from sediments was negligible ~~(Table 6)~~. In late-summer there was a steep decline in the riverine DIN load that decreased by a factor of ~3 (9.63 ± 0.80 mmol m^{-2} d^{-1}), mainly due to lower discharge and decreased NO_3^- concentrations. The amount of inorganic N recycled from sediments increased and doubled the external inputs~~, reaching values~~averaging ~~of~~ 20.18 ± 3.69 mmol m^{-2} d^{-1}, whereas N removal via denitrification accounted for 9% of total DIN load (sum of external input and internal recycling), corresponding to 2.70 ± 0.59 mmol m^{-2} d^{-1} ~~(sum of external input and internal regeneration)~~.

4 Discussion

4.1 *Temporal and spatial variability of N and O_2 dynamics in the Sacca di Goro lagoon*

Our results indicate that the lagoon was predominantly removing N through benthic denitrification under high river discharge in spring, while it was recycling N via DNRA and remineralization under low discharge in late-summer. In spring, N-cycling was strongly influenced by the high freshwater discharge and the high NO_3^- load. The nutrient loads delivered from the Po River basin and from the Po di Volano sub-basin were monitored in different studies from the 1990s (Naldi et al., 2010, Viaroli et al., 2018, Castaldelli et al., 2013, 2020). Dissolved inorganic nitrogen load displays a strong seasonality, with summer minima and extremely high late-winter peaks. Nitrate, which represents on average > 75% of total DIN load, is directly related to the water discharge, with wide inter-annual ~~oscillations~~variations, from low values in dry years to peaks in wet years (Naldi et al., 2010; Viaroli et al., 2018). The spring load determined in this study was in the higher range of values previously reported ~~in previous literature~~ for the same season (Castaldelli et al., 2013, Viaroli et al., 2013). Under these circumstances denitrification represented the leading process (Fig. 6), with total rates similar to values reported for other shallow estuarine systems in the wet season (Seitzinger, 1988; Ogilvie et al., 1997; Dong et al., 2000)~~. High denitrification rates were primarily related to increased NO_3^- availability, as reported for other shallow estuarine systems in the wet season (Bruesewitz et al., 2013)~~. Coastal lagoons act as benthic filters and regulate the supply of N both via denitrification and via the uptake of benthic primary producers (Risgaard-Petersen, 2003). Even though in this study only processes under dark conditions were analyzed, during spring photosynthetic activity of microphytobenthos and its nutrient uptake were~~activity was~~ likely suppressed by the enhanced water column turbidity, due to the delivery of suspended solid matter, and dissimilative processes represented the main pathway of N removal (Anderson et al., 2013). Despite the elevated NO_3^- availability in the water column, approximately 60% of denitrification was coupled to nitrification, indicating high sediment nitrification rates. At Giralda the elevated nitrification is demonstrated to be associated with the high abundances of *C. insidiosum*, which via continuous ventilation of its ‘U’-shaped burrows, pumps oxic water into the sediments, leading to the oxidation of pore water NH_4^+ in the upper sediment layers (Pelegrì and Blackburn, 1994; Moraes et al., 2018). Also, the presence of *A. succinea*, through the construction of dense burrow networks could enhance NH_4^+ mobilization from deep to surface sediments, stimulating nitrification (Nizzoli et al., 2007). Compared to Giralda, Gorino and Farmed

Area were characterized by lower denitrification rates, due both to the lower NO_3^- availability and to the limited bioturbation activity. These two sites, however, showed similar values of total denitrification, suggesting no effects of clam biomass on this process, a result that is in agreement with previous studies conducted in the same sites of the Sacca di Goro (Murphy et al., 2018). Despite a small amount of NO_3^- ~~was~~ being reduced to NH_4^+ via DNRA, denitrification was the main pathway of NO_3^- reduction. At Farmed Area the contribution of DNRA to nitrate reduction processes slightly increased compared to the two other sites, probably due to the larger availability of labile organic matter in the form of clam biodeposits (Nizzoli et al., 2006). However, the increase in NH_4^+ efflux derived from DNRA was negligible if compared to direct clam excretion, which was estimated to contribute from 63 to 154% of total NH_4^+ fluxes. Such percentages were calculated multiplying biomass-specific excretion rates of clams (Welsh et al., 2015 and Murphy et al., 2018) by the biomass of the clams retrieved in our experiments. During spring therefore, high freshwater discharge resulted in the dominance of denitrification over recycling via mineralization, clam excretion and DNRA.

After few months of low river discharge, during late summer, the elevated temperatures and the low O_2 concentration led to a shift of N processes from the dominance of removal to recycling. Generally, the reduced state of sediments was evidenced by an increased sediment O_2 uptake and a higher net release of NH_4^+. The latter was due to a combination of factors, including high mineralization rates, the disconnection between N removal (via coupled nitrification–denitrification) and mineralization, and the enhancement of DNRA (Kemp et al., 2005; Roberts et al., 2012). The direct contribution of clam metabolism accounted for 21–42% of the net NH_4^+ fluxes, suggesting the dominance of microbial processes also at Farmed Area. Denitrification rates dropped compared to spring values and showed a decreasing trend along with the 4-folds drop in NO_3^- concentration from the western ~~more freshwater~~ corner towards the mouth of the lagoon, mainly driven by D_w. At Giralda the decrease in the coupled nitrification-denitrification was mainly due to the decline in bioturbators abundance, likely due to high temperatures and low O_2 concentration (Pitacco et al., 2018). At Gorino and Farmed Area the simultaneous decrease in denitrification rates and increase in the relative contribution of DNRA may depend on several factors including the increment of salinity (Giblin et al., 2010), the ~~increase~~ higher ratio of labile organic carbon to NO_3^- electron acceptors ~~concentration ratio~~ (Tiedje, 1988; Nizzoli et al., 2010), and the availability of reductants as sulfides (Brunet and Garcia-Gil, 1996, Caffrey et al., 2019) and Fe^{2+} (Robertson et al., 2016). Despite all these factors would be expected to favor DNRA over denitrification, the~~DNRA rates and their~~ ~~relative~~ contribution of this process to total NO_3^- reduction was lower compared to ~~rates~~ values previously reported for temperate shallow estuaries, where it can equal or exceed denitrification (An and Gardner, 2002; Gardned et al., 2006; Murphy et al., 2018). As a consequence, denitrification remained the dominant process (Murphy et al., 2018).

Interestingly, denitrification efficiency (DE) shifted from a maximum of 100% in spring to a minimum of 4% in summer. Eyre and Ferguson (2009) reported the highest DE (~70%) in sediments with moderate organic carbon enrichment and inorganic carbon fluxes ranging between 500 and 1,500 µmol m^{-2} h^{-1}. Since in the Sacca di Goro respiration rates were always higher than 1,500 µmol O_2 m^{-2} h^{-1}, much lower DE and elevated N recycling were expected. However, in spring DE ranged from 92 to 100%, likely sustained by the high rates of D_w. High NO_3^- concentrations in the water column ~~sustained therefore~~determined high DE, despite ~~and contrasted the effects of~~the elevated sediment organic content~~organic enrichment~~. The latter did not significantly affect the macrofauna community, which is composed by tolerant species supporting elevated nitrification rates. In summer DE was lower at the three sites, with values ranging from 4 to 54%, suggesting higher N recycling over denitrification. The increased temperatures, the inhibition of nitrification, the increase in DNRA rates and the lower macrofauna activity were likely the main factors determining this drop in summer. Similar results were found by Bartoli et al. (2012) in an annual study, with data from Gorino and Giralda.

Sediments at the three sites displayed elevated sediment O_2 uptake, in the higher range of those reported for other temperate estuaries (Cabrita and Brotas, 2000; Nizzoli et al., 2007; Gardner and McCarthy, 2009). At Giralda the high organic load was derived mainly from settled particles of fluvial origin, as demonstrated by the higher C:N ratio and by the lower C and N isotopic values, within the range reported for terrestrial organic matter (~-27‰ and of 3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, Lamb et al., 2006). ~~was allochthonous and generated from settled particles of fluvial origin.~~ However, even during the high discharge period, the C:N ratio of Giralda surface sediments suggested high organic matter quality, whereas material of terrestrial origins usually displays values significantly above 12 (Yamamuro, 2000). At this site in spring the highest O_2 uptake was associated with dense burrow network of amphipod *C. insidiosum*, where O_2 was likely employed for NH_4^+ oxidation via nitrification (Pelegrì and Blackburn, 1994; Moraes et al., 2018), whereas decreased in summer. Gorino and Farmed Area were characterized by C:N ratios close to the Redfield one and by higher $\delta^{13}C$ and $\delta^{15}N$ values, closer to values reported for marine systems, suggesting a progressive increase in the proportion of organic matter from autochthonous origins ~~, sedimentary organic matter pool was primarily dominated by settled primary producers~~(Yamamuro, 2000, Liu et al., 2006)~~ and by clam biodeposits in the cultivated area~~. The isotopic values, however, were more depleted compared to marine phytoplankton, particularly relative to $\delta^{13}C$ values, usually ranging from -22 to -19 ‰ (Lamb et al., 2006), suggesting that sedimentary organic matter still derived from the mixture of terrestrial derived material and marine material and from the accumulation of clam biodeposits ($\delta^{13}C$ value of about -23,2 ‰, Mazzola and Sarà, 2001). Sediment O_2 uptake in these sites showed a distinct seasonal pattern, with higher rates in summer likely regulated by water temperature (Vidal et al., 1997; Trimmer et al., 1998; Cabrita and Brotas, 2000). At Farmed Area the higher benthic respiration measured in summer was not related to clam density, since clam contribution to O_2 demand accounted for 21–42% of the total benthic respiration, whereas in spring it represented a major fraction (62–127%) (clam biomass-specific respiration rates were derived from Welsh et al. (2015) and Murphy et al. (2018)).

An inventory of sediment O_2 uptake rates measured with the same approach over the last 26 years does not suggest significant temporal trends likely due to the extremely variable contribution of macrofauna, including cultivated and naturally present species (Fig. 7~~6~~a). It is expected that 30 years of clams farming may have enriched the sediments with labile organic matter, resulting in increased microbial respiration. However, anoxic events were more frequent in the past due to macroalgal blooms that are now reduced in this and ~~in~~ other lagoons of ~~the~~ northern Italy (Sfriso et al., 2019). In the past, macroalgal blooms and their collapse determined huge inputs of organic matter to the sediments that uncoupled O_2 uptake and availability (Viaroli et al., 2006; Naldi et al., 2020). In the last years, the high frequency of heavy rainfall events and the frequent digging or silting operations, likely washed out or buried clam biodeposits, limiting their impacts on lagoon aging, benthic respiration and algal growth. Analogously, denitrification measurements performed with the IPT over the last 26 years do not reveal significant temporal trends (Fig. 7~~6~~b). This suggests either that the large variability of macrofauna density and community composition is responsible for the variability of measured rates, or that natural or anthropogenic disturbances as hydrological extremes set to zero lagoon aging, as reported for oxygen. In the heavily impacted Sacca di Goro, the seasonal shift between high and low discharge periods seems a better predictor of dominant processes regulating N benthic metabolism than long term chronosequences.

4.2 Projections in the context of climatic anomalies and hydrological extremes

The drivers of macroalgal blooms in the Sacca di Goro were studied for nearly three decades and were ~~addressed~~ associated to anthropogenic nutrient loads (Viaroli et al., 2018), nutrient recycling by clams (Bartoli et al., 2001, 2003; Naldi et al., 2020) and introduction of alien species (Milardi et al., 2020). This study provides evidence of a new possible driver of algal blooms. Hydrological extremes, which are expected to increase in the future, ~~Future changes in climatic conditions will~~ may ~~in fact~~ reduce the role of the Sacca di Goro as biogeochemical filter, with implications for NH_4^+ availability, in particular during prolonged dry periods and heat waves. Different studies targeting the effect of climate changes on nutrients focused on processes at the watershed scale (e.g. increased or decreased runoff) and the implications on hydrology (e.g. increased erosion, sharp reduction or increase in water residence time) (Marshall and Randhir, 2008; Howarth et al., 2012; Wagena et al., 2018); the present study has analyzed the overlooked effects of two climatic extremes on sedimentary N biogeochemistry (Howarth et al., 2000; Anderson et al., 2013; Bruesewitz et al., 2013).

Climate projections forecast the increment of frequency and severity of heavy rainfalls (Vezzoli et al., 2015). The timing of these events is crucial and may determine different effects on the dynamics of transitional areas. These events may contribute to alleviate hypoxia, for example by increased discharge, lower residence time and interruption of water column stratification (Rabalais et al., 2007). High freshwater discharge may contribute to flush phytoplankton downstream, even out of the estuary, and control algal blooms (Scavia et al., 2002; Phlips et al., 2020) or may enhance sediment resuspension resulting in the release of nutrient, favoring pelagic production, or in the oxidation of reduced pools (Vidal-Durà et al., 2018; Niemistö and Lund-Hanses, 2019). The increase in riverine runoff enhances the amount of N exported from the river watershed to the coastal areas, whereas high solid transport and turbidity limit primary producers assimilative N pathways; as a consequence with microbial denitrification remains the most important N-removing dissimilative process (Anderson et al., 2013). A positive relationship between NO_3^- availability and removal capacity has been found across a range of estuaries (Seitzinger et al., 2006). The consequent decrease in water residence time, however, determines a reduction of denitrification potential.

Historical data on discharge or residence time for the Sacca di Goro are not available, but they can be inferred from variations in salinity values (Fig. 8~~7~~a). Data from the last three decades suggest large variability in the mixing of fresh and marine waters, reflecting multiple management measures implemented to improve the hydrodynamic conditions of the lagoon. The salinity decrease recorded since 2015 was probably due to the opening of locks connecting the Po di Goro to improve water circulation. However, the low salinities of May, June, November and December 2019 were far below average values, suggesting the occurrence of heavy rainfalls likely affecting the water budget, residence time and nutrient concentrations at the whole lagoon ecosystem scale (Fig. 8~~7~~b).

Different models show that the N fraction that is denitrified may be estimated from the residence time (Nixon et al., 1996; Dettmann, 2001; Seitzinger et al., 2006). These models were usually developed with data at the monthly or annual scales, whereas over short time frames the relation between denitrification efficiency and water residence times is more challenging. The annual average residence time of the Sacca di Goro lagoon varies between 1 and 12 days, with minimal values in spring in the western portion and at the lagoon-sea interface (<5 days) (Arpae-Emilia Romagna, Bologna University, CNR-Ismar, 2019). The calculations presented in this study, based on a simple mass balance, show that in spring, despite high denitrification rates, nearly half of the N load entering the lagoon was removed via denitrification. According to the models proposed by Nixon et al. (1996) and Seitzinger et al. (2006), however, a residence time of 5 days determines the removal of 15% of the total N load and this amount may be even lower considering heavy precipitation and the high runoff detected in spring. Many factors, such as depth, water temperature, salinity, O_2 and NO_3^- concentrations, organic carbon in sediments, bioturbation and presence of primary producers may affect denitrification efficiency and produce significant deviations from expected values (Eyre and Ferguson, 2009). Shallow lagoons with marked zonation as the Sacca di Goro are paradigmatic examples where multiple, co-occurring factors regulate locally and set the upper limits of processes as denitrification and where the same factors may undergo sharp spatial (e.g. among stations) and temporal variations (e.g. among wet and dry periods).

A further increase in river discharge and, consequently, in nutrient amount, may also determine the saturation of the denitrification capacity of transitional areas. Nitrate removal capacity increases with N load, up to the saturation concentrations and asymptotic rates were reported in different studies at 200 µM (Ogilvie et al., 1997), 400 µM (Trimmer et al., 1998) and 600 µM (Dong et al., 2000). These values are much higher than those reported in this study in spring (56–113 µM). The threshold values, however, may be related to local variations in biological and environmental variables or due to differences in acute or chronic nutrient load. Future works should be aimed at quantifying the saturating NO_3^- concentrations for different areas of the Sacca di Goro lagoon and determine the factors that may influence these thresholds.

Different models reported for the Po River Basin that the most significant effects of climate changes are expected in summer, with a strong increase in very dry and low flow periods, followed by significant water deficit, and a large increase in temperature and heatwaves (Cozzi and Giani, 2011; Vezzoli et al., 2015). The analysis of available, historical data supports such predictions as water temperatures underwent an increasing trend since 1987, more pronounced in the summer months, from June to September. (Fig. 8~~7~~c).

Extremes in low summer discharge may stimulate river and estuarine eutrophication and large conversion of inorganic nutrients into phytoplankton and in its transfer to coastal areas (Howarth et al., 2000; Rossetti et al., 2009). Under these circumstances, most N would be delivered to sediment in particulate form, also due to ~~active ~~filter-feeders activity. Consequently, labile organic matter inputs may fuel sediment respiration, reducing O_2 concentration in the water column and the heath-dependent water column stratification may determine the onset of bottom water hypoxia. Depletion of electron acceptors such as O_2 and NO_3^- leads to the dominance of sulfate reduction with subsequent sulfide accumulation, determining the suppression of nitrification and denitrification and the further increase in DNRA rates (An and Gardner, 2002; Gardner et al., 2006; Giblin et al., 2010). The sulfide build-up may also derive by the increase in salinity, due to high temperature and low freshwater discharge. Oxygen depletion and sulfides affect also macrofauna diversity and abundance and produce positive feedbacks towards more chemically reduced sediment conditions and towards N-recycling dominance over denitrification (Diaz and Rosenberg, 1995; Magni et al., 2005). This was evidenced in a recent study carried out in the nearby Valli di Comacchio lagoon where it was demonstrated that heatwaves pose serious threats to the resilience capacity of the macrobenthic community, favoring short-lived, opportunistic forms (Pitacco et al., 2018). If elevated residence time and heatwaves promote large NH_4^+ recycling through the described cascade mechanisms, assimilation by primary producers may represent an important temporary retention of nutrients. Naldi et al. (2020) have demonstrated that in the shallow water of the Sacca di Goro, clams control phytoplankton primary production and displace the pelagic production at the benthic level. Under low discharge and high residence time, such top-down control can be even more efficient, resulting in transparent water, enriched by excreted nutrient, which may favor macroalgal growth, with ~~a~~ negative effects~~feedback~~ on the lagoon functioning and clam farming (Bartoli et al., 2001; Viaroli et al., 2003; Naldi et al., 2020).

5 Conclusions

In the eutrophic Sacca di Goro, as reported in other estuaries and coastal systems, hydrological extremes result in sharp seasonal transitions among dominant microbial processes driving benthic N cycle. The spring, high discharge period is dominated by denitrification due to high NO_3^-, high bioturbation and likely turbidity-limited primary producers-bacteria competition. However, a further increase in river discharge and ~~However,~~ N loads may determine the saturation of denitrification capacity, and the excess N may be partly exported to the Adriatic Sea, also due to low water residence time~~N loads may saturate denitrification and be exported to the Adriatic Sea, also due to low water residence time~~. The summer, low discharge period is dominated by NH_4^+ internal recycling, also sustained by increased DNRA, largely exceeding watershed N inputs. Superimposed to and interacting with the effects of hydrological extremes are local regulations of benthic N processes. At Giralda denitrification always dominated over DNRA as NO_3^- reduction pathway; this was particularly evident during spring mainly due to higher riverine influence, bioturbation and elevated NO_3^- concentrations. At Gorino and Farmed Area, in particular during summer, the higher salinity and microbial respiration likely explained the increase in DNRA contribution to NO_3^- demand and large NH_4^+ fluxes. Such NH_4^+ mobilization may increase the intensity, duration, and extent of algal blooms.

Results of this study suggest that both the spring and summer hydrological extremes scenarios reduce the effectiveness of lagoons as benthic filter and increase the amount of N exported to the open sea, either in form of NO_3^- or NH_4^+, with implications for coastal eutrophication. Management actions aiming at the reduction of eutrophication in transitional and coastal areas have to date targeted the decrease in nutrient loads from agriculture and civil sources. Further management actions should include new and effective tools for mitigating expected nutrient increase due to climate change.

Acknowledgements

We are grateful to Edoardo Turolla of the Centre for Mollusc Research (Goro) for the provision of laboratory facilities. We acknowledge the Consorzio di Bonifica Pianura di Ferrara for the provided data. We also wish to thank Dr. Fabio Vincenzi for valuable assistance in laboratory work at Ferrara University.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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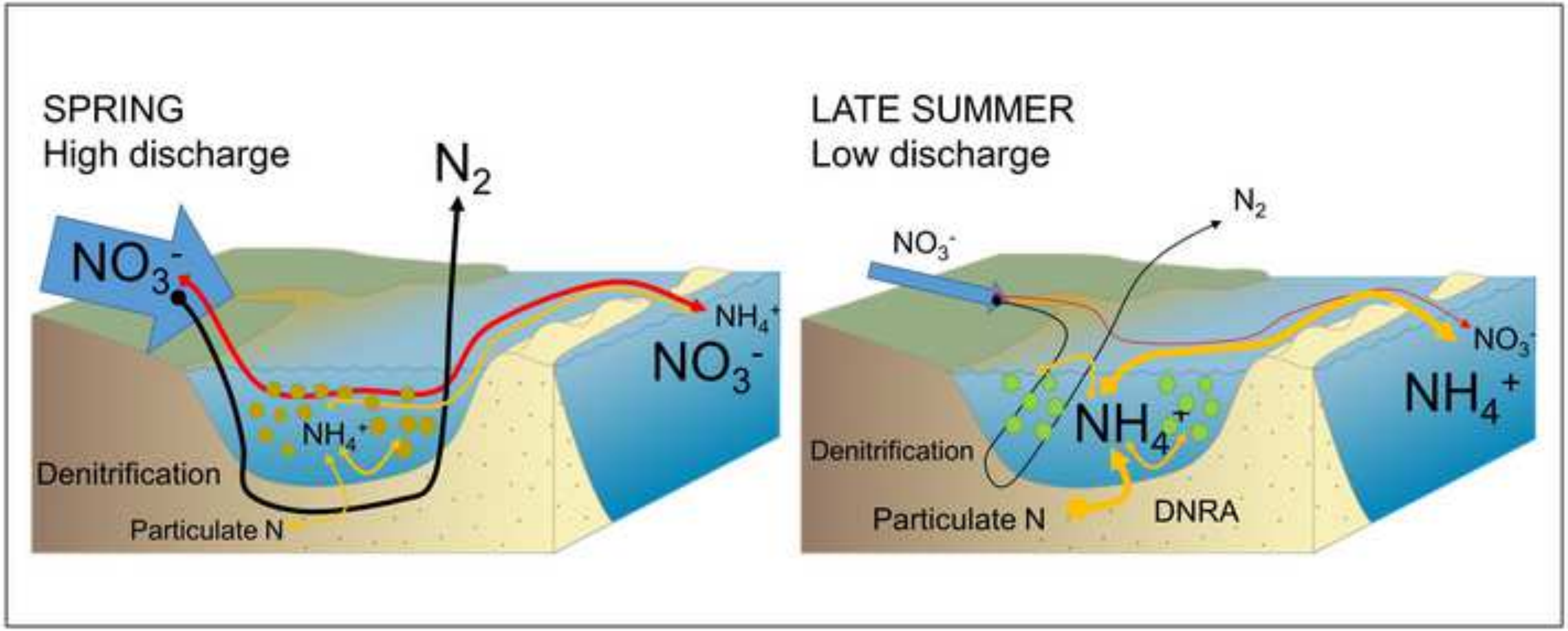
***Graphical Abstract**



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

- Benthic N dynamics were analyzed under high and after prolonged low river discharge
- The seasonal transition induced a shift from N removal to recycling dominance
- Heavy rainfalls reduce the residence time and the denitrification efficiency
- Dry periods favor large N regeneration and may favor algal blooms
- Hydrological extremes deeply alter benthic N cycling in coastal lagoons

The effects of hydrological extremes on denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and mineralization in a coastal lagoon

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Abstract

Hydrological extremes of unusually high or low river discharge may deeply affect the biogeochemistry of coastal lagoons, but the effects are poorly explored. In this study, microbial nitrogen processes were analyzed through intact core incubations and ^{15}N-isotope addition at three sites in the eutrophic Sacca di Goro lagoon (Northern Adriatic Sea) both under high discharge (spring) and after prolonged low discharge (late-summer) of the main freshwater inputs.

Under high discharge/nitrate load, denitrification was the leading process and there was no internal recycling. The site located at the mouth of the main freshwater input and characterized by low salinity exhibited the highest denitrification rate (up to 1150 ± 81 µmol N m-2 h-1), mostly sustained by nitrification stimulated by burrowing macrofauna. In contrast, we recorded high internal recycling under low discharge, when denitrification dropped at all sites due to low nitrate concentrations, reduced bioturbation and nitrification. The highest recycling was measured at the sites close to the sea entrance and characterized by high salinity and particularly at the clams cultivated area (up to 1003 ± 70 µmol N m-2 h-1). At this site, internal recycling was sustained by ammonification of biodeposits, bivalve excretion and dissimilatory nitrate reduction to ammonium (DNRA), which represented 30% of nitrate reduction.

Flash floods and high nitrate loads may overwhelm the denitrification capacity of the lagoon due to the reduced residence time and to the saturation of microbial enzymatic activity, resulting in high transport of nitrate to the sea. Prolonged dry periods favor large internal recycling, due to a combination of high temperatures, low oxygen solubility and low bioturbation, which may prolong the extent of algal blooms with negative effects on lagoon biogeochemical services. We conclude that hydrological extremes, which are expected to become more frequent under climate change scenarios, strongly alter N cycling in coastal sediments.

Keywords: coastal lagoon, hydrological extremes, benthic fluxes, nitrate respiration, nitrogen recycling

1 Introduction

Human activities, through increased fertilizer production and combustion, have more than doubled the load of bioavailable nitrogen (N) to coastal areas (37-66 Tg total N yr^{-1}), that have led to widespread eutrophication, hypoxia, and anoxia (Nixon, 1995; Cloern, 2001; Diaz and Rosenberg, 2008). Management policies have been more effective in regulating point nutrient sources of aquatic ecosystems compared to diffuse ones (Boesch, 2002; Palmeri et al., 2005). Recent analyses suggest that in most European watersheds the total N exported to the sea has either stayed the same or even increased, despite the nitrate reduction directive which was established some 30 years ago (Vybernaite-Lubiene et al., 2017; Viaroli et al., 2018). This situation can be worsened by climate change, which affects the magnitude and the seasonal pattern of precipitation, increasing the frequency of high discharge, flash flood periods as well as phases with no precipitation and minimum river flow, with negative impacts on the ecosystem functioning (Trenberth, 2005; Lehner et al., 2006; Zhang et al., 2019). Different studies reported that the consequences of these sharp fluctuations of water supply, combined with increasing temperature and changes in the pattern of salinity due to sea level rise, may be amplified in transitional systems, such as estuaries and coastal lagoons (Anthony et al., 2009; Ferrarin et al., 2014). Understanding the net effect of such changes in these systems is particularly important, as they play a crucial role in the retention and transformations of nutrients by removing approximately 25% of the total reactive N delivered from the watershed (Nixon, 1981; Asmala et al., 2017; Sharples et al., 2017).

Extreme rainfall events are predicted to increase the amount and affect the composition of nutrients exported from the watershed to transitional systems (Fig. 1) (Howarth et al., 2006; Chen et al., 2018). The enhanced load of nutrients may stimulate some processes, such as N removal through denitrification, whose rates increase with increasing nitrate (NO_3^-) in the water column, until saturating concentrations (Ogilvie et al., 1997; Dong et al., 2000). Following these events, however, the high river discharge leads to the decrease in water residence time, shortening the processing time during which N can be repeatedly cycled through uptake by primary producers, sedimentation of organic matter and mineralization or removal through coupled nitrification-denitrification (Nixon et al., 1996; Dettmann, 2001; Seitzinger et al., 2006). High runoff increases water column turbidity and reduces light penetration, affecting benthic primary producers activity

(Pratt et al., 2014) and depressing their ability to regulate nutrient fluxes at the water-sediment interface (Risgaard- Petersen et al., 1994; Sundbäck et al., 2000). Enhanced transport of fluvial material may also alter significantly the structure and the functioning of the macrobenthic community, decreasing the biodiversity and the total biomass and favoring the establishment of opportunistic species (Ellis et al., 2002; Cardoso et al., 2008). The loss of specific functional groups strongly influences the ecosystem biogeochemistry because macrofauna, through bioturbation, feeding activity, excretion and biodeposition of labile organic matter, significantly alter N dynamics (Laverock et al., 2011; Stief, 2013). Burrowing benthic animals have contrasting effects on benthic processes stimulating N removal via nitrification and denitrification (Rysgaard et al., 1995; Moraes et al., 2018), or enhancing processes leading to ammonium (NH_4^+) recycling, as recently demonstrated for deep burrowing alien worms (Bonaglia et al., 2013; Benelli et al., 2019).

At the opposite situation, low freshwater discharge after prolonged drought seasonally decreases the amount of nutrients delivered to coastal areas and may decline the relative importance of external inputs compared to internal recycling (Fig.1) (Howarth et al., 2000; Feyen and Dankers, 2009). Internal recycling is here defined as the sum of dissolved inorganic nitrogen fluxes (NH_4^+, nitrite and NO_3^-) directed from the benthic system to the water column and is the net result of different processes as ammonification, nitrification, denitrification, DNRA and excretion by macrofauna. Higher temperatures combined with low freshwater inflow, which characterize low rainfall periods, lead to the increase in water residence time and contribute to water stratification, which in turn increases the extent of hypoxia or anoxia (Statham, 2012; Du et al., 2018; Hallett et al., 2018). The decrease in oxygen (O_2) concentration in the bottom water negatively affects biological communities and biogeochemical processes (Diaz and Rosenberg, 1995, 2008). Shift in redox conditions, which takes place under O_2 shortage, determines an increase in mineralization rates and release of NH_4^+ from the sediment (Nunnally et al., 2012; Roberts et al., 2012). Reduction of oxic layer stimulates denitrification by shortening the physical distance that nitrate (NO_3^-) must go through to reach the anoxic layer (Hietanen and Lukkari, 2007), but at the same time decreases the occurrence of nitrification and coupled nitrification-denitrification (Kemp et al., 2005; Conley et al., 2007; Roberts et al., 2012). Oxygen shortage may favor DNRA, a NO_3^- reduction pathway alternative to denitrification (McCarthy et al., 2008; Jäntti and Hietanen, 2012). DNRA is a process that does not remove N from the system but instead recycles it to the water column in a bioavailable form (Burgin and Hamilton, 2007). This process is also favored by increased salinity and sulphate reduction, which occur as a result of long periods of low river discharge (Rysgaard et al., 1999; An and Gardner, 2002; Gardner et al., 2006; Giblin et al., 2010). The increment of water temperature and NH_4^+ concentration due to the combined effect of DNRA, increase in mineralization rates and decrease in nitrification, may favor primary producers activity offsetting efforts to contrast eutrophication and extending the duration of blooms (Conley et al., 2007).

The effects of climatic extremes on benthic N cycling are therefore multifaceted, site-specific and thus difficult to forecast (Najjar et al., 2010; Statham, 2012). In this study, we analyzed microbial N transformations in the Sacca di Goro, a eutrophic coastal lagoon connected to the Adriatic Sea (Northern Mediterranean Sea). The lagoon is located downstream of the Po River watershed, one of the most impacted areas in Europe (Viaroli et al., 2018) and in the past years it was affected by macroalgal blooms, followed by anoxic events (Viaroli et al., 2006). Combined retrospective analysis and model simulations allow to predict for the Po River basin an increase in average and maximum temperatures (Coppola and Giorgi, 2010; Tibaldi et al., 2010) and a general decline in runoff (30-40% reduction), mainly in summer (June-August) (Coppola and Giorgi, 2010; Cozzi and Giani, 2011). Simultaneously, it is expected that the frequency and the intensity of extreme events will increase, with prolonged droughts and peaks of river discharge due to more severe and less frequent precipitation (Vezzoli et al., 2015). In this study, investigations were conducted to evaluate variations in benthic N dynamics in response to the recent climatic anomalies of 2019. A spring sampling was conducted in May, which was characterized by unusually heavy rainfall with values of cumulative precipitation nearly 2.5 times higher compared to the past 20 years. A late-summer campaign was conducted at the beginning of September, following a period characterized by high temperature and low river discharge. Summer temperatures in the Po River basin show a clear increasing trend from the 1970s (Brunetti et al.,2006). In the Sacca di Goro, during summer 2019, water temperatures exceeded 30°C for 8 days, whereas during the 2006 - 2018 period such threshold was exceeded for 3.5 days. We hypothesized that: (1) low salinity and high NO_3^- availability, together with high densities of burrowing macrofauna lead to high denitrification efficiency and low N recycling during spring; (2) high salinities and low NO_3^- availability, together with low bioturbation lead to decreased denitrification efficiency and high N recycling during late-summer; (3) hydrological extremes lead to the loss of ecosystem services such as N removal.

2 Material and methods

2.1 Study area

The Sacca di Goro is a shallow (average depth 1.5 m) microtidal lagoon (27 km^2) located in the southern part of the Po River Delta (NE Italy) (Fig. 2). The lagoon is connected to the Adriatic Sea through a 3 km wide mouth and receives freshwater inputs from the Po di Volano and Po di Goro, and from three minor artificial channels (Collettore Giralda, Canal Bianco, Canale Bonello). The salinity is highly variable due to fluctuations in freshwater and marine inflows, with the widest daily variations in the area near the sea mouth, which can be up to 10. The lagoon is intensively exploited for clam farming (*Ruditapes philippinarum*) at present covering 41% of the bottom surface with densities higher than 500 ind. m^{-2} (Bartoli et al., 2016). Heavy loads of NO_3^- generated in the Po River basin have been considered the main cause for seaweeds blooms (*Ulva* sp., *Gracilaria* sp. and *Cladophora* sp.) that characterize the lagoon since the 1980s (Viaroli et al., 2006). Besides external loads, different studies stressed the importance of clams activity on internal recycling. High densities of filter feeders determine the delivery of high amounts of organic matter on the sediment surfaces, as faeces and pseudofaeces. This labile substratum fuels microbial activity, increasing benthic O_2 uptake and nutrient recycling, which in turn sustain primary producers activity (Bartoli et al., 2001; Nizzoli et al., 2006; Viaroli et al., 2006; Naldi et al., 2020). During periods characterized by high temperature and low wind, macroalgal blooms might be followed by a sudden collapse of their production. The decomposition of macroalgal mats leads to anoxia and determines the onset of anaerobic processes and the release of sulfides to the water column. These phenomena, in the last 30 years, caused massive damage to the ecosystem and the local economy (Viaroli et al., 2006).

The lagoon is generally divided into three areas based on sedimentary and hydrological characteristics (Marinov et al., 2006). The western portion is located at the mouth of the Po di Volano and is characterized by the highest nutrients concentration and the lowest salinity; the sediments are muddy-clayish, with a high organic matter content and are highly bioturbated by surface and deep burrowers, such as *Corophium insidiosum* and *Alitta succinea* (Bartoli et al., 2012; Politi et al., 2019). The eastern part, called Valle di Gorino, is shallow (average depth 0.6 m), it is characterized by muddy-sandy sediments and it receives freshwater inputs from different locks connecting the lagoon with the Po di Goro. This sheltered area is characterized by slow water exchange and generally by higher temperature compared to the rest of the lagoon. The central portion, which hosts most of the licensed areas for clams farming, is affected by tidal exchanges that determine more intense water circulation and prevent the organic matter accumulation. Specific meteorological conditions, for example prevailing Scirocco winds, may contrast tidal forcing and increase stagnation also in this area, with anoxic risk for clams.

In the present study, sampling was carried out at three sites located within these representative areas: Giralda ("GI", 44° 49' N 12°16' E) in the western area, Gorino ("GO", 44° 48' N 12°19' E) at the edge of the Valle di Gorino, and a site near the sea mouth within the farmed area ("FA", 44° 48' N 12°18' E) (Fig. 2). The distance between stations was between 1.5 and 4.5 Km.

To understand ongoing changes in the Sacca di Goro lagoon in the context of climate anomalies, historical monthly averages of water temperature and salinity were retrieved for Gorino from samplings carried out by Parma University (1987 to 1991; Bencivelli et al., 1991, 1993) and from the Regional Agency of Environmental Protection of the Emilia Romagna Region – ARPAE (https://simc.arpae.it/dext3r/) (2006 to 2019). Precipitation data related to the area of the Po River Delta were also retrieved for the period 2000-2019 from ARPAE.

2.2 Sediments sampling and benthic flux measurements

Samplings were carried out on May 27th (spring campaign) and on September 2nd (summer campaign) 2019. At each site intact sediment cores (Plexiglass liners, i.d. 8.4 cm, length 30 cm) were randomly collected by hand for benthic fluxes (8 cores at Farmed Area and 4 cores per site at Giralda and Gorino in spring; 8 cores per site in summer) and denitrification and DNRA measurements (8 cores per site at Giralda and Farmed Area and 4 cores at Gorino in spring; 8 cores per site in summer). Concurrently, 6 intact sediment cores (Plexiglass liners, i.d. 4.6 cm, length 20 cm) were collected at each site for the sediment characterization and the determination of pore water NH_4^+ concentration. Water column temperature, pH, salinity and O_2 concentration were measured at the three sites by means of a multiple probe (YSI Instruments, Mod 556). In addition, from each site, 80 L of water was collected for cores maintenance, pre-incubation, and incubation periods. The intact cores were immediately submerged with the top open in a box filled with *in situ* water, cooled with ice packs to slow microbial activity and transferred to the laboratory within a couple of hours.

Once in the laboratory, the cores were placed into three large tanks, one for each site, filled with unfiltered water, maintained at *in situ* temperature and they were left to settle overnight (Dalsgaard et al., 2000). The water in the tanks was continuously aerated by aquarium pumps. Each core was equipped with a Teflon-coated magnet rotating at 40 rpm driven by a central magnet. Each magnet was suspended about 6 cm above the sediment surface to mix the water column, avoiding resuspension.

After overnight pre-incubations, the water within the tanks was replaced and the larger cores were incubated in the dark (Dalsgaard et al., 2000). Incubations for aerobic respiration and net N_2 and nutrient fluxes lasted 2-3 hours in order to keep O_2 concentration within 20% of initial values and started when gas-tight lids were positioned on the top of the cores (Dalsgaard et al., 2000). Dissolved O_2 concentration was measured with a microelectrode (OX-50, Unisense A/S, DK), whereas water samples were collected from each tank (4 replicates) at the beginning of incubation and from the water phase of each core at the end of incubation. In both cases, an aliquot of water was transferred and flushed to 12-ml exetainers (Exetainer®, Labco Limited, UK), and fixed with 100 µL of 7 M $ZnCl_2$ to stop microbial activity for N_2 determination. Another aliquot of 20 ml was filtered (Whatman GF/F glass fiber filters) and transferred to scintillation vials to analyze dissolved inorganic N compounds via standard spectrophotometric techniques. Samples for N_2 were analyzed to determine changes in N_2:Ar ratios via a membrane inlet mass spectrometer (MIMS) equipped with a copper reduction column maintained at 600 °C (Bay instrument, MD, USA) (Kana et al., 1994). Ammonium was determined using salicylate and hypochlorite in the presence of sodium nitroprussiate (Bower and Holm-Hansen, 1980). Nitrate was determined after reduction to nitrite (NO_2^-) in the presence of cadmium, and NO_2^- was determined using sulphanilamide and N-(1-naphthyl)ethylenediamine (APHA, 1992; Golterman et al., 1978). Gas and nutrient fluxes at the sediment-water interface were calculated according to the equation below:

$$F_x = \frac{(C_f - C_i) \times V}{A \times t}$$

where F_x is the flux of the chemical species x expressed in µmol or mmol m^{-2} h^{-1}, C_i and C_f (µM or mM) are concentration values of the chemical species x at the beginning and at the end of incubation, respectively, V is the water column volume (L), A (m^2) is the sediment surface and t (h) is the incubation time.

2.3 *Measurement of denitrification and DNRA rates*

After the first incubation, the water in the tanks was renewed and the open cores were left submerged for 2 hours in *in situ* and well-mixed water. Thereafter, a second incubation was performed to quantify the denitrification rates with the isotope pairing technique (IPT, Nielsen, 1992). The water in the tanks was lowered just below the top of the cores and $^{15}NO_3^-$ from a stock solution of 20 mM $^{15}NO_3^-$ ($Na^{15}NO_3^-$, Sigma Aldrich) was added to the water phase of each core. When 8 cores were collected, labelled NO_3^- was added to have a final ^{15}N atom % of 50% (4 cores) and 100% (4 cores); when 4 cores were collected a 50% labelling was carried out. A water sample was collected from each core before and after the $^{15}NO_3^-$ addition to determine the ^{15}N-enrichment of the NO_3^- pools. Thereafter, the cores were capped and incubated for 2-3 hours in dark conditions as described for nutrient flux measurements. At the end of the incubation, the whole sediment column was mixed with the water column and homogenized. An aliquot of the slurry was transferred to 12-ml exetainers, allowing abundant overflow and fixed with 200 µL of 7 M $ZnCl_2$ to stop the microbial activity. The abundance of $^{29}N_2$ and $^{30}N_2$ was determined via MIMS. As the genuine $^{28}N_2$ production was independent from the $^{15}NO_3^-$ level we assumed that anammox contribution to N_2 production was negligible, as reported in previous denitrification measurements in the Sacca di Goro sediments (Moraes et al., 2018). Denitrification rates were calculated from the production of $^{29}N_2$ (p29) and $^{30}N_2$ (p30) as follows:

$$D_{15} = p29 + 2p30$$

$$D_{14} = D_{15} \times (p29/2p30)$$

where D_{15} is the denitrification rate of the $^{15}NO_3^-$, whereas D_{14} is the denitrification rate of $^{14}NO_3^-$. From the total denitrification rate, the denitrification of nitrate diffusing to the anoxic layer from the water column (D_w) and the denitrification of nitrate produced within the sediments due to nitrification (D_n) were calculated as described by Nielsen (1992):

$$D_w = (^{14}NO_3^- / ^{15}NO_3^-) \times D_{15}$$

$$D_n = D_{14} - D_w$$

where $^{14}NO_3^-$ is the ambient nitrate concentration (µM) and $^{15}NO_3^-$ is the concentration of labelled nitrate added to the cores.

Denitrification efficiency (DE), defined as the percentage of total processed inorganic N released as N_2, was calculated according to Eyre and Ferguson (2009) as:

$$DE = \frac{D_{tot}}{DIN + D_{tot}}$$

where D_{tot} is total denitrification (D_w+D_n) and DIN represents the sum of dissolved inorganic N fluxes (NH_4^++NO_2^-+NO_3^-) directed from the sediment to the water column (effluxes).

Within the same denitrification experiment, an additional aliquot of the slurred sediment (30 ml) was collected to determine the rates of DNRA. The samples were transferred to 50-ml falcon tubes and treated with KCl (2 M) for the determination of the exchangeable ammonium pool and the $^{15}NH_4^+$ fraction. Briefly, tubes were shaken for 1 h, then centrifuged (1800 rpm for 15 min) and the supernatant was filtered (GF/F glass fiber filters) into 20-ml scintillation vials for later analyses. These samples were purged with helium for 10 minutes, to eliminate $^{29}N_2$ and $^{30}N_2$ pools produced during the incubations. Samples were then transferred to exetainers and treated with alkaline hypobromite solution, to oxidize NH_4^+ to N_2 (Warembourg, 1993). The abundance of $^{29}N_2$ and $^{30}N_2$ was determined via MIMS. Assuming that DNRA occurs in the same sediment horizon as denitrification, total DNRA rates were calculated from the production of $^{15}NH_4^+$ ($p^{15}NH_4^+$), according to the equation reported in Risgaard-Petersen and Rysgaard (1995):

$$DNRA \quad = p^{15}NH_4^+ \times (D_{14}/D_{15})$$

Total DNRA rates were divided into direct DNRA of NO_3^- from the water column ($DNRA_w$) and coupled DNRA ($DNRA_n$) and were calculated as follows:

$$DNRA_w = (^{14}NO_3^- / ^{15}NO_3^-) \times \ p^{15}NH_4^+$$

$$DNRA_n = DNRA \quad - DNRA_w$$

At the end of the incubation, sediments from all cores were sieved (0.5 mm mesh size) to retrieve the macrofauna. Organisms were sorted under a stereomicroscope (Leica S8 APO, amplification 8x), identified by dichotomous keys (http://species-identification.org/identify_species.php) and by scientific papers (Wägele et al., 1981) to the lowest possible taxonomic level and counted. The identification was strengthened by the comparison with previous studies on the macrobenthic community of the Sacca di Goro (Mistri et al., 2001, Ludovisi et al., 2013, Politi et al., 2019). For each species, the dry weight was determined after drying at 80°C for 48 h. For the clams, shells were removed, and only flesh weight was measured.

2.4 Sediment and pore water characterization

The six additional sediment cores were extruded and sliced in five layers: 0-1, 1-2, 2-3, 3-5 and 5-10 cm for physical and chemical sediment characterization. Briefly, in half of the cores, the slices were rapidly homogenized, and subsamples of 5 ml were collected using cut-off syringes, to determine physical properties. Sediment porosity was determined from the loss of wet weight after 48 h at 70 °C. Later, sediments were analyzed for carbon (C) and N content and their isotopic composition in the upper 0-2 cm sediment layer with a mass spectrometer (Thermo Scientific Delta V) coupled with element analyzer (FlashEA 1112, Thermo Electron Corporation) at the Center for Physical Sciences and Technology (Lithuania). Before measurements samples were grinded and acidified with 1 N HCl in order to remove carbonates. The last three cores were sliced to analyze the vertical distribution of pore water NH_4^+ concentration. Water was extracted by centrifugation of wet sediment (1800 rpm for 15 min), the supernatant was then filtered (Whatman GF/F glass fiber filters) and analyzed to determine NH_4^+ concentration as described in the section 2.2.

2.5 Rivers discharge and reactive N loadings

The Consorzio di Bonifica Pianura di Ferrara provided data on Po di Volano, Collettore Giralda, Canal Bianco, and Canale Bonello discharges. This authority continuously monitors the water discharge and provides daily or weekly average values. River discharges for Po di Goro were not available, then mean annual data derived from the Final Report of the Hydrodynamic Modelling System of the Sacca di Goro lagoon (Arpae-Emilia Romagna, Bologna University, CNR-Ismar, 2019) were used. It was assumed that other diffuse sources were negligible. At each tributary, water samples were collected in triplicates in May and September and immediately filtered into 20-ml vials for NH_4^+, NO_2^- and NO_3^- determination as described in the section 2.2. Sampling stations were located at a certain distance from the mouth of the canals to minimize the variability due to marine water intrusion. The daily load of dissolved inorganic N was obtained by multiplying the concentration measured at each sampling date by the mean daily discharge. The latter was calculated from monthly data of May and September.

2.6 Statistical analysis

Two-way analysis of variance (ANOVA) was used to assess the significance of sites and seasons in explaining differences among benthic net fluxes, denitrification and DNRA rates. The normality and the homogeneity of variance were checked using the Shapiro-Wilk test and the Levene median test, respectively. If significant heteroscedasticity was found, data were log-transformed. Pairwise multiple comparison of means was carried out using the Tukey's test for all the significant factors. Statistical significance was set at p level lower than 0.05. All statistical analyses were performed with R software v. 3.5.1 (R Core Team, 2018). Graphs were made with Sigma Plot 11.0.

3 Results

3.1 General features of water column, sediments and macrofauna

The concentration of dissolved inorganic N, temperature and salinity displayed strong spatial and temporal variability influenced by different hydrological regimes. During spring the high freshwater discharge associated with unusually heavy rainfall, resulted in low salinity, low temperatures and high NO_3^- concentrations (Table 1). During summer drought, water temperatures increased by 3-6 °C as compared to spring, O_2 saturation decreased by 20% and NO_3^- concentrations decreased at all sites by a factor of 4. Salinities reflected limited riverine discharge with values close to marine measured at Gorino and Farmed Area (Table 1).

Sediment properties revealed sharp differences between Giralda and the other two sites, mainly due to the riverine influence. This site was characterized by muddy-clayish sediments with higher porosity and higher C and N content, which decreased in summer. Particulate matter displayed more depleted $\delta^{13}C$ and $\delta^{15}N$ signatures and higher C:N compared to sites located closer to the sea entrance (Table 2).

At Giralda, vertical profiles of pore water NH_4^+ revealed an increasing trend, with the highest values observed in summer, when concentration peaked at ~600 µM at the 5-10 cm layer (Fig. 3). At the other sites, NH_4^+ concentration was less variable along the depth profiles, and increased from spring to summer at Gorino, whereas it showed similar seasonal values at Farmed Area. In both seasons, pore water NH_4^+ concentration exceeded that in the bottom water, suggesting upwards diffusive fluxes, generally increasing from spring to summer and with gradients peaking in the warmest season at the sediment-water interface (Fig. 3).

The abundance of dominating macrofaunal taxonomic groups differed among sites and seasons. In spring at Giralda the sediments appeared heavily bioturbated, particularly by *C. insidiosum* and *A. succinea*, with densities of 7,071 ± 260 and 2,226 ± 69 ind. m^{-2} (averages ± standard errors), respectively, which accounted on average for 80% of the total biomass. In summer the densities of these organisms drastically dropped to 105 ± 10 and 270 ± 17 ind. m^{-2} for *C. insidiosum* and *A. succinea*, respectively. At Gorino the biodiversity and the abundance of the macrobenthic community were relatively low and mainly dominated by *A. succinea* (361 ± 20 ind. m^{-2} in spring, 135 ± 15 ind. m^{-2} in summer) and by the isopod *Cyathura carinata* (180 ± 30 ind. m^{-2} in spring and 1,865 ± 81 ind. m^{-2} in summer). Within Farmed Area *R. philippinarum* constituted more than 95% of the total macrofauna biomass, with densities of 768 ± 56 and 407 ± 10 ind. m^{-2} in spring and summer, respectively.

3.2 Inorganic N fluxes at the sediment-water interface

Inorganic N fluxes at the sediment-water interface varied among sites depending on seasons ($p < 0.001$, Table S1 in Supplementary Material). In all three sites and both seasons, sediments were net NH_4^+ sources, with Giralda as exception during spring (Fig. 4a). At all sites NH_4^+ fluxes significantly increased ($p < 0.001$, Table S1) from spring (average rate 146 ± 59 µmol m^{-2} h^{-1}) to summer (average rate 726 ± 73 µmol m^{-2} h^{-1}), and Farmed Area displayed the highest NH_4^+ recycling in both seasons.

Nitrite and NO_3^- were more erratic without clear patterns among sites and seasons (Table S1). In spring, high water column NO_3^- concentrations resulted in large uptake (average rate -730 ± 150 µmol m^{-2} h^{-1}) peaking at Giralda (Tukey pairwise comparison, $p < 0.001$; Fig. 4b). Giralda and Farmed Area in summer turned into net NO_3^- sources, with fluxes of 445 ± 135 and 168 ± 55 µmol m^{-2} h^{-1}, respectively, while Gorino displayed values comparable to the spring season. Fluxes of NO_2^- were always nearly one order of magnitude lower than those of NO_3^-. In both seasons sediments from the three sites released NO_2^- to the overlying bottom water, with Farmed Area as exception in spring (Fig. 4c).

Measured net N_2 fluxes were largely positive suggesting the dominance of denitrification over N_2-fixation (Fig. 4d). In spring sediment at Giralda displayed the highest N_2 effluxes (1,150 ± 81 µmol N m^{-2} h^{-1}), exceeding by a factor of 5 rates measured at Gorino and Farmed Area. In summer there was a general decline in net N_2 production, in particular at Giralda, which showed significant differences between seasons (Tukey pairwise comparison, $p < 0.001$).

3.3 Aerobic respiration, denitrification and DNRA rates

Benthic O_2 uptake ranged from -1.74 to -8.77 mmol m^{-2} h^{-1} and significantly varied among the three sites in the two seasons (Fig. 5a, $p < 0.001$, Table S2 in Supplementary Material). In spring, Giralda displayed the highest O_2 uptake (-6.78 ± 0.32 mmol m^{-2} h^{-1}), which almost halved in summer despite the increase in temperature. Gorino and Farmed Area were characterized by an opposite seasonal trend, with higher fluxes measured in summer, and peaking at Farmed Area (-8.77 ± 0.87 mmol m^{-2} h^{-1}; Tukey pairwise comparison, $p < 0.001$).

Total denitrification rates ($D_{tot} = D_w + D_n$) were more elevated in spring at all sites, with the highest rates measured at Giralda (Tukey pairwise comparison, $p < 0.001$, Fig. 5b). At this site denitrification was supported mainly by coupled nitrification-denitrification (625 ± 50 µmol N m^{-2} h^{-1}) and to a lesser extent by NO_3^- diffusing from the water column (442 ± 64 µmol N m^{-2} h^{-1}). Spring rates of D_n and D_w were ~5 times lower at Gorino and Farmed Area as compared to Giralda (Tukey pairwise comparison, $p < 0.001$). Despite the peak of denitrification matched with the peak of NO_3^- concentration in the water column, the D_n prevailed over the D_w in all the investigated sites, contributing nearly 60% of total denitrification (Table 3). In summer at all three sites total denitrification rates decreased compared to spring (Tukey pairwise comparison, $p < 0.001$ for Giralda and Farmed Area). The greater change occurred at Giralda, where D_w and D_n rates dropped to 85 ± 18 and 132 ± 43 µmol N m^{-2} h^{-1}, respectively. The share of denitrification supported by nitrification was more variable in summer, ranging from 53 to 73% at Gorino and Farmed Area, respectively (Table 3). In spring denitrification efficiency was generally high at all sites (>92%), whereas it substantially dropped in summer (Table 3).

The highest rates of DNRA were found at Giralda both in spring and summer (Fig. 5c). At all three sites values tended to increase in summer, but only at Gorino seasonal differences were significant (Tukey pairwise comparison, $p < 0.001$). In spring at Farmed Area DNRA represented 10% of total NO_3^- reduction pathways, whereas at Giralda and Gorino it represented a minor portion. During summer the share of DNRA to NO_3^- reduction increased at all sites and reached nearly 33% at Gorino and Farmed Area (Table 3).

3.4 External loads versus internal removal and recycling

To compare the magnitude of external loads and internal processes, total DIN delivered from the lagoon watershed were normalized by the lagoon total surface, whereas removal and recycling rates were calculated by averaging denitrification rates and DIN effluxes measured at each sampling site. During spring the load of DIN was 27.25 ± 1.30 mmol m^{-2} d^{-1} (average ± standard error). About 40% of the imported N was removed via denitrification (11.86 ± 1.02 mmol m^{-2} d^{-1}), whereas inorganic N recycling from sediments was negligible. In late-summer there was a steep decline in the riverine DIN load that decreased by a factor of ~3 (9.63 ± 0.80 mmol m^{-2} d^{-1}), mainly due to lower discharge and decreased NO_3^- concentrations. The amount of inorganic N recycled from sediments increased and doubled the external inputs, averaging 20.18 ± 3.69 mmol m^{-2} d^{-1}, whereas N removal via denitrification accounted for 9% of total DIN load (sum of external input and internal recycling), corresponding to 2.70 ± 0.59 mmol m^{-2} d^{-1}.

4 Discussion

4.1 Temporal and spatial variability of N and O_2 dynamics in the Sacca di Goro lagoon

Our results indicate that the lagoon was predominantly removing N through benthic denitrification under high river discharge in spring, while it was recycling N via DNRA and remineralization under low discharge in late-summer. In spring, N-cycling was strongly influenced by the high freshwater discharge and the high NO_3^- load. The nutrient loads delivered from the Po River basin and from the Po di Volano sub-basin were monitored in different studies from the 1990s (Naldi et al., 2010, Viaroli et al., 2018, Castaldelli et al., 2013, 2020). Dissolved inorganic nitrogen load displays a strong seasonality, with summer minima and extremely high late-winter peaks. Nitrate, which represents on average > 75% of total DIN load, is directly related to the water discharge, with wide inter-annual variations, from low values in dry years to peaks in wet years (Naldi et al., 2010; Viaroli et al., 2018). The spring load determined in this study was in the higher range of values previously reported for the same season (Castaldelli et al., 2013, Viaroli et al., 2013). Under these circumstances denitrification represented the leading process (Fig. 6), with total rates similar to values reported for other shallow estuarine systems in the wet season (Seitzinger, 1988; Ogilvie et al., 1997; Dong et al., 2000). Coastal lagoons act as benthic filters and regulate the supply of N both via denitrification and via the uptake of benthic primary producers (Risgaard-Petersen, 2003). Even though in this study only processes under dark conditions were analyzed, during spring photosynthetic activity of microphytobenthos and its nutrient uptake were likely suppressed by the enhanced water column turbidity, due to the delivery of suspended solid matter, and dissimilative processes represented the main pathway of N removal (Anderson et al., 2013). Despite the elevated NO_3^- availability in the water column, approximately 60% of denitrification was coupled to nitrification, indicating high sediment nitrification rates. At Giralda the elevated nitrification is demonstrated to be associated with the high abundances of *C. insidiosum*, which via continuous ventilation of its ‘U’-shaped burrows, pumps oxic water into the sediments, leading to the oxidation of pore water NH_4^+ in the upper sediment layers (Pelegrì and Blackburn, 1994; Moraes et al., 2018). Also, the presence of *A. succinea*, through the construction of dense burrow networks could enhance NH_4^+ mobilization from deep to surface sediments, stimulating nitrification (Nizzoli et al., 2007). Compared to Giralda, Gorino and Farmed Area were characterized by lower denitrification rates, due both to the lower NO_3^- availability and to the limited bioturbation activity. These two sites, however, showed similar values of total denitrification, suggesting no effects of clam biomass on this process, a result that is in agreement with previous studies conducted in the same sites of the Sacca di Goro (Murphy et al., 2018). Despite a small amount of NO_3^- being reduced to NH_4^+ via DNRA, denitrification was the main pathway of NO_3^- reduction. At Farmed Area the contribution of DNRA to nitrate reduction processes slightly increased compared to the two other sites, probably due to the larger availability of labile organic matter in the form of clam biodeposits (Nizzoli et al., 2006). However, the increase in NH_4^+ efflux derived from DNRA was negligible if compared to direct clam excretion, which was estimated to contribute from 63 to 154% of total NH_4^+ fluxes. Such percentages were calculated multiplying biomass-specific excretion rates of clams (Welsh et al., 2015 and Murphy et al., 2018) by the biomass of the clams retrieved in our experiments. During spring therefore, high freshwater discharge resulted in the dominance of denitrification over recycling via mineralization, clam excretion and DNRA.

After few months of low river discharge, during late summer, the elevated temperatures and the low O_2 concentration led to a shift of N processes from the dominance of removal to recycling. Generally, the reduced state of sediments was evidenced by an increased sediment O_2 uptake and a higher net release of NH_4^+. The latter was due to a combination of factors, including high mineralization rates, the disconnection between N removal (via coupled nitrification–denitrification) and mineralization, and the enhancement of DNRA (Kemp et al., 2005; Roberts et al., 2012). The direct contribution of clam metabolism accounted for 21–42% of the net NH_4^+ fluxes, suggesting the dominance of microbial processes also at Farmed Area. Denitrification rates dropped compared to spring values and showed a decreasing trend along with the 4-folds drop in NO_3^- concentration from the western corner towards the mouth of the lagoon, mainly driven by D_w. At Giralda the decrease in the coupled nitrification-denitrification was mainly due to the decline in bioturbators abundance, likely due to high temperatures and low O_2 concentration (Pitacco et al., 2018). At Gorino and Farmed Area the simultaneous decrease in denitrification rates and increase in the relative contribution of DNRA may depend on several factors including the increment of salinity (Giblin et al., 2010; Caffrey et al., 2019), the higher ratio of labile organic carbon to NO_3^- electron acceptors (Tiedje, 1988; Nizzoli et al., 2010), and the availability of reductants as sulfides (Brunet and Garcia-Gil, 1996) and Fe^{2+} (Robertson et al., 2016). Despite all these factors would be expected to favor DNRA over denitrification, the contribution of this process to total NO_3^- reduction was lower compared to values previously reported for temperate shallow estuaries, where it can equal or exceed denitrification (An and Gardner, 2002; Gardned et al., 2006; Murphy et al., 2018). As a consequence, denitrification remained the dominant process (Murphy et al., 2018).

Interestingly, denitrification efficiency (DE) shifted from a maximum of 100% in spring to a minimum of 4% in summer. Eyre and Ferguson (2009) reported the highest DE (~70%) in sediments with moderate organic carbon enrichment and inorganic carbon fluxes ranging between 500 and 1,500 μmol m^{-2} h^{-1}. Since in the Sacca di Goro respiration rates were always higher than 1,500 μmol O_2 m^{-2} h^{-1}, much lower DE and elevated N recycling were expected. However, in spring DE ranged from 92 to 100%, likely sustained by the high rates of D_w. High NO_3^- concentrations in the water column determined high DE, despite the elevated sediment organic content. The latter did not significantly affect the macrofauna community, which is composed by tolerant species supporting elevated nitrification rates. In summer DE was lower at the three sites, with values ranging from 4 to 54%, suggesting higher N recycling over denitrification. The increased temperatures, the inhibition of nitrification, the increase in DNRA rates and the lower macrofauna activity were likely the main factors determining this drop in summer. Similar results were found by Bartoli et al. (2012) in an annual study, with data from Gorino and Giralda.

Sediments at the three sites displayed elevated sediment O_2 uptake, in the higher range of those reported for other temperate estuaries (Cabrita and Brotas, 2000; Nizzoli et al., 2007; Gardner and McCarthy, 2009). At Giralda the high organic load was derived mainly from settled particles of fluvial origin, as demonstrated by the higher C:N ratio and by the lower C and N isotopic values, within the range reported for terrestrial organic matter (∼-27‰ and of 3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, Lamb et al., 2006). However, even during the high discharge period, the C:N ratio of Giralda surface sediments suggested high organic matter quality, whereas material of terrestrial origins usually displays values significantly above 12 (Yamamuro, 2000). At this site in spring the highest O_2 uptake was associated with dense burrow network of amphipod *C. insidiosum*, where O_2 was likely employed for NH_4^+ oxidation via nitrification (Pelegrì and Blackburn, 1994; Moraes et al., 2018), whereas decreased in summer. Gorino and Farmed Area were characterized by C:N ratios close to the Redfield one and by higher $\delta^{13}C$ and $\delta^{15}N$ values, closer to values reported for marine systems, suggesting a progressive increase in the proportion of organic matter from autochthonous origins (Yamamuro, 2000, Liu et al., 2006). The isotopic values, however, were more depleted compared to marine phytoplankton, particularly relative to $\delta^{13}C$ values, usually ranging from -22 to -19 ‰ (Lamb et al., 2006), suggesting that sedimentary organic matter still derived from the mixture of terrestrial derived material and marine material and from the accumulation of clam biodeposits ($\delta^{13}C$ value of about -23,2 ‰, Mazzola and Sarà, 2001). Sediment O_2 uptake in these sites showed a distinct seasonal pattern, with higher rates in summer likely regulated by water temperature (Vidal et al., 1997; Trimmer et al., 1998; Cabrita and Brotas, 2000). At Farmed Area the higher benthic respiration measured in summer was not related to clam density, since clam contribution to O_2 demand accounted for 21–42% of the total benthic respiration, whereas in spring it represented a major fraction (62–127%) (clam biomass-specific respiration rates were derived from Welsh et al. (2015) and Murphy et al. (2018)).

An inventory of sediment O_2 uptake rates measured with the same approach over the last 26 years does not suggest significant temporal trends likely due to the extremely variable contribution of macrofauna, including cultivated and naturally present species (Fig. 7a). It is expected that 30 years of clams farming may have enriched the sediments with labile organic matter, resulting in increased microbial respiration. However, anoxic events were more frequent in the past due to macroalgal blooms that are now reduced in this and other lagoons of northern Italy (Sfriso et al., 2019). In the past, macroalgal blooms and their collapse determined huge inputs of organic matter to the sediments that uncoupled O_2 uptake and availability (Viaroli et al., 2006; Naldi et al., 2020). In the last years, the high frequency of heavy rainfall events and the frequent digging or silting operations, likely washed out or buried clam biodeposits, limiting their impacts on lagoon aging, benthic respiration and algal growth. Analogously, denitrification measurements performed with the IPT over the last 26 years do not reveal significant temporal trends (Fig. 7b). This suggests either that the large variability of macrofauna density and community composition is responsible for the variability of measured rates, or that natural or anthropogenic disturbances as hydrological extremes set to zero lagoon aging, as reported for oxygen. In the heavily impacted Sacca di Goro, the seasonal shift between high and low discharge periods seems a better predictor of dominant processes regulating N benthic metabolism than long term chronosequences.

4.2 Projections in the context of climatic anomalies and hydrological extremes

The drivers of macroalgal blooms in the Sacca di Goro were studied for nearly three decades and were associated to anthropogenic nutrient loads (Viaroli et al., 2018), nutrient recycling by clams (Bartoli et al., 2001, 2003; Naldi et al., 2020) and introduction of alien species (Milardi et al., 2020). This study provides evidence of a new possible driver of algal blooms. Hydrological extremes, which are expected to increase in the future, may reduce the role of the Sacca di Goro as biogeochemical filter, with implications for NH_4^+ availability, in particular during prolonged dry periods and heat waves. Different studies targeting the effect of climate changes on nutrients focused on processes at the watershed scale (e.g. increased or decreased runoff) and the implications on hydrology (e.g. increased erosion, sharp reduction or increase in water residence time) (Marshall and Randhir, 2008; Howarth et al., 2012; Wagena et al., 2018); the present study has analyzed the overlooked effects of two climatic extremes on sedimentary N biogeochemistry (Howarth et al., 2000; Anderson et al., 2013; Bruesewitz et al., 2013).

Climate projections forecast the increment of frequency and severity of heavy rainfalls (Vezzoli et al., 2015). The timing of these events is crucial and may determine different effects on the dynamics of transitional areas. These events may contribute to alleviate hypoxia, for example by increased discharge, lower residence time and interruption of water column stratification (Rabalais et al., 2007). High freshwater discharge may contribute to flush phytoplankton downstream, even out of the estuary, and control algal blooms (Scavia et al., 2002; Phlips et al., 2020) or may enhance sediment resuspension resulting in the release of nutrient, favoring pelagic production, or in the oxidation of reduced pools (Vidal-Durà et al., 2018; Niemistö and Lund-Hanses, 2019). The increase in riverine runoff enhances the amount of N exported from the river watershed to the coastal areas, whereas high solid transport and turbidity limit primary producers assimilative N pathways; as a consequence with microbial denitrification remains the most important N-removing dissimilative process (Anderson et al., 2013). A positive relationship between NO_3^- availability and removal capacity has been found across a range of estuaries (Seitzinger et al., 2006). The consequent decrease in water residence time, however, determines a reduction of denitrification potential.

Historical data on discharge or residence time for the Sacca di Goro are not available, but they can be inferred from variations in salinity values (Fig. 8a). Data from the last three decades suggest large variability in the mixing of fresh and marine waters, reflecting multiple management measures implemented to improve the hydrodynamic conditions of the lagoon. The salinity decrease recorded since 2015 was probably due to the opening of locks connecting the Po di Goro to improve water circulation. However, the low salinities of May, June, November and December 2019 were far below average values, suggesting the occurrence of heavy rainfalls likely affecting the water budget, residence time and nutrient concentrations at the whole lagoon ecosystem scale (Fig. 8b).

Different models show that the N fraction that is denitrified may be estimated from the residence time (Nixon et al., 1996; Dettmann, 2001; Seitzinger et al., 2006). These models were usually developed with data at the monthly or annual scales, whereas over short time frames the relation between denitrification efficiency and water residence times is more challenging. The annual average residence time of the Sacca di Goro lagoon varies between 1 and 12 days, with minimal values in spring in the western portion and at the lagoon-sea interface (<5 days) (Arpae-Emilia Romagna, Bologna University, CNR-Ismar, 2019). The calculations presented in this study, based on a simple mass balance, show that in spring, despite high denitrification rates, nearly half of the N load entering the lagoon was removed via denitrification. According to the models proposed by Nixon et al. (1996) and Seitzinger et al. (2006), however, a residence time of 5 days determines the removal of 15% of the total N load and this amount may be even lower considering heavy precipitation and the high runoff detected in spring. Many factors, such as depth, water temperature, salinity, O_2 and NO_3^- concentrations, organic carbon in sediments, bioturbation and presence of primary producers may affect denitrification efficiency and produce significant deviations from expected values (Eyre and Ferguson, 2009). Shallow lagoons with marked zonation as the Sacca di Goro are paradigmatic examples where multiple, co-occurring factors regulate locally and set the upper limits of processes as denitrification and where the same factors may undergo sharp spatial (e.g. among stations) and temporal variations (e.g. among wet and dry periods).

A further increase in river discharge and, consequently, in nutrient amount, may also determine the saturation of the denitrification capacity of transitional areas. Nitrate removal capacity increases with N load, up to the saturation concentrations and asymptotic rates were reported in different studies at 200 µM (Ogilvie et al., 1997), 400 µM (Trimmer et al., 1998) and 600 µM (Dong et al., 2000). These values are much higher than those reported in this study in spring (56–113 µM). The threshold values, however, may be related to local variations in biological and environmental variables or due to differences in acute or chronic nutrient load. Future works should be aimed at quantifying the saturating NO_3^- concentrations for different areas of the Sacca di Goro lagoon and determine the factors that may influence these thresholds.

Different models reported for the Po River Basin that the most significant effects of climate changes are expected in summer, with a strong increase in very dry and low flow periods, followed by significant water deficit, and a large increase in temperature and heatwaves (Cozzi and Giani, 2011; Vezzoli et al., 2015). The analysis of available, historical data supports such predictions as water temperatures underwent an increasing trend since 1987, more pronounced in the summer months, from June to September. (Fig. 8c).

Extremes in low summer discharge may stimulate river and estuarine eutrophication and large conversion of inorganic nutrients into phytoplankton and in its transfer to coastal areas (Howarth et al., 2000; Rossetti et al., 2009). Under these circumstances, most N would be delivered to sediment in particulate form, also due to filter-feeders activity. Consequently, labile organic matter inputs may fuel sediment respiration, reducing O_2 concentration in the water column and the heath-dependent water column stratification may determine the onset of bottom water hypoxia. Depletion of electron acceptors such as O_2 and NO_3^- leads to the dominance of sulfate reduction with subsequent sulfide accumulation, determining the suppression of nitrification and denitrification and the further increase in DNRA rates (An and Gardner, 2002; Gardner et al., 2006; Giblin et al., 2010). The sulfide build-up may also derive by the increase in salinity, due to high temperature and low freshwater discharge. Oxygen depletion and sulfides affect also macrofauna diversity and abundance and produce positive feedbacks towards more chemically reduced sediment conditions and towards N-recycling dominance over denitrification (Diaz and Rosenberg, 1995; Magni et al., 2005). This was evidenced in a recent study carried out in the nearby Valli di Comacchio lagoon where it was demonstrated that heatwaves pose serious threats to the resilience capacity of the macrobenthic community, favoring short-lived, opportunistic forms (Pitacco et al., 2018). If elevated residence time and heatwaves promote large NH_4^+ recycling through the described cascade mechanisms, assimilation by primary producers may represent an important temporary retention of nutrients. Naldi et al. (2020) have demonstrated that in the shallow water of the Sacca di Goro, clams control phytoplankton primary production and displace the pelagic production at the benthic level. Under low discharge and high residence time, such top-down control can be even more efficient, resulting in transparent water, enriched by excreted nutrient, which may favor macroalgal growth, with negative effects on the lagoon functioning and clam farming (Bartoli et al., 2001; Viaroli et al., 2003; Naldi et al., 2020).

5 Conclusions

In the eutrophic Sacca di Goro, as reported in other estuaries and coastal systems, hydrological extremes result in sharp seasonal transitions among dominant microbial processes driving benthic N cycle. The spring, high discharge period is dominated by denitrification due to high NO_3^-, high bioturbation and likely turbidity-limited primary producers-bacteria competition. However, a further increase in river discharge and

N loads may determine the saturation of denitrification capacity, and the excess N may be partly exported to the Adriatic Sea, also due to low water residence time. The summer, low discharge period is dominated by NH_4^+ internal recycling, also sustained by increased DNRA, largely exceeding watershed N inputs. Superimposed to and interacting with the effects of hydrological extremes are local regulations of benthic N processes. At Giralda denitrification always dominated over DNRA as NO_3^- reduction pathway; this was particularly evident during spring mainly due to higher riverine influence, bioturbation and elevated NO_3^- concentrations. At Gorino and Farmed Area, in particular during summer, the higher salinity and microbial respiration likely explained the increase in DNRA contribution to NO_3^- demand and large NH_4^+ fluxes. Such NH_4^+ mobilization may increase the intensity, duration, and extent of algal blooms.

Results of this study suggest that both the spring and summer hydrological extremes scenarios reduce the effectiveness of lagoons as benthic filter and increase the amount of N exported to the open sea, either in form of NO_3^- or NH_4^+, with implications for coastal eutrophication. Management actions aiming at the reduction of eutrophication in transitional and coastal areas have to date targeted the decrease in nutrient loads from agriculture and civil sources. Further management actions should include new and effective tools for mitigating expected nutrient increase due to climate change.

Acknowledgements

We are grateful to Edoardo Turolla of the Centre for Mollusc Research (Goro) for the provision of laboratory facilities. We acknowledge the Consorzio di Bonifica Pianura di Ferrara for the provided data. We also wish to thank Dr. Fabio Vincenzi for valuable assistance in laboratory work at Ferrara University.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Tables

Table 1. Physical and chemical features of the water column measured at the three sampling sites in spring and summer in the Sacca di Goro lagoon. For inorganic N concentrations averages ± standard errors are reported.

		GI	**GO**	**FA**
T (°C)	Spring	18	22	19
	Summer	23	25	25
Salinity	Spring	8	3	10
	Summer	5	28	29
O_2 (% sat)	Spring	82	87	90
	Summer	62	72	63
NH_4^+ (µM)	Spring	20.7 ± 0.1	4.2 ± 0.1	27.7 ± 0.3
	Summer	28.8 ± 0.9	9.9 ± 0.2	14.9 ± 0.4
NO_2^- (µM)	Spring	17.2 ± 0.1	4.7 ± 0.1	5.1 ± 0.1
	Summer	8.1 ± 0.4	1.8 ± 0.1	1.7 ± 0.1
NO_3^- (µM)	Spring	113.2 ± 2.7	84.6 ± 1.1	56.1 ± 2.4
	Summer	31.7 ± 3.2	22.6 ± 0.6	12.3 ± 0.6

Table 2. Sediment characteristics at the three sampling sites in spring and summer in the Sacca di Goro lagoon. Averages ± standard errors are reported.

		Sediment layer	**GI**	**GO**	**FA**
Type			Muddy-clayish	Muddy-sandy	Sandy
Porosity	Spring	0 – 1 cm	0.71 ± 0.03	0.42 ± 0.00	0.43 ± 0.00
	Summer	0 – 1 cm	0.82 ± 0.01	0.57 ±0.03	0.53 ± 0.02
TN (%)	Spring	0 – 2 cm	0.26 ± 0.01	0.01 ± 0.01	0.02 ± 0.00
	Summer	0 – 2 cm	0.20 ± 0.00	0.04 ± 0.01	0.04 ± 0.01
δ^{15}N (‰)	Spring	0 – 2 cm	4.56 ± 0.18	5.05 ± 0.38	5.83 ± 0.36
	Summer	0 – 2 cm	4.16 ± 0.39	6.87 ± 0.58	5.30 ± 0.13
C_{org} (%)	Spring	0 – 2 cm	2.81 ± 0.15	0.09 ± 0.01	0.15 ± 0.00
	Summer	0 – 2 cm	1.79 ± 0.04	0.24 ± 0.08	0.35 ± 0.02
δ^{13}C (‰)	Spring	0 – 2 cm	-26.79 ± 0.04	-21.64 ± 0.10	-24.73 ± 0.20
	Summer	0 – 2 cm	-27.23 ± 0.01	-23.42 ± 0.30	-24.25 ± 0.10
C:N (mol:mol)	Spring	0 – 2 cm	12.41 ± 0.50	7.85 ±0.26	7.61 ± 0.16
	Summer	0 – 2 cm	10.31 ±0.19	7.82 ± 0.22	9.22 ± 0.18

Table 3. Relative proportion of D_n to total denitrification rates (D_{tot}), denitrification efficiency and relative proportion of DNRA to total NO_3^- reduction (D_{tot}+DNRA) at the three sampling sites in spring and summer. Averages ± standard errors are reported.

		D_n/D_{tot} (%)	**Denitrification Efficiency (%)**	**DNRA/(D_{tot}+DNRA) (%)**
Spring	**GI**	59 ± 5	100 ± 0	4 ± 1
	GO	58 ± 5	92 ± 8	4 ± 1
	FA	58± 3	97 ± 3	10 ± 1
Summer	**GI**	55 ± 7	25 ± 11	21 ± 5
	GO	53 ± 4	54 ± 13	33 ± 4
	FA	73 ± 4	4 ± 1	33 ± 3

Figure captions

Figure 1. Potential impacts induced by hydrological extremes on benthic N processes in a coastal lagoon.

Figure 2. Map of the Sacca di Goro lagoon (NE Italy) and location of the three sampling sites.

Figure 3. Vertical profiles of pore water NH_4^+-N concentration at the three sampling sites measured in spring (a) and summer (b) in the Sacca di Goro lagoon. Averages ± standard errors are reported.

Figure 4. Benthic dark fluxes of NH_4^+-N (a), NO_3^--N (b), NO_2^--N (c), and N_2-N (d) measured at the three sampling sites in spring (light grey bars) and summer (dark grey bars) in the Sacca di Goro lagoon. Averages ± standard errors are reported. Fluxes are expressed in µmol N m^{-2} h^{-1}.

Figure 5. Benthic dark O_2 fluxes (a), denitrification (b) and DNRA rates (c) measured at the three sampling sites in spring (light grey bars) and summer (dark grey bars) in the Sacca di Goro lagoon. Total denitrification (D_{tot}) and DNRA rates include the portion coupled to nitrification, D_n and $DNRA_n$ (hatched bars) and the portion sustained by NO_3^- from the water column, D_w and $DNRA_w$ (solid bars). Averages ± standard errors are reported. Fluxes of O_2 are expressed in mmol m^{-2} h^{-1}, whereas D_{tot} and DNRA rates are expressed in µmol N m^{-2} h^{-1}.

Figure 6. Graphic representation of benthic N cycling in spring and summer at the three sampling sites. Fluxes and process rates were derived from direct measurements and calculations. Net O_2 fluxes were converted into theoretical rates of organic N mineralization. The absolute values of O_2 fluxes were assumed to be equivalent to CO_2 fluxes (RQ, Respiratory Quotient, $|O_2|/|CO_2|=1$) (Strickland and Parson, 1972) and were divided by the measured C:N molar ratios of the organic matter in surface sediments. Nitrification rates were estimated, as minimum rates, from the sum of D_n and $DNRA_n$. The contribution of clam respiration and excretion was calculated multiplying biomass-specific excretion rates reported in Welsh et al. (2015) by the biomass of the clams retrieved in our experiments. Mean rates (averages ± standard errors) are expressed in µmol N m^{-2} h^{-1}, C_{org} content is expresses as percentage value (averages ± standard errors). Denitrification efficiency (DE) was calculated as the ratio between dinitrogen (N_2) flux and the sum of N_2 and DIN effluxes.

Figure 7. Inventory of sediment O_2 uptake (a) and total denitrification rates (b) measured in the Sacca di Goro lagoon in the last 26 years. White and grey symbols represent spring and summer values, respectively. Averages ± standard deviations are reported. Fluxes of O_2 are expressed in mmol m^{-2} h^{-1}, whereas D_{tot} rates are expressed in µmol N m^{-2} h^{-1}.

Figure 8. Average values of salinity (a) from 1987 to 1991 (brown lines), from 2006 to 2010 (dotted orange lines), from 2011 to 2014 (dotted light green lines) and from 2015 to 2018 (dotted dark green lines). For 2019 averages ± standard errors are reported (red dots), values of March and April are missing. Average monthly cumulative precipitation (mm) (b) from 2000 to 2018 (grey bars, averages ± standard errors), 2019 values are indicated by black dots. Average values of temperature (c) from 1987 to 1991 (brown lines), from 2006 to 2010 (dotted orange lines), from 2011 to 2014 (dotted light green lines) and from 2015 to 2018 (dotted dark green lines). For 2019

averages ± standard errors are reported (red dots). In the three figures, black squares represent our sampling dates (spring and summer).

Figure 1

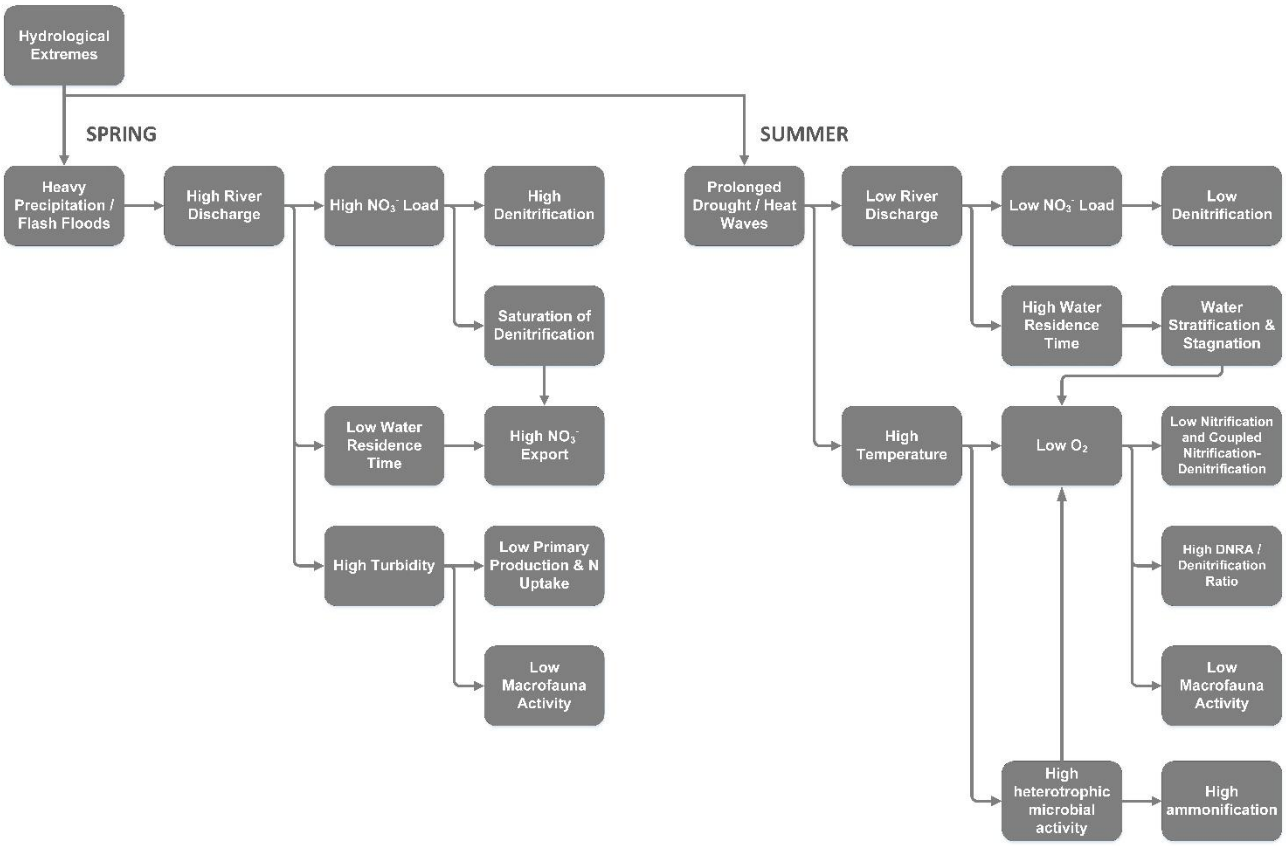


Figure 2

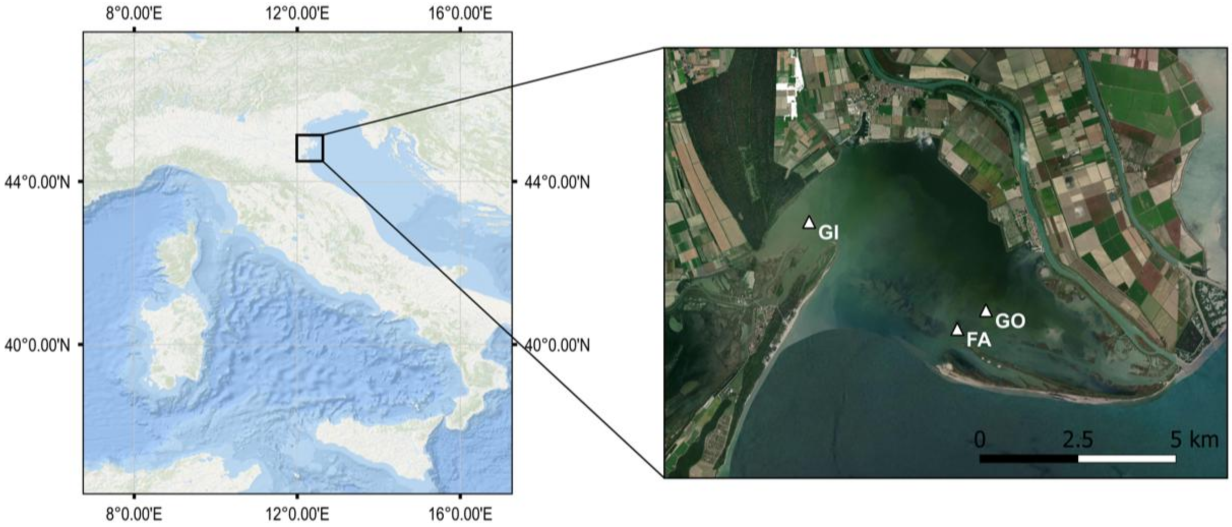
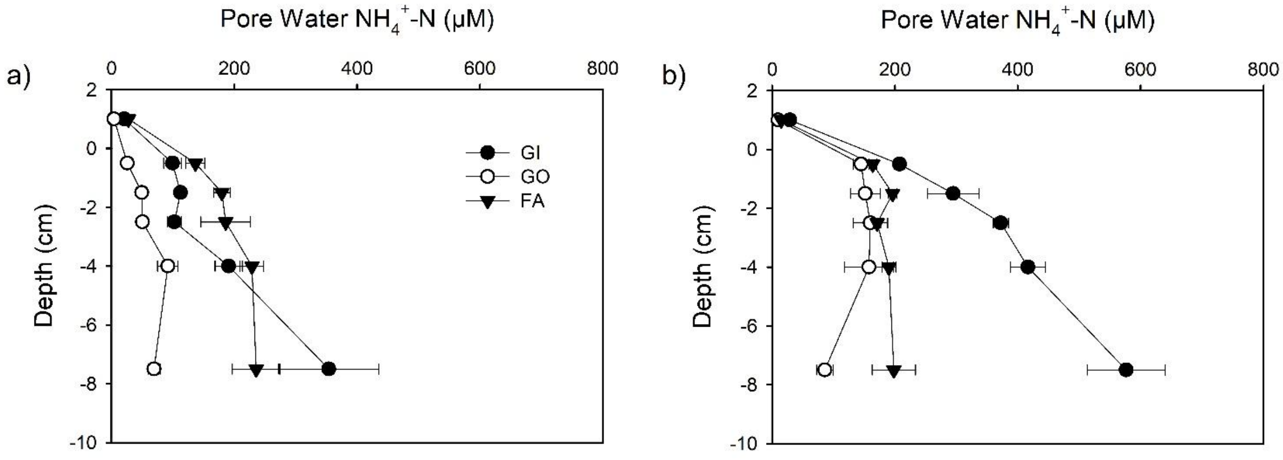


Figure 3



Spring

Summer

Figure 4

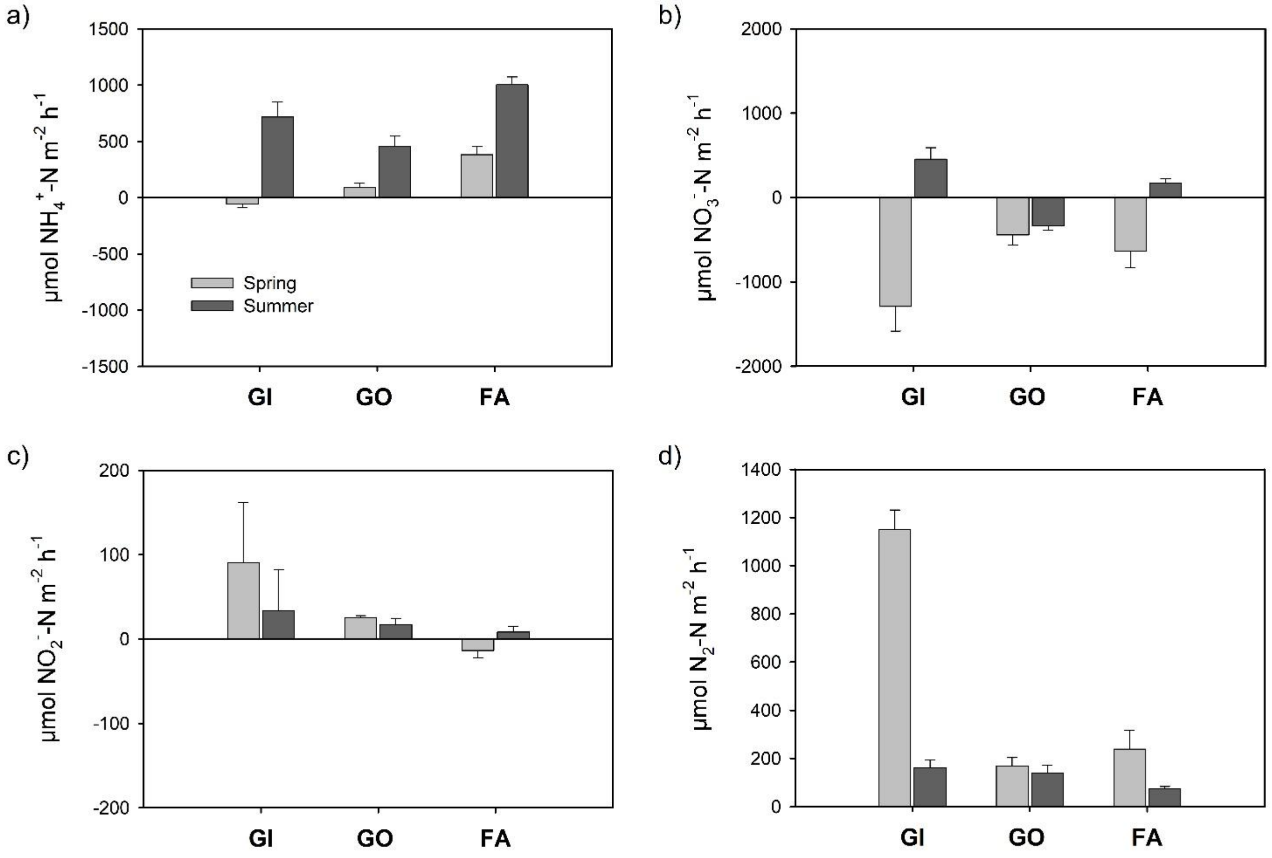


Figure 5

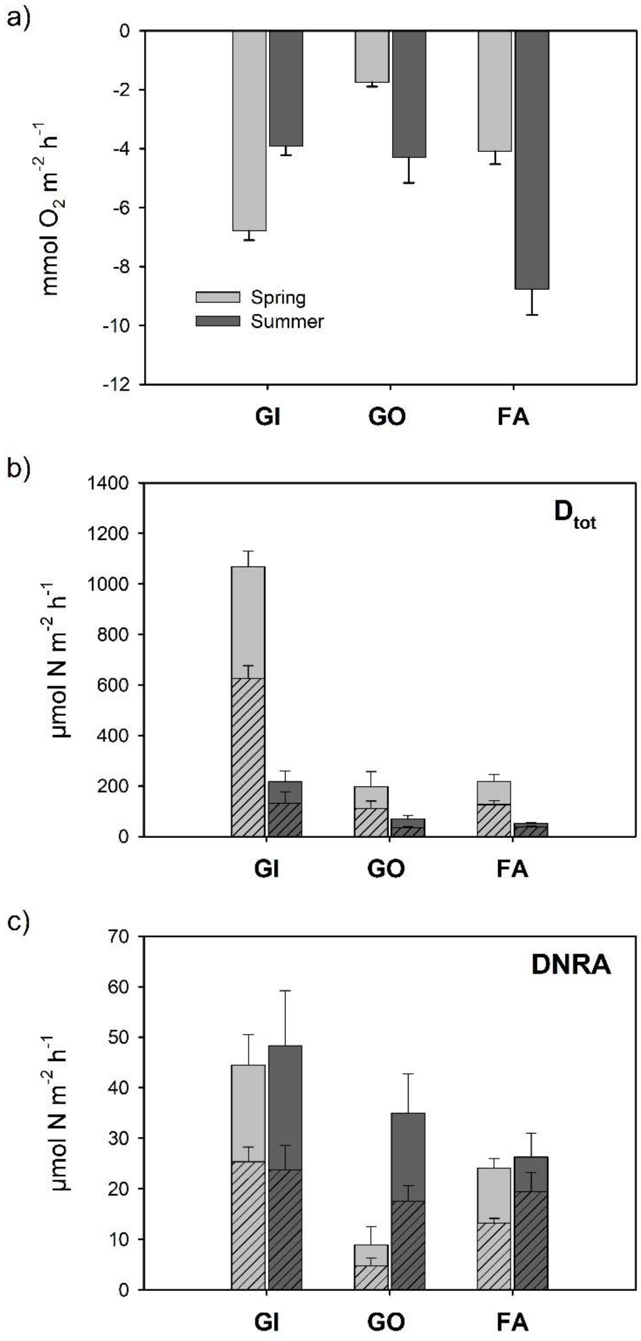

a)
mmol O$_2$ m^{-2} h^{-1}
0
-2
-4
-6
-8
-10
-12
Spring
Summer
GI
GO
FA
b)
μmol N m^{-2} h^{-1}
1400
1200
1000
800
600
400
200
0
D$_{tot}$
GI
GO
FA
c)
μmol N m^{-2} h^{-1}
70
60
50
40
30
20
10
0
DNRA
GI
GO
FA

Figure 6

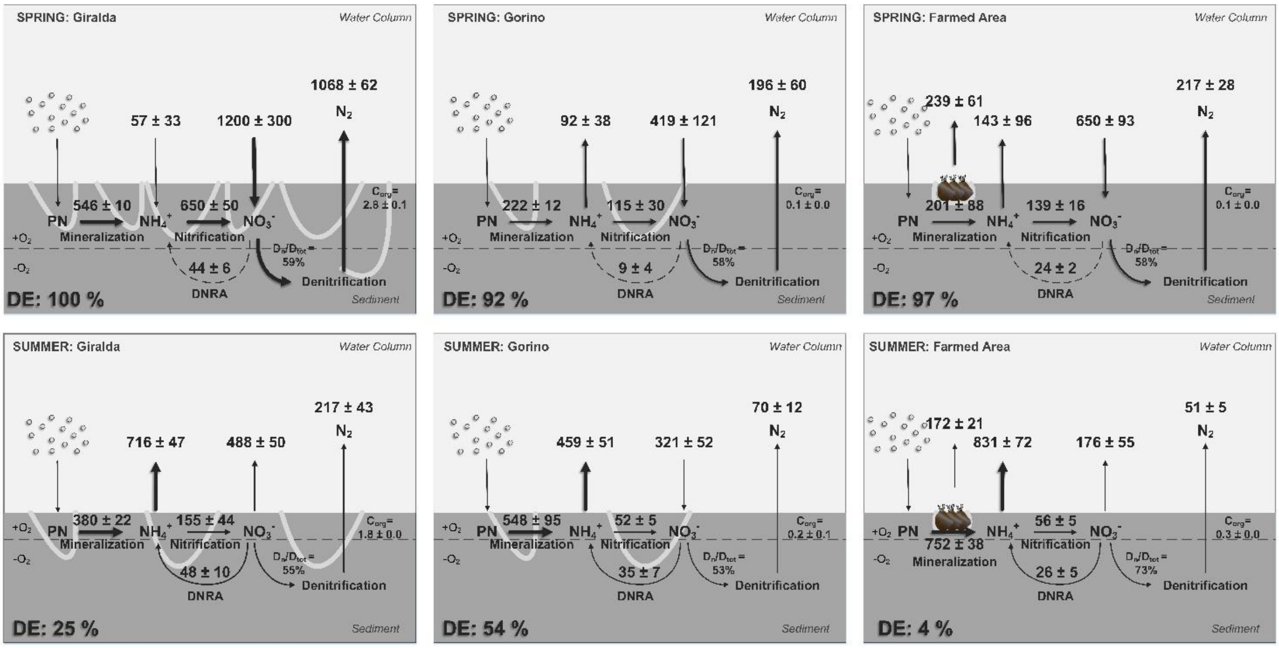

SPRING: Giralda
Water Column
1068 ± 62
N_2
57 ± 33
1200 ± 300
546 ± 10
650 ± 50
C_{org}=
2.8 ± 0.1
PN
NH_4^+
NO_3^-
+O_2
Mineralization
Nitrification
D_n/D_{tot} =
59%
-O_2
44 ± 6
DNRA
Denitrification
DE: 100 %
Sediment
SPRING: Gorino
Water Column
196 ± 60
N_2
92 ± 38
419 ± 121
222 ± 12
115 ± 30
C_{org}=
0.1 ± 0.0
PN
NH_4^+
NO_3^-
+O_2
Mineralization
Nitrification
D_n/D_{tot} =
58%
-O_2
9 ± 4
DNRA
Denitrification
DE: 92 %
Sediment
SPRING: Farmed Area
Water Column
217 ± 28
N_2
239 ± 61
143 ± 96
650 ± 93
201 ± 88
139 ± 16
C_{org}=
0.1 ± 0.0
PN
NH_4^+
NO_3^-
+O_2
Mineralization
Nitrification
D_n/D_{tot} =
58%
-O_2
24 ± 2
DNRA
Denitrification
DE: 97 %
Sediment
SUMMER: Giralda
Water Column
217 ± 43
N_2
716 ± 47
488 ± 50
+O_2
PN
380 ± 22
NH_4^+
155 ± 44
NO_3^-
C_{org}=
1.8 ± 0.0
Mineralization
Nitrification
-O_2
D_n/D_{tot} =
55%
48 ± 10
DNRA
Denitrification
DE: 25 %
Sediment
SUMMER: Gorino
Water Column
70 ± 12
N_2
459 ± 51
321 ± 52
+O_2
PN
548 ± 95
NH_4^+
52 ± 5
NO_3^-
C_{org}=
0.2 ± 0.1
Mineralization
Nitrification
-O_2
D_n/D_{tot} =
53%
35 ± 7
DNRA
Denitrification
DE: 54 %
Sediment
SUMMER: Farmed Area
Water Column
51 ± 5
N_2
172 ± 21
831 ± 72
176 ± 55
+O_2
PN
NH_4^+
56 ± 5
NO_3^-
C_{org}=
0.3 ± 0.0
752 ± 38
Mineralization
Nitrification
-O_2
D_n/D_{tot} =
73%
26 ± 5
DNRA
Denitrification
DE: 4 %
Sediment

Figure 7

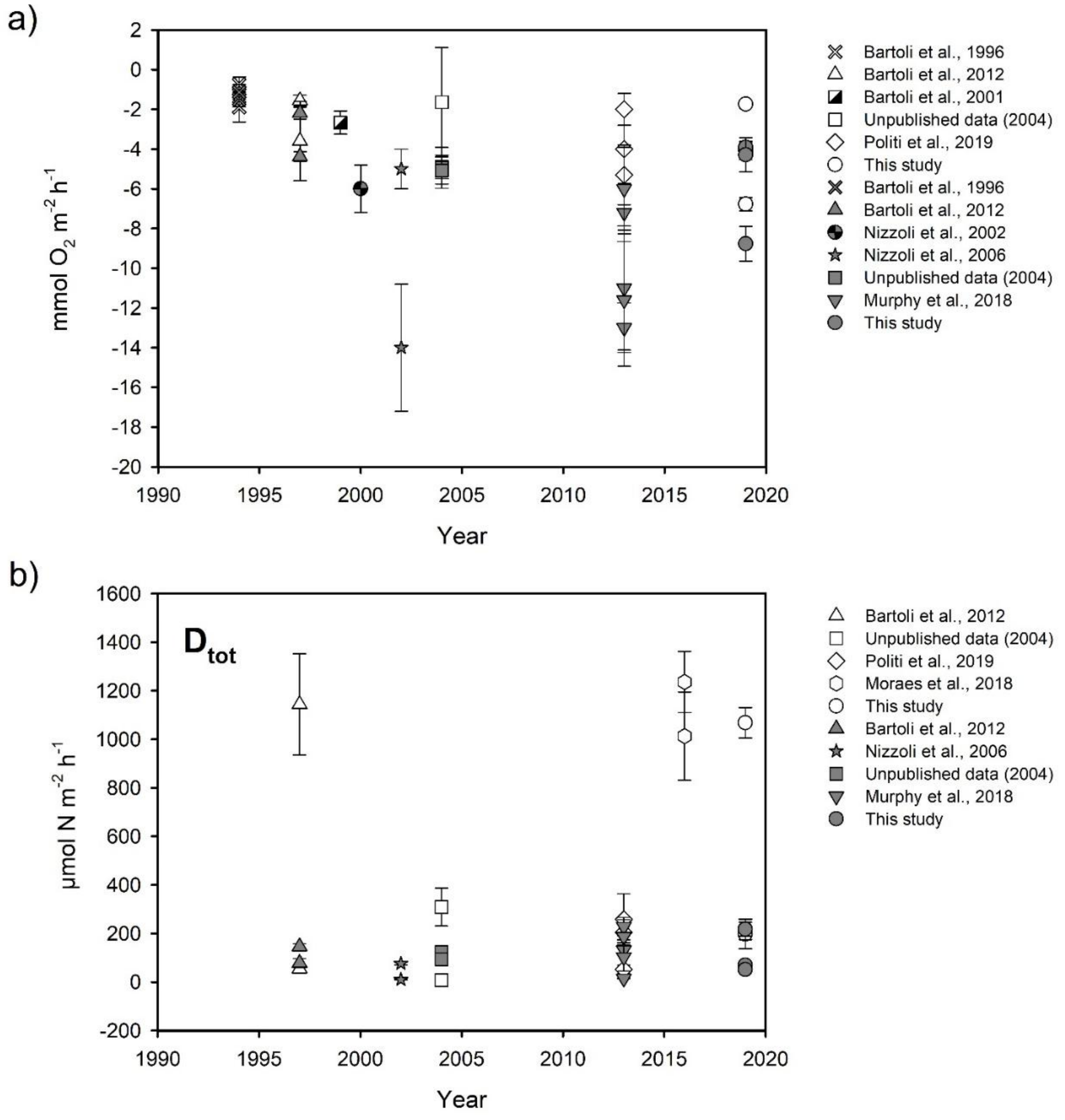
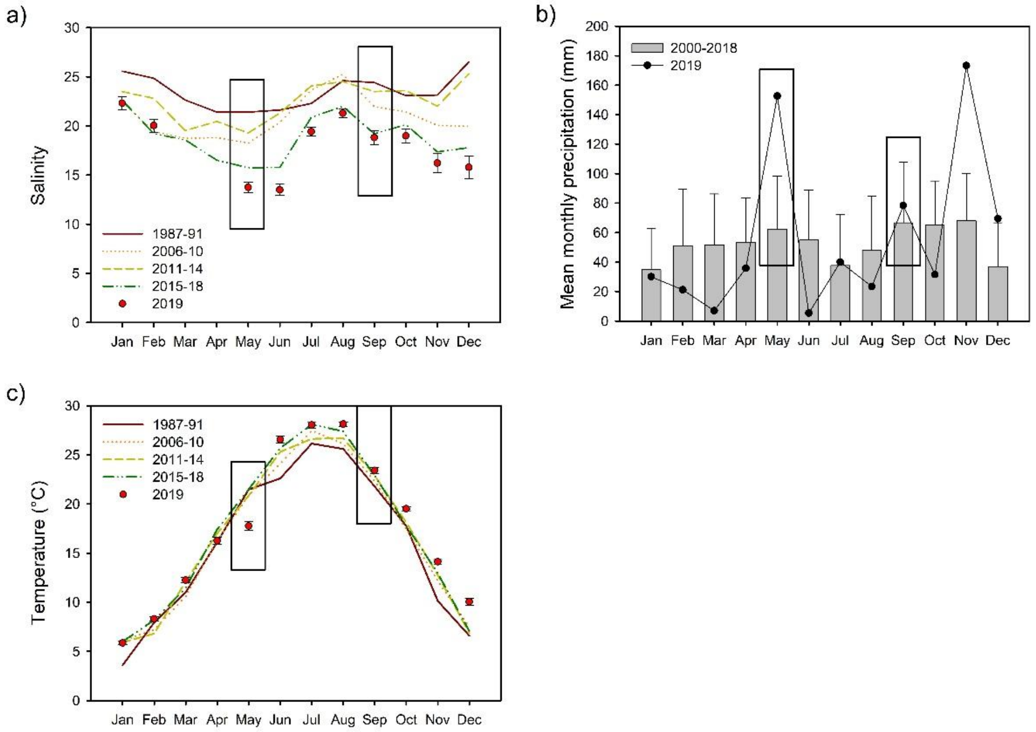
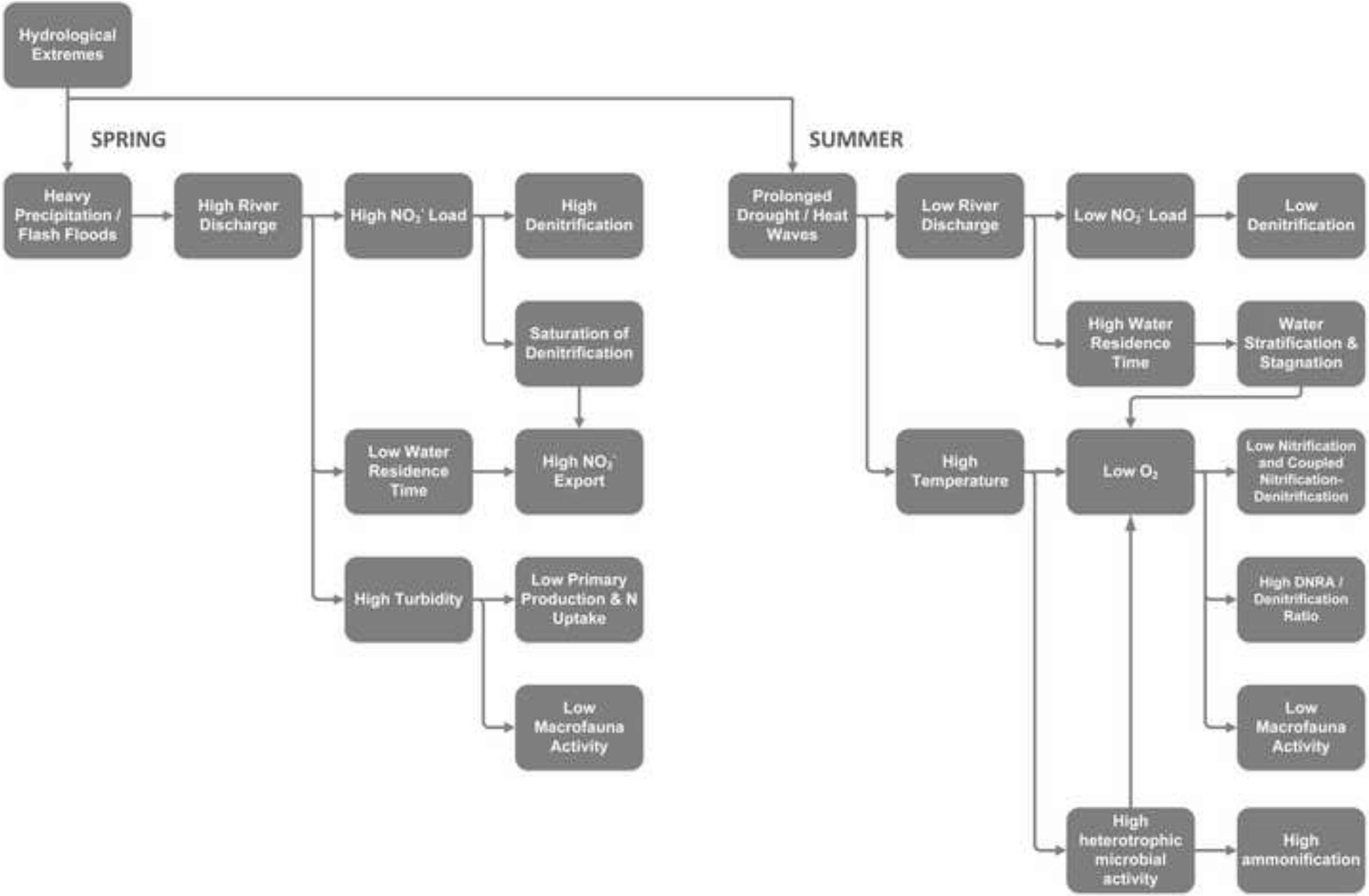

a)
mmol O$_2$ m^{-2} h^{-1}
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
1990
1995
2000
2005
2010
2015
2020
Year
Bartoli et al., 1996
Bartoli et al., 2012
Bartoli et al., 2001
Unpublished data (2004)
Politi et al., 2019
This study
Bartoli et al., 1996
Bartoli et al., 2012
Nizzoli et al., 2002
Nizzoli et al., 2006
Unpublished data (2004)
Murphy et al., 2018
This study
b)
D$_{tot}$
µmol N m^{-2} h^{-1}
1600
1400
1200
1000
800
600
400
200
0
-200
1990
1995
2000
2005
2010
2015
2020
Year
Bartoli et al., 2012
Unpublished data (2004)
Politi et al., 2019
Moraes et al., 2018
This study
Bartoli et al., 2012
Nizzoli et al., 2006
Unpublished data (2004)
Murphy et al., 2018
This study

Figure 8


a)
Salinity
0
5
10
15
20
25
30
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
1987-91
2006-10
2011-14
2015-18
2019
b)
Mean monthly precipitation (mm)
0
20
40
60
80
100
120
140
160
180
200
2000-2018
2019
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
c)
Temperature (°C)
0
5
10
15
20
25
30
1987-91
2006-10
2011-14
2015-18
2019
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec


Hydrological Extremes
SPRING
SUMMER
Heavy Precipitation / Flash Floods
High River Discharge
High NO_3^- Load
High Denitrification
Saturation of Denitrification
Low Water Residence Time
High NO_3^- Export
High Turbidity
Low Primary Production & N Uptake
Low Macrofauna Activity
Prolonged Drought / Heat Waves
Low River Discharge
Low NO_3^- Load
Low Denitrification
High Water Residence Time
Water Stratification & Stagnation
High Temperature
Low O_2
Low Nitrification and Coupled Nitrification-Denitrification
High DNRA / Denitrification Ratio
Low Macrofauna Activity
High heterotrophic microbial activity
High ammonification

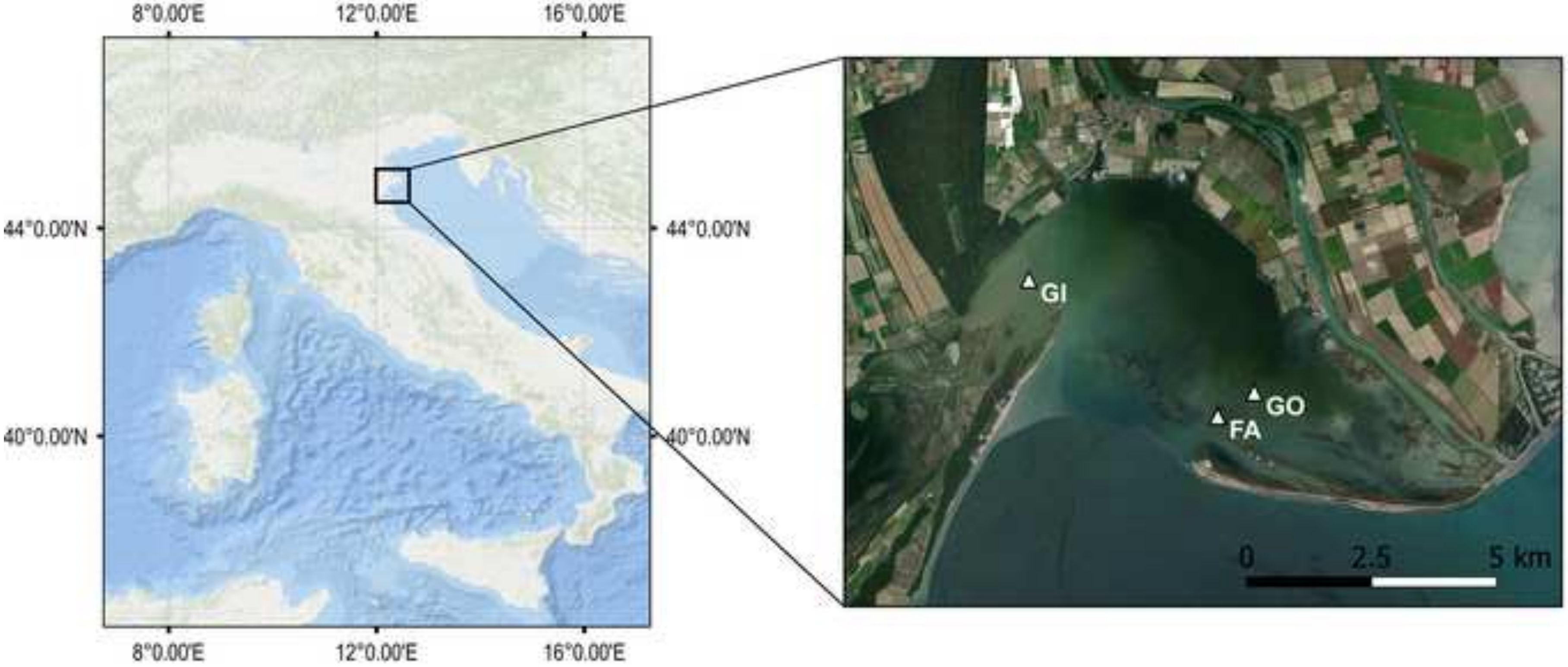

8°0.00'E
12°0.00'E
16°0.00'E
44°0.00'N
40°0.00'N
44°0.00'N
40°0.00'N
8°0.00'E
12°0.00'E
16°0.00'E
GI
GO
FA
0
2.5
5 km

Fig_3_jpg

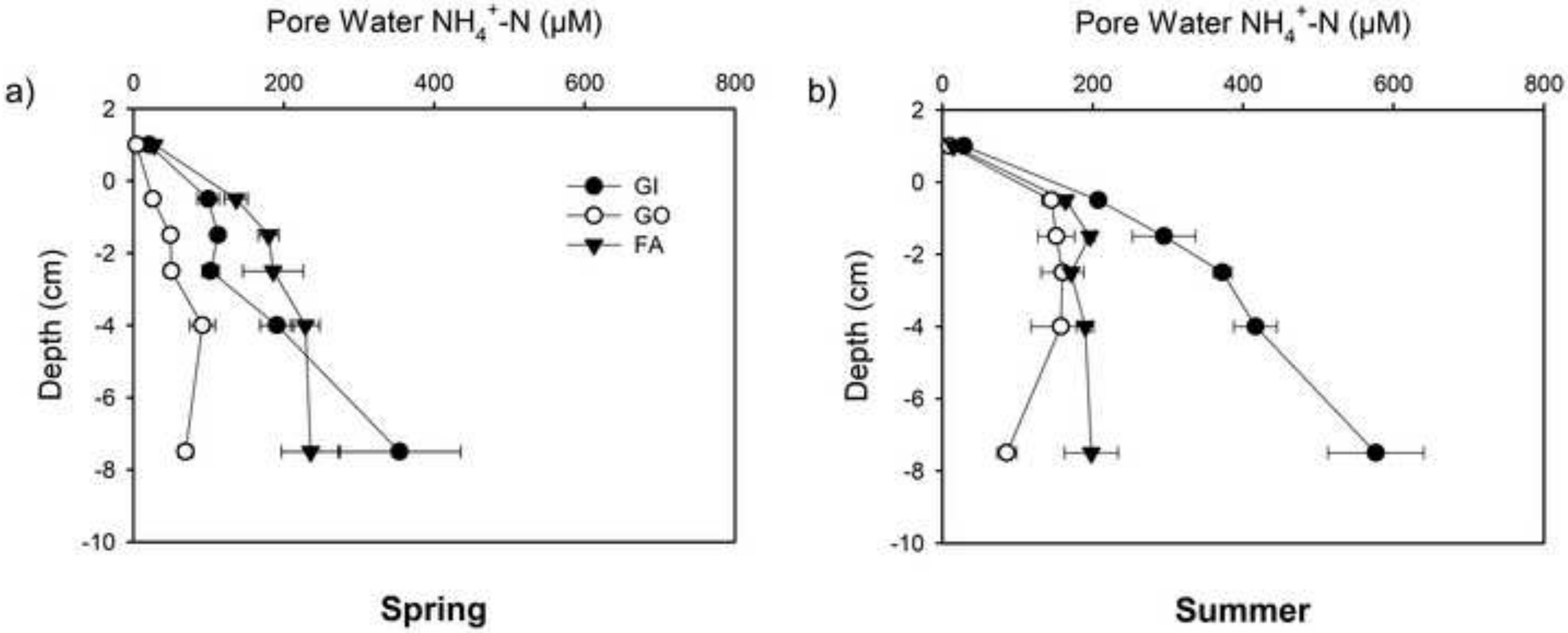
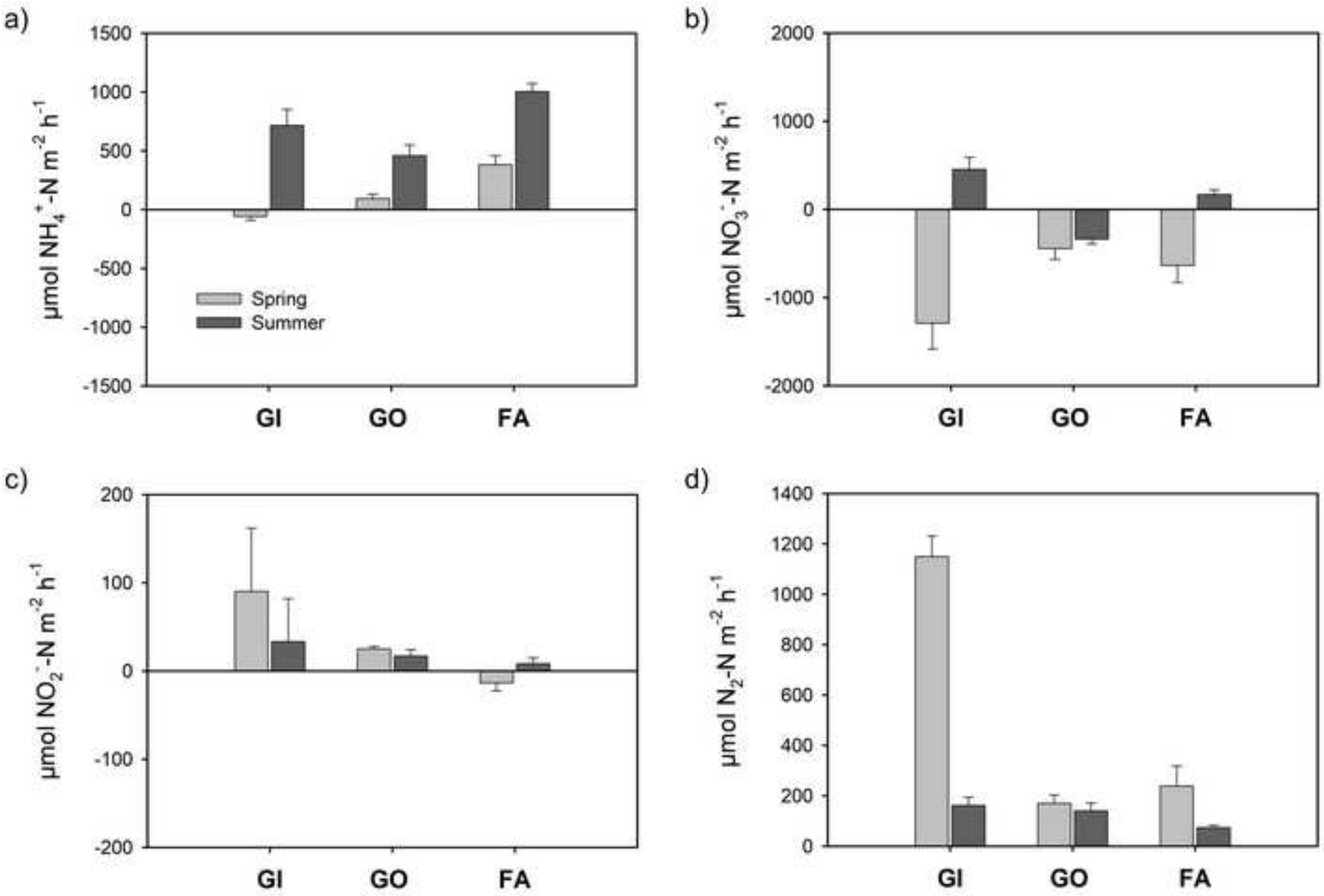

a)
Pore Water NH4+-N (μM)
0
200
400
600
800
2
0
-2
-4
-6
-8
-10
Depth (cm)
GI
GO
FA
Spring
b)
Pore Water NH4+-N (μM)
0
200
400
600
800
2
0
-2
-4
-6
-8
-10
Depth (cm)
Summer

Fig_4_jpg


a)
1500
1000
500
0
-500
-1000
-1500
µmol NH_4^+-N m^{-2} h^{-1}
Spring
Summer
GI
GO
FA
b)
2000
1000
0
-1000
-2000
µmol NO_3^--N m^{-2} h^{-1}
GI
GO
FA
c)
200
100
0
-100
-200
µmol NO_2^--N m^{-2} h^{-1}
GI
GO
FA
d)
1400
1200
1000
800
600
400
200
0
µmol N_2-N m^{-2} h^{-1}
GI
GO
FA

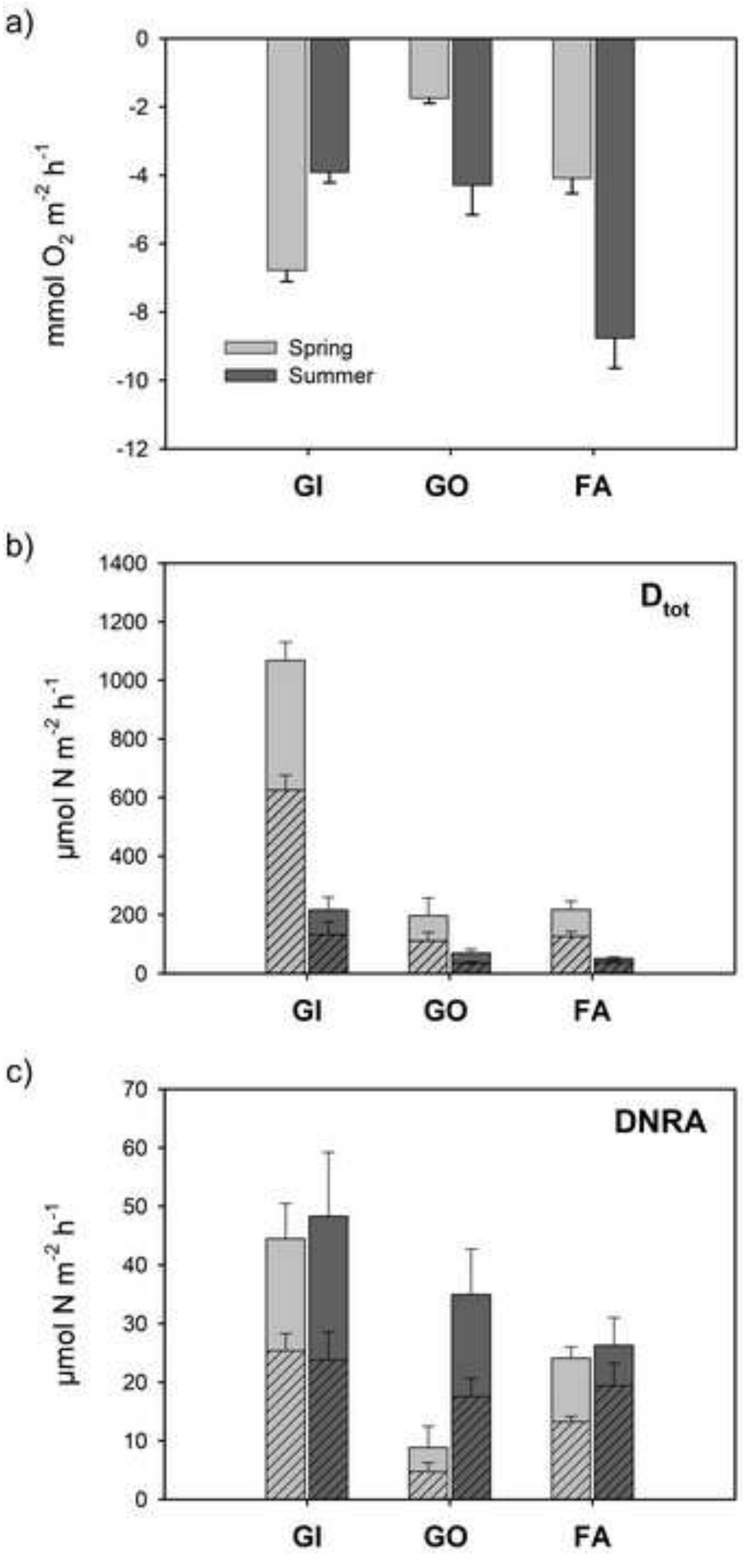

a)
0
-2
-4
-6
-8
-10
-12
mmol O_2 m^{-2} h^{-1}
Spring
Summer
GI
GO
FA
b)
1400
1200
1000
800
600
400
200
0
µmol N m^{-2} h^{-1}
D_{tot}
GI
GO
FA
c)
70
60
50
40
30
20
10
0
µmol N m^{-2} h^{-1}
DNRA
GI
GO
FA

Fig_6_jpg

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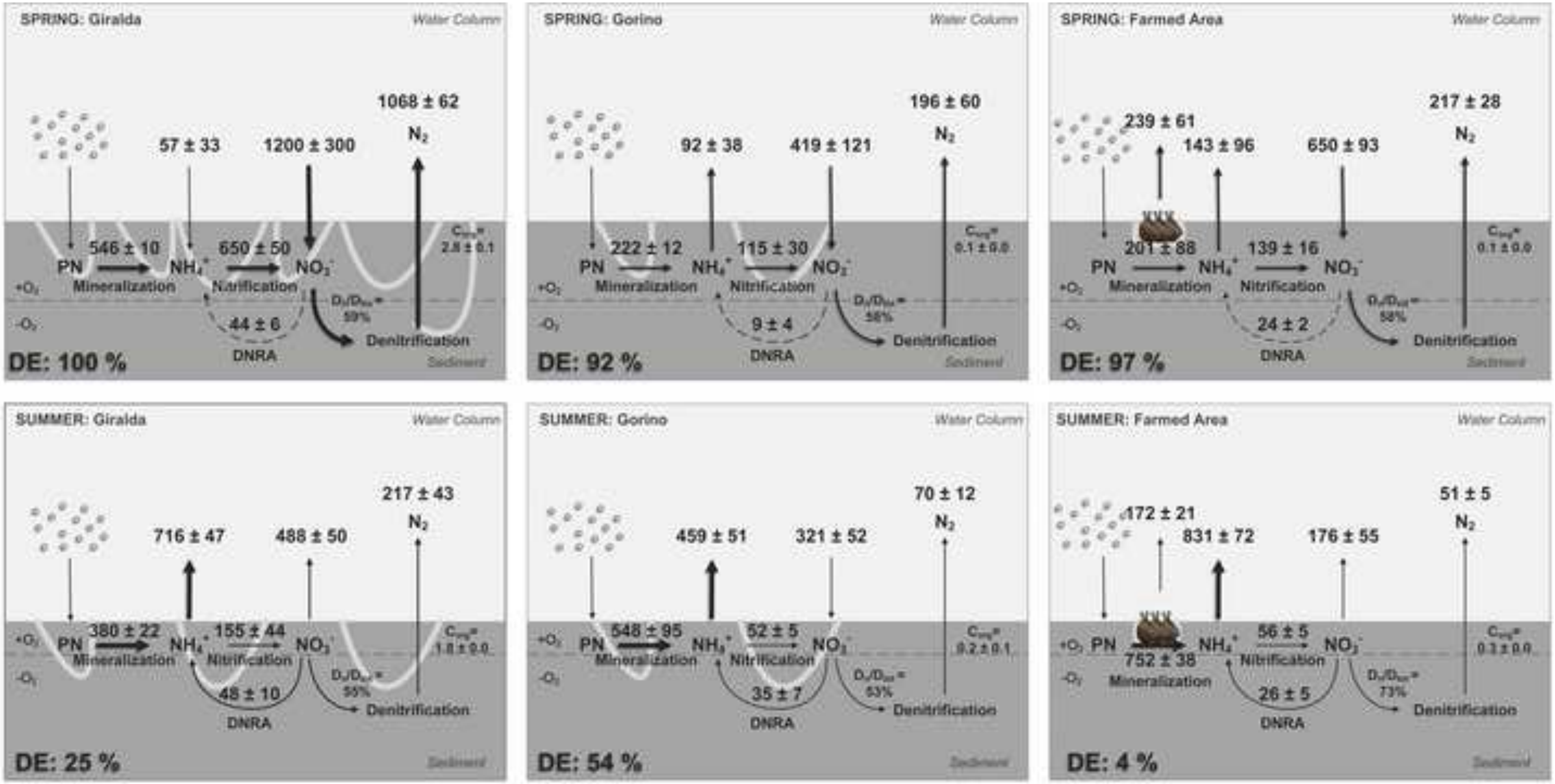


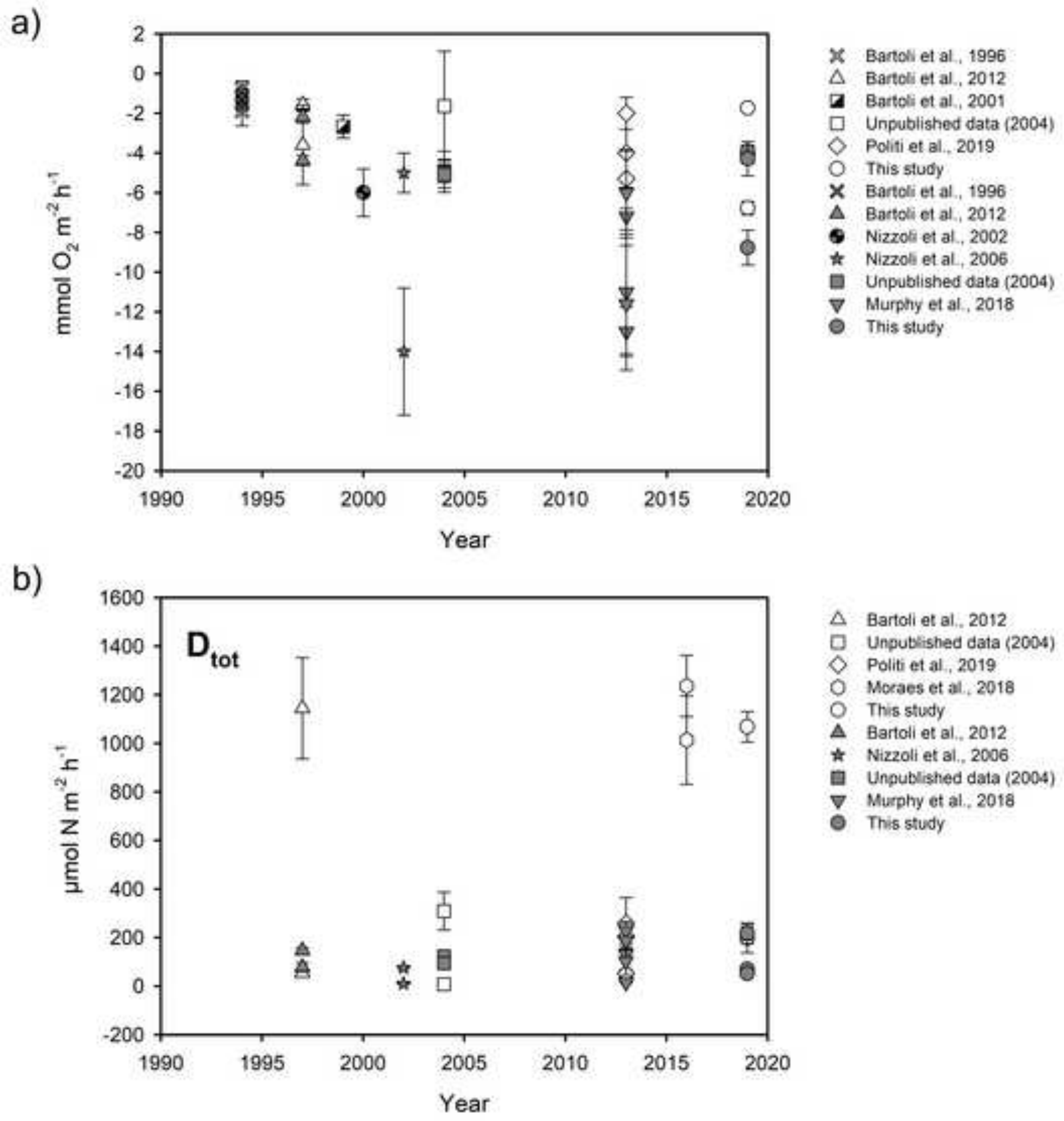
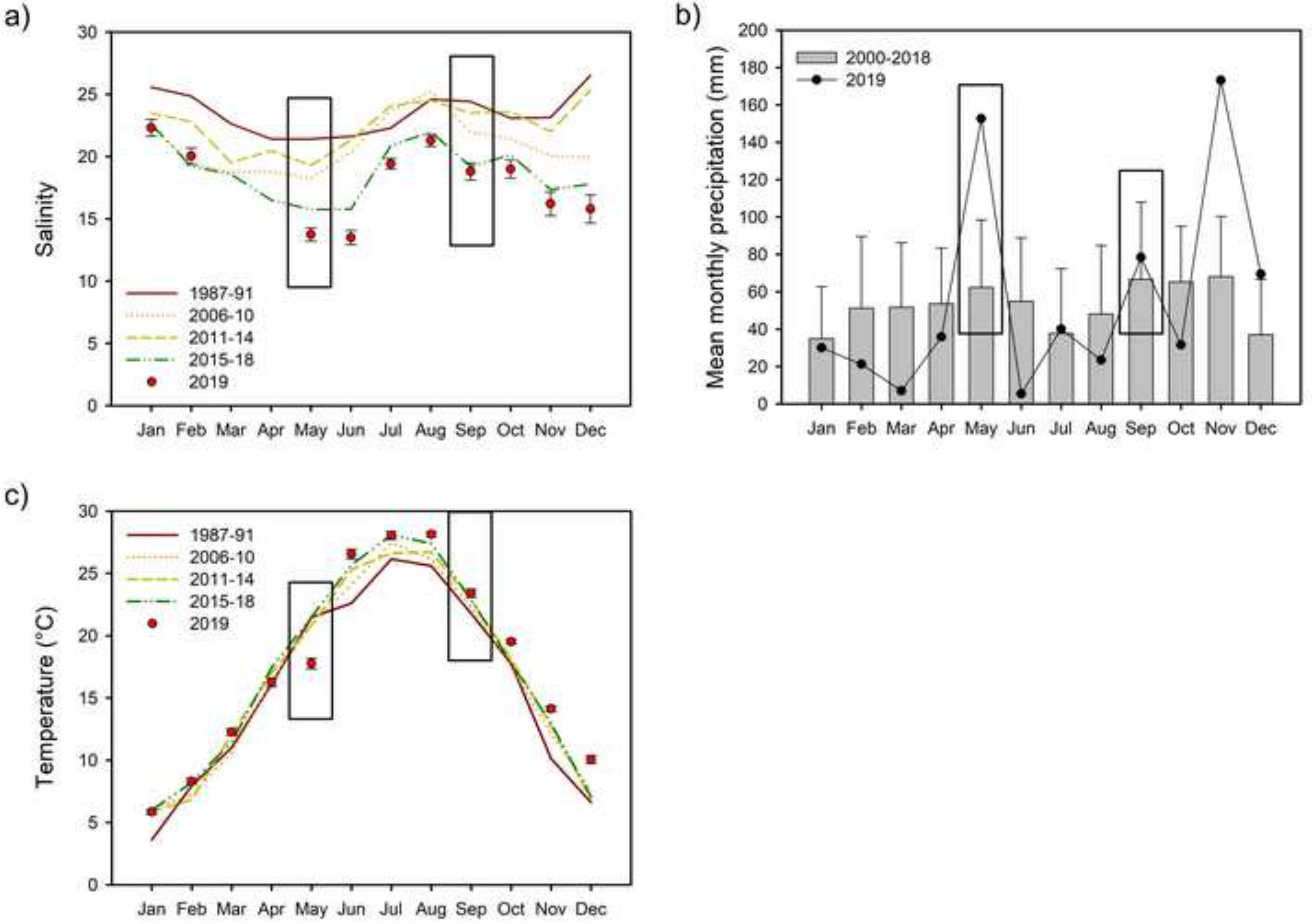

a)
mmol O_2 m^{-2} h^{-1}
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
1990
1995
2000
2005
2010
2015
2020
Year
Bartoli et al., 1996
Bartoli et al., 2012
Bartoli et al., 2001
Unpublished data (2004)
Politi et al., 2019
This study
Bartoli et al., 1996
Bartoli et al., 2012
Nizzoli et al., 2002
Nizzoli et al., 2006
Unpublished data (2004)
Murphy et al., 2018
This study
b)
D_{tot}
µmol N m^{-2} h^{-1}
1600
1400
1200
1000
800
600
400
200
0
-200
1990
1995
2000
2005
2010
2015
2020
Year
Bartoli et al., 2012
Unpublished data (2004)
Politi et al., 2019
Moraes et al., 2018
This study
Bartoli et al., 2012
Nizzoli et al., 2006
Unpublished data (2004)
Murphy et al., 2018
This study

Fig_8_jpg


a)
Salinity
30
25
20
15
10
5
0
1987-91
2006-10
2011-14
2015-18
2019
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
b)
Mean monthly precipitation (mm)
200
180
160
140
120
100
80
60
40
20
0
2000-2018
2019
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
c)
Temperature (°C)
30
25
20
15
10
5
0
1987-91
2006-10
2011-14
2015-18
2019
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

***conflict of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

*Credit Author Statement

Monia Magri: Conceptualization, Investigation, Writing – Original Draft, Writing – Review & Editing;

Sara Benelli: Conceptualization, Investigation, Writing – Original Draft, Writing – Review & Editing;

Stefano Bonaglia: Validation, Investigation, Writing – Review & Editing;

Mindaugas Zilius: Validation, Writing – Review & Editing;

Giuseppe Castaldelli: Resources, Writing – Review & Editing;

Marco Bartoli: Conceptualization, Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Supervision