



Influence of conventional and organic rice farming on aquatic biodiversity and greenhouse gas emissions in a protected Mediterranean wetland

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ABSTRACT

Coastal wetlands face numerous threats from human activities, including habitat alteration for rice cultivation and contamination by agrochemicals. In this study, we evaluated the impact of different rice production practices on the structure of aquatic communities and greenhouse gas emissions in the Albufera Natural Park (Valencia, Spain), one of the most important Mediterranean coastal wetlands. We monitored the diversity of aquatic bacteria and archaea, zooplankton, and macroinvertebrates over the rice cultivation period at four sampling sites: a water spring representing a natural (unimpacted) area, an organic rice field, and two conventional rice fields. Additionally, we measured CO₂ and CH₄ emissions at various stages of the rice cultivation period. Our study shows that areas dedicated to ecosystem preservation, such as water springs, support a higher diversity of bacteria and archaea and provide habitat for endemic and endangered macroinvertebrate species. Moreover, these areas exhibit the lowest relative greenhouse gas emissions from the water column, offering significant benefits for climate change mitigation. The study also reveals that the structure of aquatic communities is heavily influenced by rice farming practices, with organic rice farming supporting a larger abundance of pollution-resistant zooplankton and a higher diversity and biomass of emerging insects. However, organic rice farming was associated with the highest prevalence of fecal microorganisms and contributed more to greenhouse gas emissions during the rice cultivation period due to its manure fertilization regime. These findings underscore the need to carefully consider both the benefits and challenges of different farming practices, balancing ecological conservation and GHG emissions with sustainable agricultural production.

1. Introduction

Mediterranean coastal wetlands provide a broad array of ecosystem services, including biodiversity conservation, carbon sequestration, climate regulation, and nutrient cycling (Pérez-Ruzafa et al., 2011; Newton et al., 2018). These wetlands are recognized as biodiversity hotspots for aquatic species. However, despite their ecological significance, they are continuously impacted by human activities (Perennou et al., 2018; Martínez-Megías and Rico, 2022; Morant et al., 2021). One of the primary threats facing these ecosystems is the progressive loss of habitat. Between 1970 and 2013, 48 % of the surface area of Mediterranean wetlands was lost (Mediterranean Wetlands Observatory, 2018),

and this trend continues due to water scarcity and agricultural expansion, with rice production being a major contributor (Davidson and Finlayson, 2018).

Besides land transformation, the use of synthetic pesticides in conventional rice farming poses significant risks to non-target aquatic species (Martínez-Megías et al. 2023), with toxic effects documented in numerous studies (Gaget et al., 2020; Amador et al., 2023). These impacts range from changes in community composition (Xiao et al., 2017) to reductions in density, abundance, and biodiversity among zooplankton and macroinvertebrates (Cochard et al., 2014; Plangklang and Athibai, 2021). Mesocosm studies conducted on aquatic communities representative of Mediterranean coastal lagoons and ponds

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demonstrate that current exposure levels significantly alter the structure of primary producer and invertebrate communities, leading to the population decline of endangered crustacean species. Moreover, these changes can substantially affect ecosystem functions, such as biomass production and organic matter decomposition (Amador et al., 2023; Grillo-Avila et al., 2024). However, limited research has specifically explored the long-term effects of pesticides and conventional rice farming practices—such as intermittent drainage or fertilization—on biodiversity in rice paddies.

Land use and ecosystem health are critical determinants of the carbon sequestration capacity and greenhouse gas (GHG) emissions of Mediterranean wetlands (Morant et al., 2020a, 2024). Hydrological patterns, which are extensively altered in rice paddies, play a key role in shaping GHG emissions (Camacho-Santamans et al., 2024). Research on Mediterranean freshwater and brackish wetlands has shown that wetlands receiving high nutrient inputs sequester more carbon. However, these nutrient-enriched wetlands also experience elevated metabolic degradation, including aerobic respiration and methane (CH_4) emissions, compared to those unaffected by agricultural runoff (Morant et al., 2020b). This connection highlights the link between eutrophication, conservation status, and increased warming potential, which supports the development of environmental policies aimed at the conservation and climate change mitigation of these ecosystems (Camacho et al., 2017).

Rice paddies are recognized as significant global sources of CH_4 , with emissions ranging from 5 to 90 g $\text{CH}_4 \text{ m}^{-2}$ during the cultivation period (Cao et al., 1996). Research suggests that organic rice farming practices—such as reducing organic fertilizers and synthetic pesticides while minimizing tillage—can enhance soil carbon sequestration (Gangopadhyay et al., 2022; Haque et al., 2020; Komatsuzaki and Syuaib, 2010). However, most studies have focused on (sub-)tropical regions in Asia, with limited investigation into the effects of converting to organic rice farming on aquatic biodiversity and GHG emissions in Mediterranean contexts (but see Pérez-Méndez et al., 2023). Recent studies also underscore that specific management practices in Mediterranean rice paddies—such as post-harvest flooding patterns, straw management, and the type of wetlands previously present on reclaimed land—significantly affect the GHG emissions associated with rice cultivation (Belenguer-Manzanedo et al., 2022a, 2022b, 2023).

Another important habitat within Mediterranean littoral wetlands is water springs, where stable groundwater flow, temperature, and other abiotic factors create unique ecological conditions. These springs are biodiversity hotspots (Soria, 1993; Fernández-Martínez et al., 2024) and host numerous endemic species (Oliva-Paterna et al., 2009), some of which are extinct or critically endangered in rice paddies and other environments. Many springs are relatively isolated from agricultural areas and experience minimal human impact, making them suitable cases for studying the effects of land-use transformation, such as the conversion of wetlands into rice paddies, on biodiversity and ecosystem services like carbon sequestration.

This study aimed to assess the effects of different land uses and rice cultivation practices on the biodiversity of aquatic organisms and GHG emissions under Mediterranean conditions. To achieve this, we monitored the diversity of bacteria and archaea, zooplankton, and macro-invertebrates (including aquatic and emergent insects) throughout the rice cultivation period in four areas within a protected Mediterranean wetland: a water spring representing good ecological conditions, an organic rice field, and two conventional rice fields. Additionally, we measured the GHG emissions at these sites, focusing on CO_2 and CH_4 fluxes, at different stages of the cultivation cycle. The specific objectives of this study were: (1) to investigate differences in aquatic biodiversity among the sampled areas and identify representative taxa at each; (2) to examine how agricultural practices and pesticide use impact the temporal dynamics of microbial and invertebrate communities in the rice fields; and (3) to evaluate how land use and agricultural management interplays with GHG emissions during the rice cultivation period. Our

sampling efforts focused on bacteria, archaea, and aquatic invertebrates for several key reasons. First, bacteria and archaea were selected because they are particularly sensitive to certain agrochemicals with antimicrobial modes of action, as well as to specific fertilization regimes (Yen et al., 2009). Moreover, their role in carbon sequestration processes and greenhouse gas fluxes, which are central to this study, makes them critical indicators (Belenguer-Manzanedo et al., 2022a; Morant et al., 2024). Aquatic invertebrates, on the other hand, were chosen due to their high vulnerability to agrochemicals such as insecticides and fungicides, particularly zooplankton (Rico et al., 2018; Amador et al., 2023). These organisms typically spend most, if not all, of their lifespan within rice paddies, making them excellent integrative indicators of the ecological status of aquatic ecosystems in response to agricultural management practices.

2. Materials and methods

2.1. Sampling sites, rice cultivation practices and pesticide applications

The study was conducted at the Albufera Natural Park (ANP), which is located in the province of Valencia, eastern Spain. The ANP is a Ramsar-listed wetland and a key ecological and economic resource in the region. It consists of a polymictic shallow coastal lake surrounded by rice paddies (Romo et al., 2005; Fig. 1). Rice farming in the ANP significantly grew in the 18th century (Soria, 2006) thanks to the conversion of part of the lake's area into rice paddies (Vallés et al., 2018). Today, these rice fields serve as important habitats and refuges for aquatic organisms, including a variety of local and migratory birds (Picazo-Tadeo et al., 2009).

The study considered four sampling sites within the ANP: a water spring, an organic rice field, and two conventional rice fields (Rice Field 1 and Rice Field 2; Fig. 1). The selected water spring (Font del Forner) is located at the edge of the ANP and consists of a low-flow pond and a water channel with submerged macrophytes. The organic rice field is located near the ANP edge and covers 0.5 ha. It was fertilized with horse manure (10,000 kg/ha) on May 8, 2021, and manually seeded (240 kg seeds/ha) on May 21, 2021. Weeds were removed manually once per month, and an inorganic fungicide (23 % copper, 0.5 % zinc, and 0.5 % manganese) was applied during the final cultivation stages (August–September).

Conventional Rice Field 1 located is in the northern part of the ANP and is irrigated with reclaimed water from a wastewater treatment plant (WWTP). Seeding took place on May 15, 2021, using the "Omega" rice variety, which is more sensitive to fungal diseases than other varieties. This plot also exhibited a higher sensitivity to weed proliferation, leading to more frequent pesticide applications and higher herbicide dosages compared to Rice Field 2 (Fig. 2). Rice Field 2, located in the southern part of the ANP, was also seeded on May 15, 2021, with the "J-Sendra" variety, the most common rice variety in the ANP, and was irrigated with water from the Albufera Lake. Germination in both conventional fields occurred about one week after seeding. Both conventional rice fields were continuously irrigated throughout the growing cycle, maintaining an approximate water depth of 6 cm during the first month and 10 cm for the remainder of the season. Herbicides were applied two weeks after seeding, for weed control. In July, the fields were dried for about 7 days to prevent the spread of certain competing plants (e.g., *Echinochloa* species) and to facilitate further herbicide and insecticide applications. Fungicides were applied at the end of the summer in both fields. The rice harvest was completed on September 20, 2021, in Rice Field 1 and on October 2, 2021, in Rice Field 2. Detailed information on pesticide active ingredients, application dosages, and application methods is provided in Fig. 2 and the Supplementary Material (Table S1). Information on basic physico-chemical parameters of water is shown in Table S2.

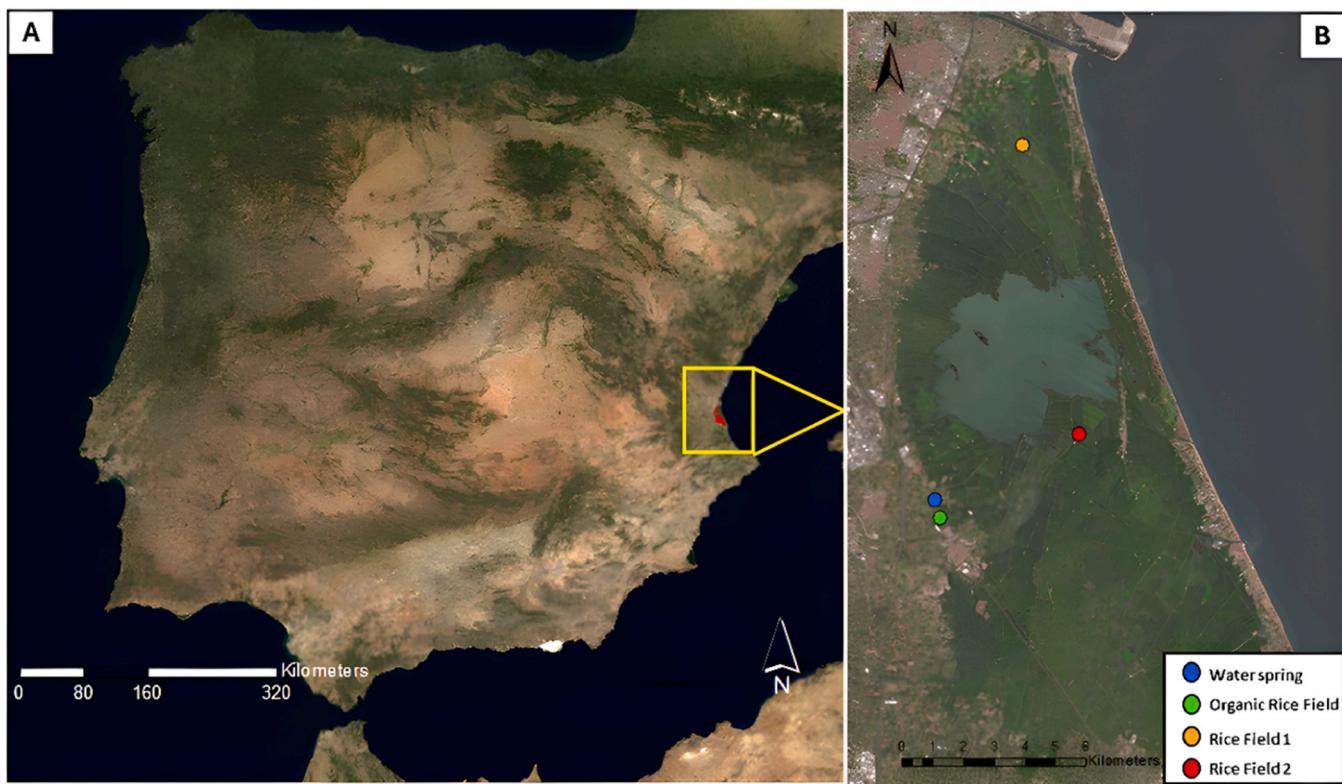


Fig. 1. Location of the Albufera Natural Park (ANP, red spot) in the Iberian Peninsula (A) and approximate location of the sampling sites within the ANP (B). Within each sampling site, three sampling points were randomly selected for the analysis of the biological variables.

2.2. Microbial sampling and analysis

Water and sediment samples for prokaryote analysis were collected in triplicate (distributed randomly within the sampling sites) at the four studied sites across five sampling campaigns, between May 24th and October 13th 2021 (for details on the sampling dates see Table S3). They were quickly transported to the laboratory, where they were immediately homogenized and aliquoted in subsamples and frozen at -80°C until further processing.

DNA extraction for each sample was performed using the EZNA soil DNA isolation kit (Omega Bio-Tek, Inc., Norcross, GA, USA). The V4 region of the 16S rRNA gene was sequenced using the Illumina MiSeq system (2x250bp) at the genomics facilities of the Research Technology Support Facility, Michigan State University, USA. Dual-indexed amplicon libraries of the 16S-V4 rRNA hypervariable region were created for each sample using primers 515 f/806r. Libraries were batch normalized using Invitrogen SequalPrep DNA Normalization Plates and then pooled together. The pool was quality-checked and quantified using Qubit dsDNA HS, Agilent 4200 TapeStation High Sensitivity DNA, and Kapa Illumina Library Quantification qPCR assays. It was then loaded onto an Illumina MiSeq v2 flow cell and sequenced in a 2x250bp paired end format using a MiSeq v2 500 cycle reagent cartridge. Custom sequencing and index primers complementary to the 515/806 target sequences were added to appropriate wells of reagent cartridge. Base calling was performed using Illumina Real Time Analysis (RTA) v1.18.54, and the output was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.19.1.

Sequences were processed using the UPARSE pipeline using USEARCHv11.0.667 (Edgar, 2013). After merging read pairs, the dataset was filtered with a maximum expected error of 0.5. Chimeric sequences were removed using UCHIME in USEARCH v11.0.667 (Edgar, 2013). Filtered sequences were clustered in zero-radius Operational Taxonomic Units (ZOTUs), which are sequences with 100 % identity. Alignment and taxonomic assignment were done with SINA v1.2.1152

using SILVA 138.1 database (Pruesse et al., 2012). SINA uses Lowest Common Ancestor method (LCA). A “Min identity” of 0.8 and a maximum of one search result per sequence were set. Sequences with low alignment quality (<75 %) and those identified as mitochondria or chloroplasts were removed. ZOTUs were normalized by rarefying reads of all samples to a minimum threshold of 2697 reads/sample. Rarefactions were repeated 100 times to preserve less abundant ZOTUs and then combined in three average rarefied ZOTUs tables.

Phylogenetic diversity based on Hill numbers (Chao et al., 2014), total richness (R_t) and Shannon's diversity index (H') were calculated for each sample using the phylogenetic tree inferred from all ZOTUs. Sequences from all samples were aligned with MAFFT version7 (Katoh and Standley, 2013), trimmed with trimAI (Capella-Gutiérrez et al., 2009) and the phylogeny constructed with IQ-TREE (Nguyen et al., 2015) using the maximum likelihood method with 10000 bootstrap samples. The resulting phylogenetic tree was midpoint-rooted with iTOL (Letunic and Bork, 2021). Phylogenetic diversity was calculated with the phylogenetic tree and the ZOTUs abundance matrix with hillR package in RStudio (Li et al., 2014). All sequence data have been deposited in the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI) BioProject PRJNA1183724.

2.3. Invertebrate sampling, identification and counting

Zooplankton samples were taken six times, in triplicate (randomly distributed), in each of the four sampling sites between June 17th and September 10th, 2021 (sampling dates are shown in Table S3). Samples were taken by filtering 5 L of water through a 55 μm zooplankton net. Samples were stored in 100 mL sterile Anaclin ® bottles and fixed with Lugol (I_3K) solution. Identification of individuals was done with a stereomicroscope and was restricted to the macrozooplankton (crustaceans) group. Species identification was performed to the lowest practical taxonomic resolution following taxonomic guidelines (Amoros, 1984; Bledzki and Rybak, 2016).

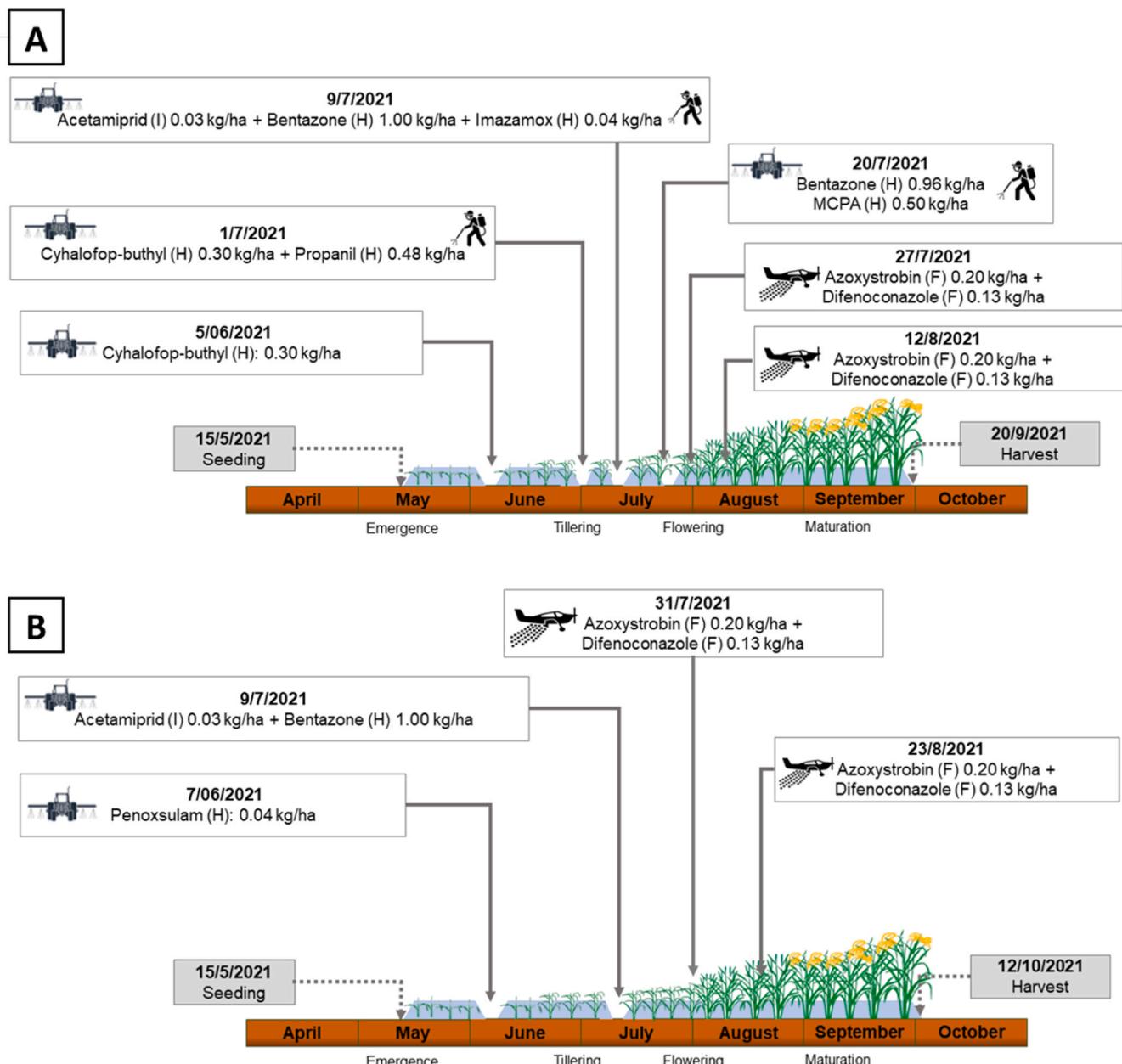


Fig. 2. Rice cultivation cycle, water levels in the rice paddies, and pesticide applications in the conventional Rice Field 1 (A) and Rice Field 2 (B), including date, dose and mode of application (H: herbicide; I: insecticide; F: fungicide). Pesticide application data was provided by farmers.

Macroinvertebrates were taken in triplicate in the same sampling sites and dates as the zooplankton samples. They were collected with a kick net (500 µm mesh size) using lineal transects in each plot section and covering a total area of 5 m per sample. The collected macroinvertebrates were fixed with ethanol (96 %) until further processing. In the laboratory, samples were sieved and cleaned prior to identification and counting. Specimen identifications were carried out with an Olympus SZ40 stereomicroscope following Tachet et al. (2010).

Emerging insects traps were placed in triplicate in each sampling site for two weeks and collected on the dates shown in Table S3. Insect traps were constructed by sewing a 250 µm mesh size net in a pyramidal structure (0.152 m) made with metal rods. Emerging insects were collected in a container filled with a solution that served both as bait and preservative of samples (ethanol 70 %, saccharose 40 g/L, and glycerin 40 mL/L). Identification and counting were done to the lowest taxonomic resolution possible according to Tachet et al. (2010).

After identification and counting, several diversity indices were

calculated in each sample: richness, total abundance and Shannon index. Moreover, the biomass of aquatic macroinvertebrates and emerging insects was assessed by drying the samples at 105 °C for 48 h. For macroinvertebrates, all Gastropoda shells were excluded from the biomass assessment.

2.4. Greenhouse gas emissions

Sediment cores for assessing the exchange of CO₂ and CH₄ gases with the atmosphere were taken between May 17th and October 13th, 2021 (4 times), from the four sampling sites in triplicate (randomly distributed, Table S3). The cores were made of transparent methacrylate tubes with 50 cm height and 4 cm diameter. Intact sediment (5–10 cm) and water (15–25 cm) were collected with the tubes, leaving a headspace above the water. The tubes were subsequently incubated for 2–5 days at field temperature within a thermostatic climatic chamber, with regulated light/dark cycles.

Incubation of sediment cores over several days have been successfully employed in comparable studies to estimate GHG emissions from wetland sediments (e.g., Brooker et al., 2014; Camacho et al., 2017; Morant et al., 2024; Miralles-Lorenzo et al., 2024; Breavington et al., 2025). This approach allows the calculation of potential GHG emissions by using standardized incubation conditions across sites and extending the duration of the measurements. Compared to the *in-situ* chamber method, longer incubations using this method facilitate the integration of both diffusive and ebullitive CH₄ emissions, the latter being especially sporadic and potentially underrepresented in short-term *in situ* measurements.

CO₂ and CH₄ concentrations in the headspace of cores were measured at the beginning and end of the incubation period, and the difference represented the net gas potentially emitted. To measure the CO₂ accumulated in the headspace, a small air volume was extracted from the tubes using a syringe equipped with a valve. The air was then injected into a circulation system connected to the LICOR LI-850 gas analyzer. After this, the accumulated CH₄ in the headspace was measured using an Aeroqual Gas A200 sensor equipped with a gas-sensitive semiconductor (GSS), appropriately calibrated by gas chromatography (for further details, see Camacho et al., 2017). The extended incubation period allowed sufficient gas accumulation in the headspace to ensure robust comparative estimates across sites, despite the lower sensitivity of the GSS sensor compared to higher-resolution techniques such as gas chromatography. However, to prevent any loss in precision, the methods used here had been previously calibrated by gas chromatography, as specified in Camacho et al. (2017). The gas concentration was retrieved as parts per million (ppm) from the gas analyzers. These concentrations were then converted to mass units using the ideal gas law. Measurements of pressure and temperature were recorded during sampling to adjust the calculations according to ambient conditions.

2.5. Statistical data analyses

Our study design included four sampling sites, each sampled in triplicate with sampling points randomly selected. Sampling was conducted repeatedly throughout the study period. Consequently, measurements within each sampling site were treated as spatially independent but temporally dependent. To account for this, time was included as an independent factor in our statistical analysis.

The influence of sampling site and time on the microbial community structure (at the phylum and family level), the zooplankton community, and the aquatic and emergent macroinvertebrate datasets were evaluated by a Permutation Multivariate Analysis of Variance (PERMANOVA) test based on Euclidean distances with 999 Monte Carlo permutations (Anderson, 2001). Prior to the PERMANOVA tests, an appropriate transformation method, *i.e.*, square root or log ($x + 1$), was used. Sample grouping and taxa correlations were visualized by a Principal Coordinates Analysis (PCoA). The PERMANOVA and the PCoA tests were done with the SIMPER software version 7 (Clarke and Gorley, 2015). To test differences in biological indices, a repeated measures ANOVA (or its non-parametric alternative) was performed with sampling site and time as independent variables, followed by a Tukey post-hoc test. These tests were conducted with the Jamovi software (The Jamovi Project, 2023).

To study the impact of agricultural management and pesticide applications on the temporal dynamics of the microbial and invertebrate communities, a Principal Response Curve (PRC) analysis was performed. The PRC is a statistical method used in ecological studies to analyze and visualize changes in community composition over time or across treatments. The PRC allows visualizing the variation between the treatments and the control at the different sampling times (C_{dt}), so that the larger the C_{dt} the larger the structural variation of the evaluated communities at a given sampling time, while also quantifies the influence of time on the whole analysis. It also allows the calculation of the affinity of each taxon for the PRC (b_k), so that the taxa with the highest b_k values show a population decline, and the taxa with negative b_k values displays a

population increase in the treatments (Van den Brink and Braak, 1999). In our study, we set the Organic Rice field as reference control and Rice Field 1 and 2 as treatments, so that we could quantify changes in community composition across conventional rice fields and the organic field over the study period. The PRC analysis was done using the CANOCO Software, version 5 (Ter Braak and Smilauer, 2012).

The influence of land use and agricultural management on the emission of greenhouse gases during the rice cultivation period was analyzed with a repeated measures ANOVA, as described above. In all cases, a significance value of 0.05 was used to determine statistically significant differences related to the tested variables.

3. Results

3.1. Microbial communities

In total, 13,192 ZOTUs of prokaryotes (bacteria and archaea) were identified in the water and the sediment samples. For water samples, significant differences were found between sampling days for ZOTUs richness (Friedman, $\chi^2 = 8.78$, $p = 0.032$, $df = 3$) and the Shannon's index (ANOVA, $F_{3,21} = 4.99$, $p = 0.009$). Site differences were only statistically significant for richness (Kruskal-Wallis, $\chi^2 = 14.3$, $p = 0.003$, $df = 3$) between Rice Field 1 and Rice Field 2, with the latter displaying the highest number of identified ZOTUs (Fig. 3). The PERMANOVA analyses showed statistically significant differences by sampling day as well as by sampling site for prokaryotes in water at both the phylum and family levels (Table S5). The PCoA performed with the water microbial community showed a gradient (x-axis) with a higher relative abundance of bacteria of the phyla Proteobacteria, Acidobacteria, Chloroflexi, Myxococcota and Deinococcota in the water spring, contrasting with the dominance of Bacteroidota Patescibacteria, Actinobacteriota and Cyanobacteria in the Rice Field 2. The Organic Rice field and the Rice Field 1 showed an intermediate abundance of the above-mentioned phyla, with dominance of Bacteroidota and Campylobacterota in some samples (Figure S1). At the family level, the separation of the sampling sites was even clearer, with Moraxellaceae, Arcobacteraceae and Comamonadaceae, dominating in the Organic Rice field, and Microbacteriaceae, Burkholderiaceae and Alcaligenaceae in the Rice Field 2 (Fig. 4). As shown by the PRC analysis, the temporal differences of the water microbial community in the rice fields were significant and maximal at the beginning of the crop cycle, with Actinobacteriota and Cyanobacteria dominating in Rice Field 2 and Campylobacterota in Rice Field 1, while it became very similar at the end of July, after the drying period (Fig. 5).

For sediment samples significant differences were observed between sites (ANOVA, $F_{3,4} = 22.6$, $p = 0.006$), with the Water Spring showing higher diversity values than the conventional rice fields, and the Organic Rice field also showing significantly higher diversity than Rice Field 2 (Fig. 3). The PERMANOVA analysis showed statistically significant differences by sampling day, and by sampling site for the sediment prokaryotes at both the phylum and family levels (Table S5). The PCoA performed with the microbial community in the sediment showed a gradient (x-axis) with a higher relative abundance of bacteria of the phyla Nitrospirota, Calditrichota, Latescibacterota, Crenarcheota, and Methylomirabilota in the water spring, and a clear separation between the different rice fields (y-axis). A higher relative abundance of Actinobacteriota, Acidobacteriota and Firmicutes was found in the Organic Rice field, while Chloroflexi and Bacteroidota showed a higher affinity for Rice Field 2. Instead, Rice Field 1 showed an intermediate relative abundance of these phyla (Figure S1). At the family level, the separation between the water spring and the rice fields was evident, with families like Acidiferrobacteraceae, Rhodocyclaceae, Methylomirabilaceae and Microscillaceae dominating in the water spring (Fig. 4). As shown by the PRC, the differences between the sediment microbial communities of the organic and the conventional rice fields were maintained during the study period, with no significant influence of the different agricultural

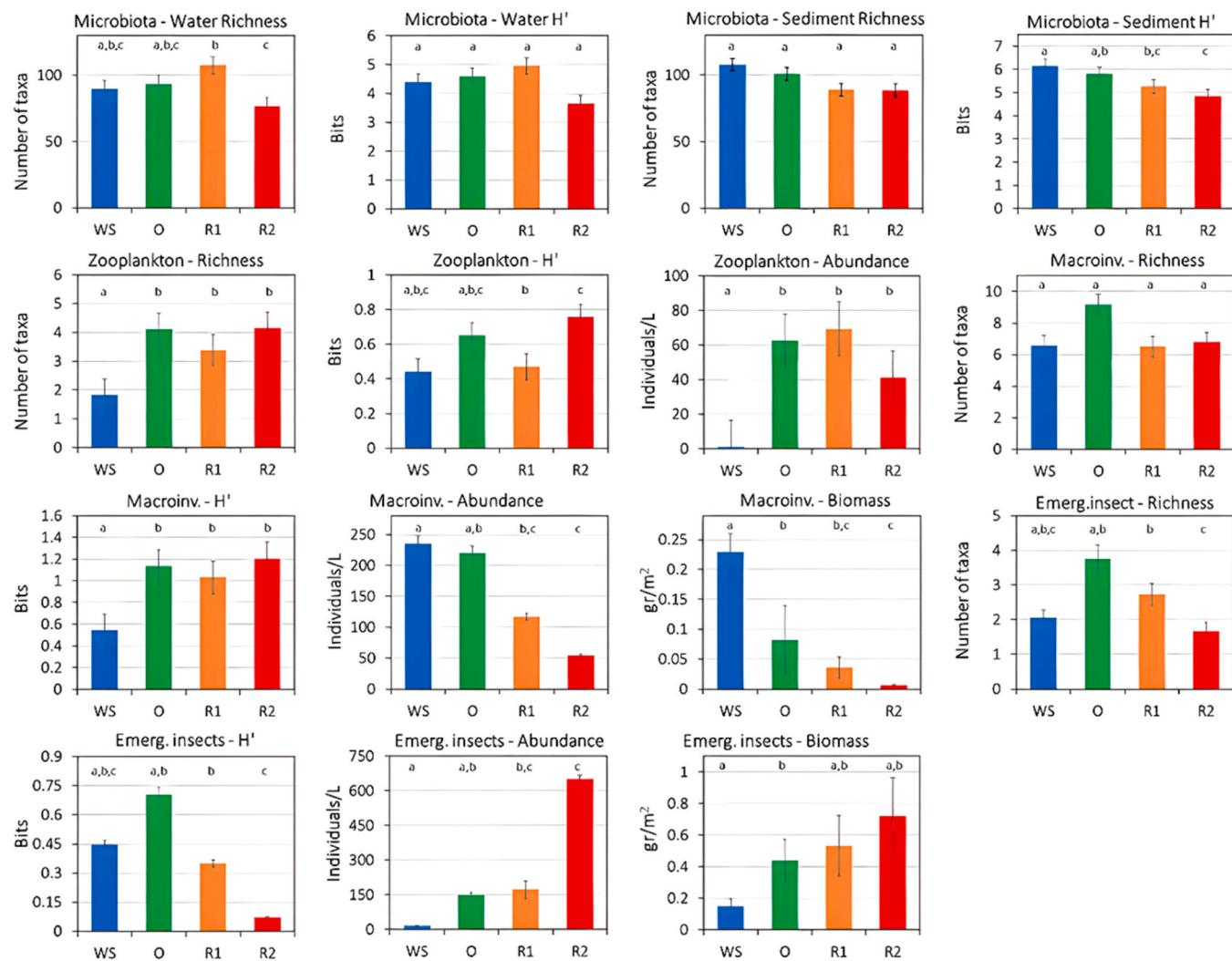


Fig. 3. Results of the calculated biological indices (richness, abundance, diversity, biomass) for the different taxonomic groups (mean \pm SE). Different letters indicate significant differences between the field plots (statistical groups) based on the Tukey post-hoc test. H': Shannon's index; Macroinv.: macroinvertebrates; Emerg: emerging insects. WS: Water Spring; O: Organic Rice field; R1: Rice Field 1; R2: Rice Field 2.

management practices on the structure of these communities (Fig. 5).

3.2. Invertebrate communities

Twelve zooplanktonic crustaceans were identified, belonging to the Ostracod, Cyclopoid and Cladocera groups. Significant differences were found between sampling days for richness (Friedman, $\chi^2 = 18.1$, $p = 0.003$, $df = 5$) and abundance (Friedman, $\chi^2 = 22.1$, $p < 0.001$, $df = 5$), but not for the Shannon's index. The Water Spring exhibited significantly lower richness (Kruskal-Wallis, $\chi^2 = 19.18$, $p < 0.001$, $df = 3$) and abundance (Kruskal-Wallis, $\chi^2 = 39.68$, $p < 0.001$, $df = 3$) as compared to the rice fields. The Shannon's index (Kruskal-Wallis, $\chi^2 = 9.48$, $p = 0.024$, $df = 3$), showed relatively homogenous results among rice fields, except for Rice Field 1, which showed significantly lower values than Rice Field 2 (Fig. 3). The PERMANOVA analyses indicated significant effects of sampling time and sampling site on the zooplankton community (Table S5). The PCoA confirms the lower relative abundance of zooplankton in the Water Spring and shows a dominance of Cyclopoida, Ostracoda and several Cladocera genera (*Simocephalus*, *Alona*, *Alonella*, *Ceriodaphnia*) in the Organic Rice field, and a prevalence of the Cladocera (genera *Moina*, *Macrothrix* and *Scapholeberis*) in the conventional rice fields (Fig. 6). The PRC analysis shows significant temporal differences between the zooplankton communities of the conventional

rice fields and the Organic Rice field, which is determined by the higher abundance of *Moina* sp. and *Alona* sp. in the conventional rice fields at the start of the culture cycle, and a decrease of these taxa towards the end of the culture cycle (Fig. 5).

Forty-three macroinvertebrate taxa were identified in the collected samples, belonging mainly to Insecta ($n = 32$), followed by Crustacea ($n = 4$), Gasteropoda ($n = 3$) and Annelida ($n = 2$). Significant differences per sampling day were identified for richness (ANOVA, $F_{5,40} = 4.26$, $p = 0.003$), abundance (Friedman, $\chi^2 = 15.7$, $p = 0.008$, $df = 5$) and the Shannon's diversity index (ANOVA, $F_{5,40} = 3.22$, $p = 0.016$), but not for biomass (Fig. 3). Notably, these differences were predominantly observed due to the higher values of these parameters on the second sampling day (Table S4). Overall, there were not significant differences for richness; however, both the Water Spring and the Organic Rice field exhibited higher macroinvertebrate abundance compared to the conventional rice plots (Kruskal-Wallis, $\chi^2 = 30.2$, $p < 0.001$, $df = 3$). The Water Spring also showed significantly lower Shannon's index values than the rice fields (ANOVA, $F_{3,8} = 6.74$, $p = 0.014$), but a significantly higher biomass (Kruskal-Wallis, $\chi^2 = 37$, $p < 0.001$, $df = 3$) due to the high abundance of large macrocrustaceans. In addition, the macroinvertebrate biomass of the Organic Rice field was significantly higher as compared to the Rice Field 2 (Fig. 3). The PERMANOVA analysis indicated significant effects of sampling time and

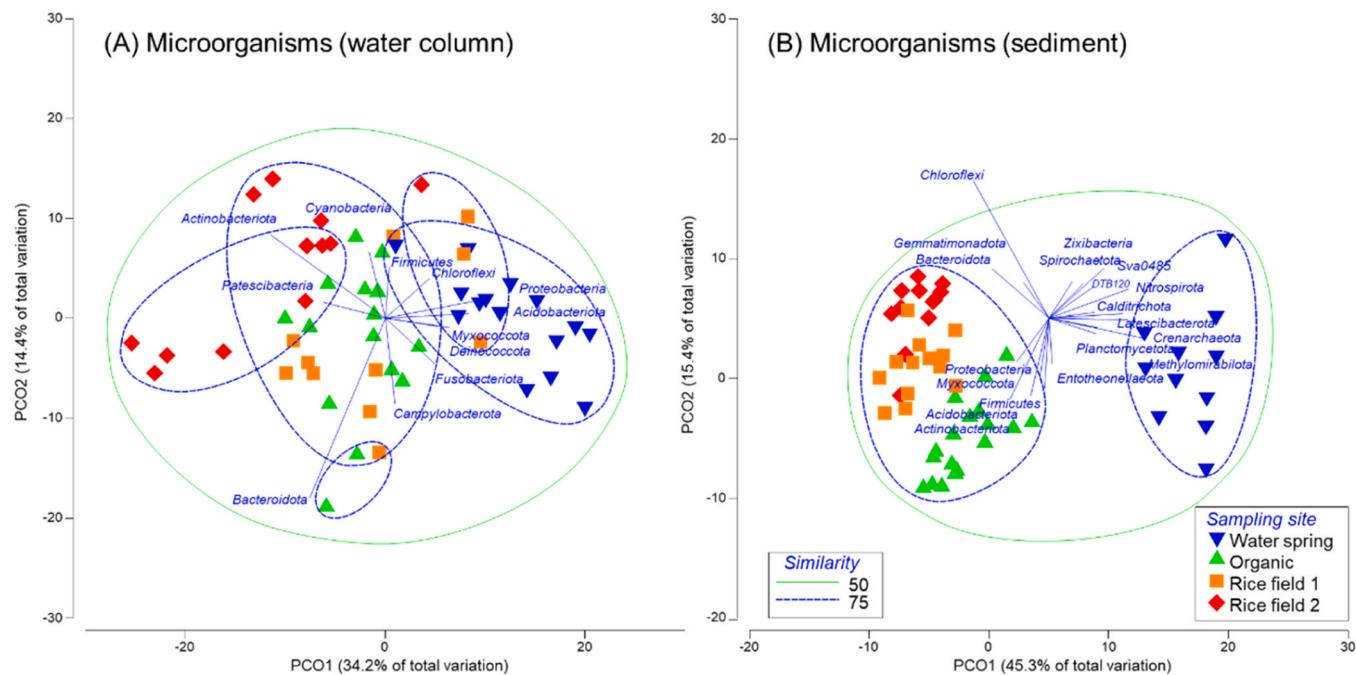


Fig. 4. Principal Coordinates Analysis (PCoA) performed with the microbial community in the water (A) and in the sediment (B) at the family level. Only the taxa that showed a percentage of correlation higher than 15 % are displayed.

sampling site on the structure of the macroinvertebrate community (Table S5). The PCoA indicated a dominance of the macrocrustaceans *Echinogammarus* sp. and the endemic shrimp species *Dugastella valentina* and *Palaeomonetes zariquieyi* in the Water Spring, and a dominance of aquatic insects in the rice fields. Among the rice fields, the PCoA indicates a notable difference (y-axis), with a higher dominance of the Hemipteran Notonectidae, adults of the Coleoptera Dytiscidae, and the Diptera genus *Chironomus* in the conventional rice fields, and a higher abundance of the Gastropoda *Galba truncatula* and the Hydrophilidae Coleoptera in the Organic Rice field (Fig. 6). The PRC analysis shows statistically significant differences between the structure of the macroinvertebrate communities in the rice fields, with differences being larger during the beginning of the culture cycle due to the higher abundance of Notonectidae and the lower abundance of small snails (*Galba truncatula*, *Gyraulus* sp.) and Coleoptera in the Rice Fields 1 and 2. However, such differences were reduced towards the end of the culture cycle (Fig. 5).

Sixteen taxa of adult insects were identified in the emergence traps, including Diptera, Trichoptera, Odonata, Ephemeroptera and Coleoptera. Significant differences in abundance (Friedman, $\chi^2 = 34.7$, $p < 0.001$, $df = 4$), Shannon's index (Friedman, $\chi^2 = 19.5$, $p < 0.001$, $df = 4$), and biomass (Friedman, $\chi^2 = 14.9$, $p = 0.001$, $df = 4$) were related to the sampling time, with higher values for abundance and biomass, and lower for diversity on D0 and D1, as compared to the other sampling days (Table S4). The Organic Rice field showed significantly higher richness (Kruskal-Wallis, $\chi^2 = 14.7$, $p = 0.021$, $df = 3$) and Shannon's diversity values (Kruskal-Wallis, $\chi^2 = 16.4$, $p < 0.001$, $df = 3$) as compared to Rice Fields 1 and 2. Particularly, Rice Field 2 showed the lowest richness and Shannon's diversity values (Fig. 3). This rice field also had a significantly higher abundance compared to the Organic Rice field (Kruskal-Wallis, $\chi^2 = 23.8$, $p < 0.001$, $df = 3$). Significant differences were also observed between the Water Spring and the conventional rice fields, with the water spring showing the lowest abundance values. A similar trend was observed for the biomass of emerging insects, although statistically significant differences were only found between the Organic Rice field and the Water Spring (Kruskal-Wallis, $\chi^2 = 11.3$, $p = 0.01$, $df = 3$; Fig. 3). The PERMANOVA analysis indicated significant effects of sampling time and sampling site on the emerging insect dataset (Table S5). The PCoA shows a higher abundance of insects in the

Chironomidae family in the conventional rice fields, and a dominance of the Diptera Ceratopogonidae in the Organic Rice field (Fig. 6). The PRC analysis indicates clear differences between the Rice Field 1 and 2, and the Organic Rice field, which are larger towards the intermediate and final part of the culture cycle. Particularly, the PRC shows a dominance of Chironomidae in the conventional rice fields, and a dominance of Ceratopogonidae and other Diptera like Psychodidae, Tipulidae, *Culex* sp., and Ephydriidae, as well as the Coleoptera Curculionidae, in the Organic Rice field (Fig. 5).

3.3. Greenhouse gas emissions

The analysis of potential CO₂ emissions based on core sample incubations showed significant differences related to sampling day (Friedman, $\chi^2 = 37.1$, $p < 0.001$, $df = 3$) and site (Kruskal-Wallis, $\chi^2 = 14.7$, $p = 0.002$, $df = 3$). The lowest mean CO₂ emissions were observed for the Rice Field 2 (276 ± 392 mg CO₂-C/m²·day; mean \pm standard deviation), shortly followed by the Water Spring (343 ± 280 mg CO₂-C/m²·day), the Rice Field 1 (699 ± 942 mg CO₂-C/m²·day) and the Organic Rice field (891 ± 699 mg CO₂-C/m²·day; Fig. 7). Temporal differences in CO₂ emissions spanned over an order of magnitude. In the Water Spring, the lowest CO₂ emissions were recorded in August, 11 mg CO₂-C/m²·day, and the highest in June, 635 mg CO₂-C/m²·day. In the rice fields, the lowest were recorded in July, 55–101 mg CO₂-C/m²·day, after the desiccation period, and peaked in October 956–2325 mg CO₂-C/m²·day, after the harvest and rice straw decomposition (Figure S2).

CH₄ potential emissions showed significant differences by sampling day (Friedman, $\chi^2 = 41.7$, $p < 0.001$, $df = 3$) and site (Kruskal-Wallis, $\chi^2 = 30.5$, $p < 0.001$, $df = 3$). The Water spring was the site with the lowest mean CH₄ emissions (1.2 ± 2.0 mg CH₄-C/m²·day), followed by the Rice Field 2 (3.6 ± 4.0 mg CH₄-C/m²·day), the Rice Field 1 (12 ± 15 mg CH₄-C/m²·day) and the Organic Rice field (11 ± 18 mg CH₄-C/m²·day; Fig. 7). In the Water Spring, the lowest CH₄ emissions were recorded in June, 0.01 mg CH₄-C/m²·day, and the highest in October, 4.1 mg CH₄-C/m²·day; while in the rice fields the lowest were recorded in June and July, 0.08–0.29 mg CH₄-C/m²·day, and the highest in August, 11–42 mg CH₄-C/m²·day (Figure S2).

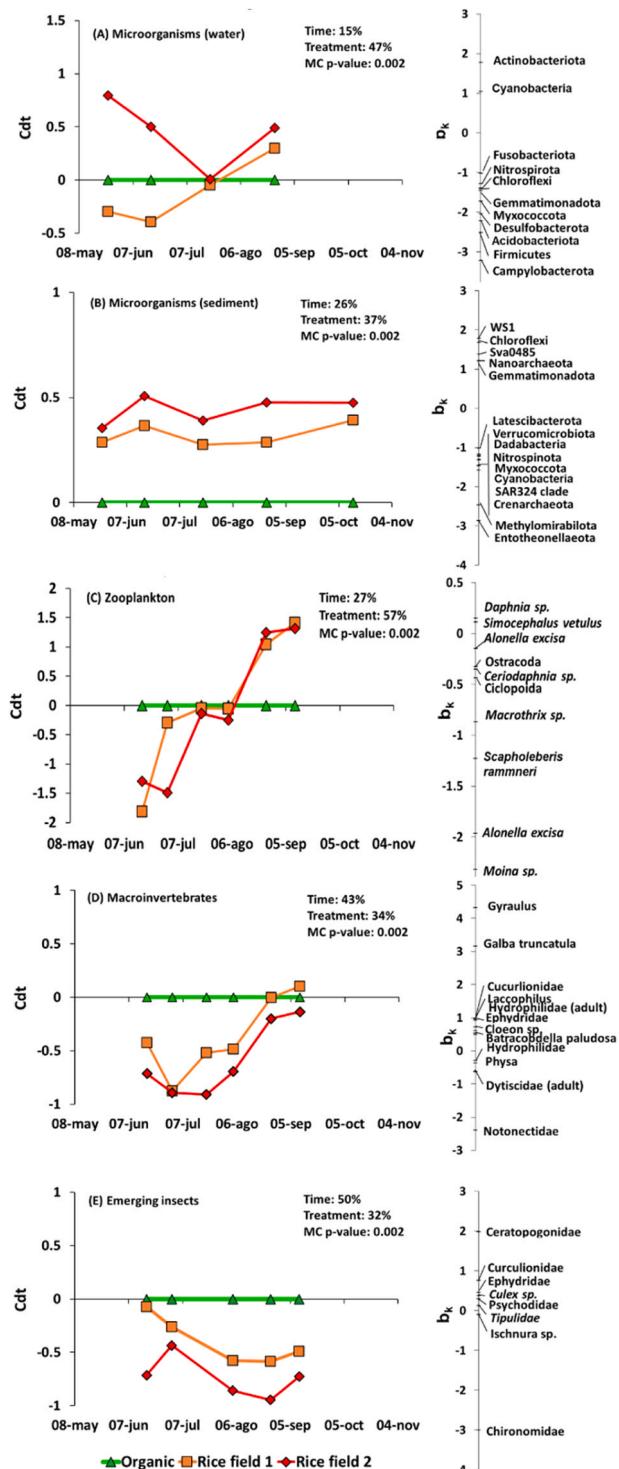


Fig. 5. Principal Response Curves (PRCs) for prokaryotes at the phylum level in water (A), and sediment (B), zooplankton (C), macroinvertebrates (D) and emerging insects (E). Cdt: sample weights (i.e., differences between treatments). b_k : species weight (i.e. affinity of taxon with the Principal Response Curve). Each figure displays the percentage of variance explained by sampling day (time), by the different rice fields (treatment) and the calculated Monte Carlo p-value. Only the taxa showing the greatest affinity to the PRC were displayed (i.e., for prokaryotes these were taxa with b_k values outside the 1 to -1 range; for zooplankton and emerging insects taxa with b_k values outside the 0.1 to -0.1 range; and for macroinvertebrates taxa with b_k values outside the 0.3 to -0.3 range).

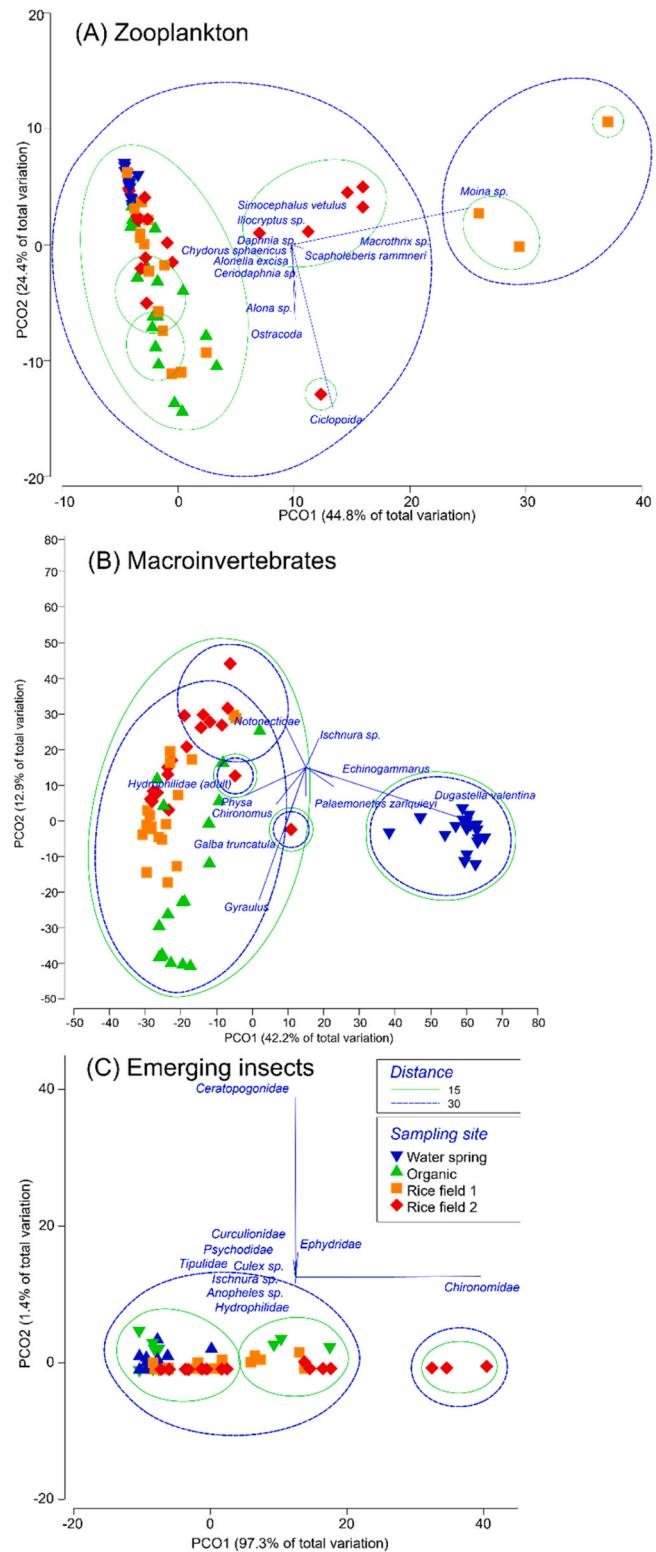


Fig. 6. Principal Coordinates Analysis (PCoA) performed with the zooplankton (A), the macroinvertebrate (B) and the emerging insects (C) datasets. For macroinvertebrates, only the taxa that showed a percentage of correlation higher than 15 % were displayed.

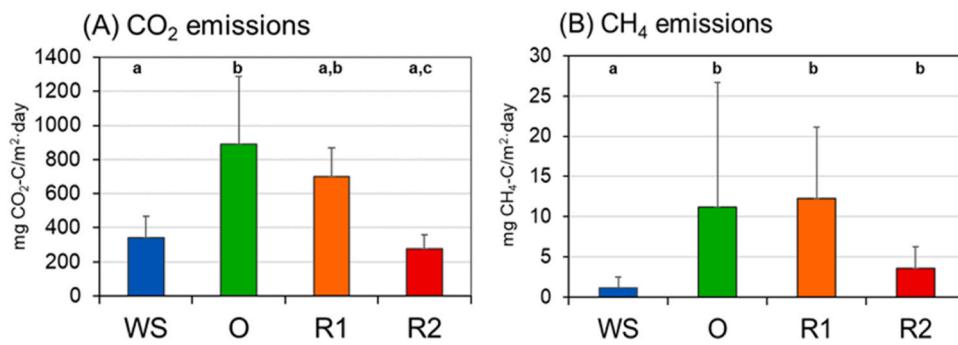


Fig. 7. Mean CO₂ (A) and CH₄ (B) emission rates based on core sample incubations in the different sampling sites during the study period. The error bars show the standard deviation. Different letters indicate significant differences (by groups) based on the repeated measures ANOVA. WS: Water Spring; O: Organic Rice field; R1: Rice Field 1; R2: Rice Field 2.

4. Discussion

4.1. Influence of land use on aquatic biodiversity

This study demonstrates that land-use changes for rice cultivation significantly influence the structure of aquatic communities in the ANP, which may be considered as a good representative of Mediterranean coastal wetlands. In the Water Spring, microbial communities in the water column exhibited the highest richness, including bacteria and archaea involved in essential ecosystem functions. Among the identified methanogens were members of the Methanobacteriaceae family (genera *Methanobacterium* and *Methanobrevibacter*) and the Methanosarcinaceae family (genus *Methanosarcina*), which are typical in such environments (Lee et al., 2014). Additionally, the study recorded a relatively high abundance of *Methylomirabilota*, a key phylum responsible for both aerobic and anaerobic methane oxidation in freshwater sediments (Su et al., 2022). This finding highlights the dual roles of methane production and methane oxidation in regulating methane emissions to the atmosphere. The balanced microbial community in the Water Spring, which includes methane-oxidizing bacteria such as *Methylomirabilota*, likely plays a crucial role in mitigating net methane emissions. The relatively oxidized sediments of the Water Spring, supported by continuous water flow and lower organic matter content, further contribute to the significantly lower methane emissions observed compared to the rice fields (Camacho et al., 2017; Morant et al., 2024). These factors underscore the importance of preserving natural wetland conditions for maintaining ecosystem balance and minimizing greenhouse gas emissions.

The microbial community structure in the water of the different rice fields showed important differences. The families Comamonadaceae, Sphingomonadaceae, Rhizobiaceae, Sporichthyaceae, Burkholderiaceae and Microbacteriaceae were predominantly found in conventional rice fields. These are common in water, abundant in soil, and do not include major pathogenic species (Gillespie et al., 2011). However, their abundance could indicate an adaptation to disturbed environments as these families are often associated with nutrient-rich and polluted habitats (Schober et al., 2025). Several families associated with fecal contamination, including Enterobacteriaceae, Arcobacteriaceae, Comamonadaceae, and Moraxellaceae, were dominant in the Organic Rice field. Their abundance is likely linked to the use of horse manure as fertilizer. Some of these families, such as Comamonadaceae, were also present in Rice Field 1, which may be related to the use of reclaimed water for irrigation. The dominant genus within Comamonadaceae in the Organic Rice field was *Comamonas*, a bacterium common in the human gut and associated with infections, indicating a potential risk of pathogen introduction through manure-based fertilization, with food safety implications (Frizzo et al., 2012). The presence of Arcobacteriaceae, often found in sewage, and resistant to standard water treatments (Venâncio et al., 2019; Ghaju Shrestha et al., 2022), is also linked to its mostly fecal

origin. On the other hand, the bacterial soil composition notably had a high occurrence of Acidobacteria, which are involved in many key biogeochemical processes (Kalam et al., 2020), indicating ongoing biogeochemical cycling and a potential influence on nutrients availability.

Overall, rice fields exhibited higher species richness, diversity, and abundance of zooplanktonic crustaceans than the Water Spring. This finding aligns with the studies by Gilbert et al. (2017), which indicate that agricultural land use factors, such as soil tillage, water runoff, and reduced habitat heterogeneity, can favor particular zooplankton taxa. The absence of large fish in the rice fields, combined with higher levels of nutrients and algae, likely fosters well-established zooplankton communities dominated by larger filter feeders during the spring and summer periods (Sahuquillo and Miracle, 2013). Additionally, the continuous water flow in the water spring compared to the almost fully stagnant conditions of rice fields explains the lower zooplankton richness and abundance compared to the rice fields (Kuczyńska-Kippen and Pronin, 2018).

The Water Spring exhibited the highest macroinvertebrate abundance and biomass, the latter primarily due to the dominance of two large macrocrustacean species (*Dugastella valentina* and *Palaemonetes zariquieyi*), both endemic to the ANP and largely restricted to these habitats (Rueda et al., 2013). This highlights the importance of conserving natural water bodies as critical habitats for endemic species and as food sources for bird populations. The predominance of macrocrustaceans and limited insect presence in the Water Spring may also explain its lower overall biodiversity.

The Organic Rice field showed a higher abundance of macroinvertebrates than Rice Fields 1 and 2, with an increased prevalence of predator groups (e.g., Hydrophilidae, Dytiscidae). The high diversity of plants in the Organic Rice field, including various competing weeds (e.g., Cyperaceae, Poaceae, and Pontederiaceae), contributed to greater habitat complexity, offering refuge, food, and nesting sites for diverse insect groups (Marshall et al., 2003). Similar findings were reported by Pérez-Méndez et al. (2023) in organic rice fields of the Ebro Delta, which also had higher predator diversity than conventional rice fields. Consistently, the Organic Rice field in our study showed higher diversity and richness of emerging insects. Huynh et al. (2021) found that pesticide-free rice paddies support greater Odonata species richness, while conventional fields often have a higher overall insect abundance. Baba et al. (2019) also observed increased insect abundance in rice fields with reduced insecticide and fungicide use. Higher insect emergence in the Organic Rice field provide food to riparian arthropod predators, amphibians, and water birds, contributing to energy transfer between aquatic and terrestrial environments.

4.2. Influence of agricultural management practices on aquatic biodiversity

Our study reveals temporal shifts in the structure of the water bacterial community early in the cultivation cycle. These shifts were associated with the application of organic fertilizers in the Organic Rice field and the periodic desiccation in Rice Fields 1 and 2 for fertilization and pesticide treatments. In contrast, sediment bacterial communities showed greater stability over time, with only minor changes in the microbial community structure and minimal influence from management practices. In our study, it was not possible to observe immediate effects of the fungicide applications on bacterial and archaeal communities in water samples. Similarly, sediment prokaryotes sampled after fungicide application showed no significant changes in community composition, which is consistent with previous studies assessing the effects of similar fungicides (tebuconazole) on sediment bacteria (Dimitrov et al., 2014).

In conventional rice fields, the zooplankton community structure exhibited a shift in dominant species, from *Daphnia* sp. and *Simocephalus* sp. at the start of the cultivation cycle, to *Moina* sp. and *Alona* sp. later in the season. These shifts may be explained by changes in water temperature and pesticide applications. Previous studies indicate that *Moina* sp. show a larger population abundance at higher water temperatures and generally shows a higher tolerance to pesticides and other agricultural stressors than large Cladocera (Gilbert et al., 2017; Vilas-Boas et al., 2021). Further, fungicides like azoxystrobin, which are commonly present in rice paddies during the second half of the cultivation cycle, have been found to reduce populations of *Daphnia* sp. and *Simocephalus* sp., contributing to the observed shifts in community structure (Amador et al., 2023).

Regarding macroinvertebrates, the most notable temporal differences between the organic and conventional rice fields were observed early in the cultivation cycle. These differences may be attributed to continuous desiccation for herbicide applications and the use of acetamiprid, an insecticide highly toxic to aquatic insects (Raby et al., 2018; Rico et al., 2018). In contrast, Dalzochio et al. (2016) reported high similarities between organic and conventional rice fields throughout most cultivation stages, with notable differences occurring only during the "off-season" when fields lie fallow. In the Organic Rice field, the gastropod *Galba truncatula* was found to have a higher dominance as compared to the conventional rice fields in the first part of the culture cycle. A review by Gustafson et al. (2015) highlighted several cases where herbicides indirectly affect herbivorous snail populations by reducing aquatic plant and biofilm biomass. Additionally, intermittent desiccation periods may adversely impact snail populations due to water absence, oxygen fluctuations, and the drying of periphytic algae, their primary food source (Collas et al., 2014).

Toward the end of the cultivation cycle, Ceratopogonidae and Curculionidae were the dominant insects in the Organic Rice field, while Chironomidae was most prevalent in conventional fields, particularly during periods of stable water levels. The Ceratopogonidae family has desiccation-tolerant eggs, providing an advantage in habitats with temporary water regimes (Talavera et al., 2011). Many species within this family are important disease vectors for livestock (Cuéllar et al., 2018) and are also considered significant pests in rice cultivation (Dembilio and Jacas, 2012). Conversely, the dominance of *Chironomus* spp. in conventional rice fields can be attributed to its high tolerance to water pollution (Alba-Tercedor and Sánchez-Ortega, 1988) and its rapid recolonization capabilities (Stenert et al., 2018). The high abundance of this taxon in Rice Field 2 likely supports robust populations of insect predators such as Notonectidae and Dytiscidae, which rely on Chironomidae larvae as a primary food source (Culler et al., 2014; Fischer et al., 2013).

4.3. Influence of land use and agricultural management on greenhouse gas emissions

The Water Spring exhibited significantly lower CO₂ and CH₄ emissions compared to the rice fields. The reduced potential CH₄ emissions are related to their continued aerobic conditions and the higher activity of methane-oxidizing bacteria (Chen et al., 2013), as it has been described above. Among the rice fields, the Organic Rice field, fertilized with manure, and Rice Field 1, irrigated with reclaimed water, exhibited the highest potential emissions. In contrast, Rice Field 2, irrigated with water from an irrigation canal connected to the Albufera Lake, produced lower carbon emissions. This is consistent with previous investigations indicating that manure-fertilized soils or soils irrigated with reclaimed waters tend to have higher CO₂ emissions due to enhanced microbial decomposition (Ellert and Janzen, 2008). Nonetheless, the GHG emissions observed in this study are relatively low compared to those reported in other regions. For example, Cao et al. (1996) noted that the highest methane emissions occur in tropical and subtropical areas, particularly in Asia, where rice cultivation practices and climatic conditions are more conducive to methane production. This makes the Mediterranean region a comparatively modest contributor to carbon emissions per unit of rice production area. However, it should be noted that the gas exchange rates quantified here mainly relate to microbial processes based on core sample incubations and do not account for other components, such as plant-mediated transport or emissions from the water column not directly connected to benthic processes.

The most pronounced differences between the Water Spring and the rice fields were observed in August, the hottest month. Elevated CH₄ emissions during this period are associated with higher water temperatures, which promote sediment anoxia and stimulate the activity of methanogenic archaea such as *Methanobacterium*, *Methanobrevibacter*, and *Methanosarcina* (Camacho et al., 2017; Miralles Lorenzo, 2023). Changes in GHG emissions were also influenced by agricultural management practices. For instance, CH₄ emissions in the rice fields increased during irrigation, likely due to the induced anaerobic soil conditions—a process widely documented in the literature (Cao et al., 1996; Kimura et al., 2004). In this moment, methanogenic archaea (e.g., acetoclastic and hydrogenotrophic methanogens) tend to decompose organic substrates like root exudates, straw and manure, enhancing CH₄ production. A way to counteract this would be the implementation of non-continuous flooding (e.g., alternate wetting and drying) which reduces methanogen activity by increasing soil oxygenation. On the other hand, this promotes the activity of nitrifying and denitrifying bacteria, leading to trade-offs between CH₄ and N₂O emissions (Qian et al., 2023).

Additionally, certain post-harvest practices could be adopted to mitigate methane emissions, such as delaying winter flooding or removing straw after harvest to reduce methanogenesis and microbial decomposition, which are key contributors to elevated CH₄ and CO₂ fluxes, respectively (Maraseni et al., 2018; Belenguer-Manzanedo et al., 2022b). Moreover, the minimal emissions observed during the Water Spring period underscore the substantial potential of restoring agricultural areas to their natural state and rehabilitating degraded wetlands. These actions could play a vital role in preserving these ecosystems as carbon sinks and enhancing their capacity to mitigate climate change (Morant et al., 2020b; Camacho-Santamans et al., 2024).

It is important to acknowledge that the study design used here had limited replication. This limitation stemmed from the challenge of identifying relatively unimpacted water springs within the ANP and the scarcity of organic rice fields in the region. In fact, our study focused on the only organic rice field actively operating in the ANP at the time the study was initiated. To build on these findings, follow-up studies should be conducted to compare the effects of different agricultural practices on biodiversity and GHG emissions at a regional scale, thereby contributing to both climate change mitigation and ecological conservation efforts.

5. Conclusions

Our study quantifies the impact of land use and rice farming practices on key ecosystem services in Mediterranean wetlands, including carbon sequestration and the conservation of local biodiversity, offering valuable insights to inform sustainable management strategies. Despite the limited number of replicates included here, the findings reveal that land use significantly shapes the structure of aquatic communities. Undisturbed areas dedicated to ecosystem preservation, such as water springs, exhibit high bacterial and archaeal diversity and harbor numerous endemic and endangered macroinvertebrate species, which serve as critical food sources for wetland fish and birds. Moreover, these areas demonstrate the greatest potential as carbon sinks, providing essential services for climate regulation. The study also highlights the influence of rice farming practices on aquatic invertebrate communities. For instance, the organic rice field examined supported a higher abundance of zooplankton taxa resistant to pollution, promoting greater biodiversity and a higher biomass of emerging insects. This increased biodiversity in the organic field can be attributed to a more diverse plant community, subtle differences in the hydrological cycle, and the absence of synthetic pesticides. However, this farming approach is associated with a higher prevalence of fecal prokaryotic species (including potentially pathogenic bacteria) and significant greenhouse gas emissions during the rice cultivation period due to the use of manure-based fertilizers. In conclusion, our study emphasizes the benefits and challenges of different farming practices concerning ecological conservation and greenhouse gas emissions, and calls for more extensive studies conducted in the Mediterranean region, including also higher animals such as amphibians and water birds, to reinforce sustainable environmental policies and agricultural management.

CRediT authorship contribution statement

Jesús Moratalla: Writing – review & editing, Formal analysis, Data curation. **Carlos Pascual-March:** Writing – review & editing, Formal analysis, Data curation. **Antonio Picazo:** Writing – review & editing, Methodology, Investigation. **Carlos Rochera:** Writing – review & editing, Methodology, Investigation. **Claudia Martínez-Megías:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Daniel Morant:** Writing – review & editing, Methodology, Investigation. **Andreu Rico:** Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Antonio Camacho:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at doi:10.1016/j.agee.2025.109835.

Data availability

Data will be made available on request.

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