

# Ecological trade-offs as a product of water saving irrigation strategies in rice fields

Sebastián Echeverría-Progulakis<sup>a,b</sup>, Maite Martínez-Eixarch<sup>a</sup>, Dani Boix Masafret<sup>c</sup>, Raul Llevat Pamies<sup>b</sup>, Lluís Jornet Torren<sup>a</sup>, Néstor Pérez-Méndez<sup>b</sup>

<sup>a</sup>*Marine and Continental Waters Program, IRTA, La Ràpita, 43540, Catalonia, Spain*

<sup>b</sup>*Sustainable Field Crops Program, IRTA, Amposta, 43870, Catalonia, Spain*

<sup>c</sup>*Aquatic Ecology Institute, University of Girona, Girona, 17003, Catalonia, Spain*

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## Abstract

Tackling climate change while enhancing biodiversity without compromising production is a main goal in agricultural management. In rice farming, water-saving irrigation techniques alternative to permanent flooding are necessary to face more severe and frequent droughts and have proven effective in reducing greenhouse gas (GHG) emissions, yet potential trade-offs with biodiversity conservation are often overlooked. Here we used a field-scale experiment to compare the effects of water management strategies representing a water use gradient on i) GHG emissions, ii) the abundance and diversity of aquatic macroinvertebrate and vertebrate (fish and amphibians) communities, and iii) crop productivity. Methane cumulative emissions decreased 92.5% under the lowest water use strategy (alternate wetting and drying, AWD) and 67.3% with medium intensity management (mid-season drainage, MSD), when compared to constant flooding (CON). Effects on aquatic biodiversity were the opposite, AWD resulted in strong negative impact in abundance of individuals (decreasing up to 51% for Decapoda and 65% for Odonata species). Species richness was 53% and 55% lower for MSD and AWD practices, respectively, in contrast to CON after re-flooding fields following drainage. Mean production decreased 12.9% with AWD management but did not vary under MSD. Through this holistic assessment approach, we were able to identify MSD as an alternative rice water-saving irrigation strategy that reconciles climate change mitigation, biodiversity conservation and crop production in rice agrosystems.

**Keywords:** Biodiversity conservation, greenhouse gas emissions, paddy rice, water management

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## 1. Introduction

Climate change mitigation and adaptation failure, as well as biodiversity loss and ecosystem collapse, are recognized among the most severe global risks over the next decade ([World Economic Forum, 2023](#)). Furthermore, extreme events attributed to climate change, such as the alternation of severe drought periods and heavy rains, are expected to continue negatively impacting food security due to their adverse effects on agricultural production and yield ([FAO, 2022c](#)). The latest Intergovernmental Panel on Climate Change (IPCC) report highlights the interdependence of climate, ecosystems and biodiversity, and human societies ([IPCC, 2023](#)). Nevertheless, it also warns that, even though mitigation policies have expanded, global warming is likely to exceed 1.5°C during the 21st century, further increasing current rates of biodiversity loss and causing adverse impacts on food security. Despite these interrelations made clear, strategies addressing global change are often disconnected and tend to follow independent courses of action, either focusing on climate change mitigation, biodiversity conservation or sustainable development policies, failing to identify potential outcome conflicts or synergistic measures ([Arneth et al., 2020](#); [Rusch et al., 2022](#)). Even though integrative approaches like degraded ecosystem restoration ([Strassburg et al., 2020](#); [Temperton et al., 2019](#)) and the protection of wetlands and non-forest carbon-rich environments ([Smith et al., 2022](#)) achieve to address multiple challenges simultaneously, some strategies within the recently developed frameworks of Nature-Based Solutions (NBS, [Seddon et al. \(2019\)](#)) and Climate-Smart Agriculture (CSA, [Tripathi et al. \(2022\)](#)) might result in conflicting outcomes if not well implemented. Among climate change mitigation measures that have been proven to hinder ecosystem biodiversity are large area afforestation ([Veldman et al., 2015](#)) and bioenergy crops ([Hof et al., 2018](#)), large-scale harvest of logging residues in managed forest landscapes ([Felton et al., 2016](#)) and the implementation of some agriculture intensification methods (through increased use of agrochemicals and synthetic fertilisers, [Cohen et al. \(2021\)](#)). Additionally, carelessly designed climate mitigation policies exclusively aiming at achieving the Paris Agreement goal of keeping global warming below 2°C, could have negative impacts on food security, foiling the achievement of the UN Sustainable Development Goal (SDG) 2 of "zero-hunger" by 2030 ([Fujimori et al., 2019](#)).

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31 Agrifood systems, and livelihoods depending on them, face the challenge of sustainably  
32 providing sufficient, accessible, affordable, safe and nutritious food under current and pro-  
33 jected climate change and biodiversity loss processes (FAO, 2022b). Rice (*Oryza sativa* L.) is  
34 a critical staple food for over 3 billion people and its production has recently reached all-time  
35 highs (FAO, 2022a, 2023). A large proportion of global natural wetland area has been mod-  
36 ified for rice cultivation, but also large surfaces of previous non-flooded farmlands have been  
37 converted into wetlands for this purpose (Hook, 1993). Rice wetlands are widely recognised  
38 for their role on greenhouse gases (GHG) emissions (Mitsch et al., 2013; Mitsch and Mander,  
39 2018) and as biodiversity hotspots (Biggs et al., 1994). Rice production is responsible for the  
40 largest agriculture related GHG emissions, particularly regarding methane (CH<sub>4</sub>) (Schaefer  
41 et al., 2016; Wang et al., 2023) due to the anaerobic decomposition of organic matter under  
42 flooded soil conditions (Burke and Lashof, 1990). Rice paddy fields are also habitat to many  
43 species of macrofauna and benthic macroinvertebrates, affected by irrigation managements  
44 modifying water permanence periods (Lupi et al., 2013). These organisms play a key role  
45 in the provision of different ecosystem services in aquatic systems, such as nutrient cycling,  
46 primary production, OM decomposition and material translocation (Wallace and Webster,  
47 1996).

48

49 Drought events affecting agricultural production are expected to duplicate their frequency  
50 at a global scale during the upcoming century in relation to the 1955-2014 period, driven  
51 by higher temperatures and precipitation deficits (Feng et al., 2023). Water-saving irriga-  
52 tion methods have been developed as alternatives to conventional constant flooding (CON),  
53 adapting rice production to lower water availability and aiming to reduce unproductive water  
54 outflows (Tuong and Bouman, 2003). These strategies are mainly based on reducing flooding  
55 periods by draining fields at different intensities and frequencies across the growing season  
56 (Kumar and Rajitha, 2019). Allowing aerobic conditions into soils disrupts methanogenic  
57 activity, due to the depletion of methane in soils by aerobic oxidation by methanotrophs  
58 (Sass et al., 1992). The positive effect that water-saving irrigation strategies in paddy-fields  
59 have on decreasing CH<sub>4</sub> emissions, has been largely identified and described (Yan et al., 2005;

60 [Fertitta-Roberts et al., 2019](#)). On the contrary, the effects of such strategies on the biodi-  
61 versity of these agro-ecosystems is less known, as policy makers and social awareness tend  
62 to focus mainly in issues concerning climate change mitigation ([Rusch et al., 2022](#)). Most  
63 species inhabiting wetland ecosystems are adapted to temporary drying-out, but some are  
64 unable to survive droughts or larger drainage periods ([Biggs et al., 1994](#)). Previous studies  
65 have already identified contrasting outcomes of rice agriculture policies on GHG mitigation  
66 and biodiversity conservation, suggesting a need for management assessments that consider  
67 potential collateral effects on these agri-ecosystems ([Pérez-Méndez et al., 2022](#)). Even though  
68 large adoption of these water-saving practices has been observed across Asia ([Lampayan et al.,](#)  
69 [2015](#)), their implementation has been scarce in western rice producing countries, mainly due  
70 to farmers' concerns regarding potential negative effects on yields ([Carrijo et al., 2017](#)).

71

72 As evidence pointing towards non-significant yield decreases due to improved water-  
73 saving strategies mounts up ([Linguist et al., 2015](#); [Martínez-Eixarch et al., 2021](#); [Monaco](#)  
74 [et al., 2021](#)), their implementation assessment should shift from agronomic to environmen-  
75 tal concerns. The present study aims to identify and assess potential ecosystem synergies  
76 or trade-offs induced by water-saving strategies. A field experiment within the Ebro Delta  
77 rice production region (North-East Spain) was established to analyze the effects of irrigation  
78 strategies on CH<sub>4</sub> emissions, macrofauna and benthic macroinvertebrates biodiversity and  
79 rice production. The hypothesis to be tested is that those strategies with higher impact  
80 reducing water inputs represent an ecosystem trade-off, having a positive impact from a  
81 climate change mitigation perspective but affecting negatively the abundance and diversity  
82 of aquatic species, when compared to constantly flooded fields. This analysis pretends to  
83 contribute to the decision making process regarding water management in rice production  
84 areas, considering the importance of optimizing water resources, but also that of implement-  
85 ing sustainable ecosystem practices able to optimise potential positive and mitigate possible  
86 negative outcomes.

## 2. Materials and methods

### 2.1. Study area

A field experiment was conducted through the 2022 rice growing season (May to September) within IRTA's Estación Experimental del Ebre facilities in Amposta, Ebro Delta, Spain (40°42'30.2"N, 0°37'56.5"E). The region has a Mediterranean climate with mean annual air temperature of 16.5°C, hot summers (mean temperature in July: 25°C) and mild winters (mean temperature in December: 9°C). Mean annual precipitation is around 550 mm, being October the wettest month (75 mm) and July the driest one (20 mm). The experimental station is located at 100 m distance from the Ebro riverbank and 20 km from the river's mouth. The Ebro Delta is one of the largest coastal wetlands in the Mediterranean, with a total area of 320 km<sup>2</sup> and a coastline of approximately 50 km (Rodríguez-Santalla and Navarro, 2021). Rice production is the main economic activity in the region, representing 63% of the total area (Genua-Olmedo et al., 2016). Sites surrounding the study area are commercial rice paddy fields, managed conventionally under a constant flooding irrigation system throughout the growing season (May to September). Rice straw incorporation is the common practice across the region, leading to re-flooding fields after dry harvests to assist straw decomposition. From October to December fields are either flooded or left to drain, depending on each farmer's management approach. Besides the rice growing season, fields are left fallow. Due to its large variety of ecosystems and wildlife abundance, the Ebro Delta is considered a biodiversity hotspot, leading to its protection as a Ramsar site, enlisted within the Natura 2000 protected sites of the European Union, and recognized as the second most important bird area in Spain (Day et al., 2006; Romagosa et al., 2013; Genua-Olmedo et al., 2016).

### 2.2. Experimental layout

Three irrigation practices were tested in a complete randomized block design with five repetitions. Each block consisted of three 10 × 11 m plots, each individually managed under one of the irrigation strategies. Treatments corresponded to tested water-saving irrigation strategies (see Figure 1): (i) conventional constant flooding (CON), where plots remained

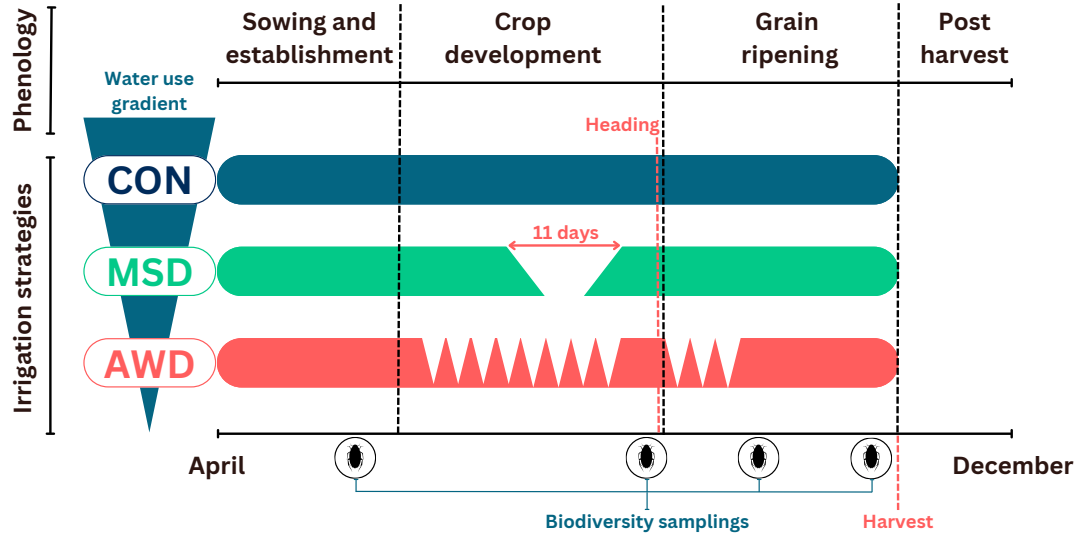


Figure 1: Water-saving irrigation strategies across the water use gradient. Horizontal bars represent reference water layer levels for each strategy through rice phenological stages. Biodiversity sampling dates are also represented across the season.

115 flooded from sowing (April - May) until two weeks before harvest (September); (ii) mid-  
 116 season drainage (MSD), draining once, for a 11 days period (from June 30<sup>th</sup> to July 11<sup>th</sup>,  
 117 2022), approximately 20 days before heading; and (iii) alternate wetting and drying (AWD),  
 118 maintaining fields flooded until the fourth leaf emerges and from heading to the end of flower-  
 119 ing (due to both processes' sensitivity to water-deficit stress), and under a flooding-drainage  
 120 cycle through all other periods within the growing season. Individual plot management was  
 121 achieved by the implementation of water input and drainage channels, independent from plot  
 122 to plot. AWD is the most widely adopted water-saving irrigation strategy, reportedly improv-  
 123 ing water use efficiency by 18-63%, depending on the saturated volumetric water threshold  
 124 considered for re-flooding (Linguist et al., 2015). The technique has been improved to min-  
 125 imize yield impacts through the establishment of underground water depletion thresholds  
 126 (usually 15 cm) to trigger field flooding (Carrijo et al., 2017), this practice is referred to  
 127 as Safe-AWD or Mild-AWD. Besides intermittent flooding, MSD was evaluated as a sec-  
 128 ond alternative practice with the objective of establishing a treatment intensity and water  
 129 use gradient. Treatment intensity is defined by the frequency of dry periods through the  
 130 growing season. This gradient presents its highest intensity with AWD management (low

131 water use), medium intensity with MSD (medium water use) and lowest intensity with CON  
132 irrigation (high water use). All three treatments followed local conventional practices regard-  
133 ing fertilization, pest and disease control, and straw incorporation. Cultivated rice variety  
134 corresponded to JSendra, a common commercial rice variety across Spain.

### 135 *2.3. Greenhouse gas flux measurement*

136 Gasses were sampled through the non-steady state gas chambers method adapted from  
137 [Altor and Mitsch \(2008\)](#) on a weekly basis from mid-May to late-September 2022. Chambers  
138 made of polyvinylchloride (PVC) squared frames and covered by transparent plastic were  
139 equipped with two ports, sealed with rubber septa, for the insertion of a thermometer and of  
140 sampling syringes. The chambers' dimensions (129 cm<sup>2</sup> basal area and 72 cm height) allowed  
141 their installation including several rice plants inside and all through the plant growth, as they  
142 were taller than plant height at harvest. Removable foam was set at the base of chambers to  
143 allow buoyancy through flooded periods and removed when fields were dry. This foam pre-  
144 vented as well gas exchange in between the chamber headspace and the exterior, wet towels  
145 were used with this purpose when foam was removed. Chambers were installed and removed  
146 for every sampling and at the same location within plots. During samples, four gas samples  
147 (30 ml) were extracted from each chamber every 10 minutes over a 30 minute period and then  
148 transferred over-pressured to pre-evacuated 12.5 ml vials. Samplings where done consistently  
149 from 10:00 to 15:00 to minimize variation derived from daily temperature variability. Gas con-  
150 centrations (i.e., CH<sub>4</sub> and N<sub>2</sub>O) were determined using a THERMO TRACE 2000 (Thermo  
151 Fisher Scientific, USA) gas chromatograph equipped with a flame ionization detector (FID,  
152 Trace GC 2000, Thermo Finnigan, Germany). The chromatograph calibration was done us-  
153 ing CH<sub>4</sub> standards in nitrogen provided by Carbueros Metalicos S.A. (Spain). Temperature  
154 variations within chambers' headspace were used to correct gas concentrations according to  
155 the ideal gas law. CH<sub>4</sub> emission rates were calculated as the slope of the linear regression  
156 between gas concentration and sampling time. Regressions were considered significant and  
157 their slopes accepted as considerable emission rates if they were positive and  $R^2 > 0.70$ , rates  
158 were considered as zero if these requirements were not met. Besides complete models, those  
159 considering all four measurements per sampling, four alternative models, each removing one  
160 measurement step-wise, were assessed for each sampling. In cases where model requirements

were met by the alternative models but not by complete models, the alternative model with higher  $R^2$  was selected to calculate emissions. This way, all calculated emission rates considered at least three measurements, the slopes corresponded to those of the best fitting linear models and any potentially altered measurements, due to possible chamber installation issues, were removed from calculations. Seasonal mean cumulative C-CH<sub>4</sub> emissions (kg ha<sup>-1</sup>) were calculated for each individual plot considering constant rate in between samplings.

#### 2.4. Biodiversity assessment

Aquatic vertebrate (fish and amphibians) communities were characterized sampling individuals fortnightly through the rice growing season installing cylindrical static nets within the flooded plots, identifying and counting trapped individuals after 24 hours. Benthic macroinvertebrate sample collection was carried out using a dip net (250  $\mu$ m). Sampled water volume was kept homogeneous by making four net collections across a 30  $\times$  30 cm plastic frame for each sub-sample. The net was submerged at 1 cm below soil level to collect individuals from the water layer as well as those living just above the soil-water interface. This process was repeated four times per plot and then all collected sub-samples were combined in one recipient, resulting in one final sample per plot. Samples were then washed and stored in 70% ethyl alcohol for later processing. Macroinvertebrate sample collection was repeated four times through the growing season (June 10<sup>th</sup>, July 15<sup>th</sup> and August 2<sup>nd</sup> and 31<sup>st</sup>, 2022) to assess potential changes in community structure and dynamics associated to the irrigation strategies. Sample processing consisted in washing each recipient's content through a 500  $\mu$ m mesh sieve and individualizing aquatic organisms (i.e., excluding plant material and terrestrial macroinvertebrates that might have fallen into the traps). Collected organisms were then identified up until the highest possible taxonomic resolution. Individuals that could only be identified up to a certain level (i.e., family, order or species), were afterwards assigned to higher levels according to the weighted proportion of individuals in these higher levels.

#### 2.5. Crop yield

All experimental plots were drained before harvesting to allow machinery entrance. Mechanical harvest was carried out and final yield (kg ha<sup>-1</sup>) was registered per plot to assess



190 potential impacts of water-saving irrigation strategies over production. Yield was calculated  
191 as wet weight, immediately after the harvest.

## 192 2.6. Data analysis

193 Water-saving treatments' effect on CH<sub>4</sub> emission rates was analysed through the appli-  
194 cation of a generalized linear mixed-modelling (GLMM) approach. The CH<sub>4</sub> emission rate  
195 was defined as the models' dependent variable, while the interactions between water-saving  
196 strategies and sampling dates were considered as fixed effects ( $n = 213$  observations). This  
197 interaction was assessed to account for potential temporal variation in the effects of these  
198 water regimes on methane emissions. Soil and water physicochemical parameters were in-  
199 cluded as covariates within the model after correlation (through Spearman's rank, see S1,  
200 and Pearson correlation coefficient, see S2) and collinearity (through Variance inflation fac-  
201 tor, VIF) assessments. Covariates with Pearson's correlation coefficient over 0.75 or VIF  
202 over 5 were not included in models. Random effects were accounted for in the model using  
203 repetitions (i.e., five replicated blocks) as grouping factor. Gaussian distribution and *identity*  
204 link function were applied in the model.

205  
206 The impact of water-saving irrigation strategies on microinvertebrate communities was  
207 evaluated from the perspectives of abundance and diversity. Accumulated abundance (num-  
208 ber of total sampled individuals) through the entire rice growing season was assessed for all  
209 experimental plots using a subset of the complete biodiversity sampling results. In this, only  
210 larvae from water beetles (Coleoptera), water bugs (Heteroptera), dragonflies and damselflies  
211 (Odonata) were considered. These three groups were selected as they presented the highest  
212 number of species and could be identified to higher taxonomic levels. All individuals in adult  
213 stadiums were excluded to avoid potential mobility in between plots, focusing the analysis on  
214 less mobile individuals which would better represent effects on biodiversity due to their higher  
215 susceptibility to drainage. Besides these three macroinvertebrate groups, abundance anal-  
216 ysis was extended considering as well decapoda (red swamp crayfish, *Procambarus clarkii*),  
217 fish and amphibians (*Pelophylax perezii* tadpoles). Individuals were grouped among orders  
218 as maximum taxonomic level to simplify analysis. This first analysis complements posterior  
219 diversity analyses, allowing the assessment of any potential effect of irrigation treatments on

the amount of individuals per taxonomic level, independent of communities' diversity. Effects on accumulated abundance of individuals were assessed through a GLMM considering abundance as dependent variable and the interaction between irrigation strategy and taxonomic identity as fixed effects. No random effects or additional covariates were considered.

Effects on the diversity of these communities were analyzed considering only macroinvertebrates from the same taxonomic subset as for the abundance analysis. Decapoda, fish and amphibians were excluded due to being composed only by one (i.e., Decapoda and amphibians) or very few species (i.e., fish) and, therefore, not representing potential diversity variations. Species richness ( $q_0$ ) and Shannon diversity (the exponential of Shannon index, or  $q_1$ ) for each experimental plot were estimated using the iNext R package (Hill numbers; Chao et al. (2014b); Hsieh et al. (2016)). Both estimations characterize biodiversity profiles within rice fields, species richness indicates the total number of observed species and Shannon diversity can be interpreted as the effective number of common or typical species in the community (Chao et al., 2014a). Data analysis was done using GLMMs considering biodiversity indexes ( $q_0$  and  $q_1$ ) as each model's dependent variable, the interaction between irrigation strategies and the square of sampling dates (as a factor) as fixed effects, and blocks to account for random effects. A quadratic relation was identified between both biodiversity indexes and sampling date, therefore, models including the square of sampling dates were selected as they better fit collected data than the alternative ones considering non-transformed sampling date. Soil and water physicochemical parameters were included as covariates after checking for correlation and collinearity, under the same criteria as for methane emission analysis. For Shannon diversity, a strong interaction was observed between conductivity and water strategies (see S3), so it was removed from the model as an explanatory variable to avoid artifacts and isolate the treatments' effect. All abundance and biodiversity models used gaussian distribution and *identity* link function.

Crop yield variations among the assessed strategies were analyzed fitting a linear model considering yield and irrigation treatment as dependant and independent variables, respectively. Physicochemical covariates were excluded from the analysis.

250

251 Data management, visualization and analysis was carried out using R software (R-4.2.2).  
 252 GLMMs were fit using the *glmmTMB* package. All models were analyzed through residual  
 253 diagnostics and  $R^2$ , collinearity and singularity analyses using the *DHARMa* and *performance*  
 254 R packages, respectively. Analyses of variance (ANOVA) were carried out for all models to  
 255 identify significant effects. For all cases where different effect were contrasted for factor levels,  
 256 the *emmeans* package was used.

### 257 3. Results

#### 258 3.1. Climate change mitigation

259 Irrigation strategies had a significant effect on C-CH<sub>4</sub> emission rates ( $\chi^2 = 59.3$ ,  $p = 1.324e^{-13}$ ;  
 260 Figure 2). Lower rates were identified for both non-continuous irrigation treatments, MSD  
 261 ( $t = -1.9$ ,  $p < 0.0001$ ) and AWD ( $t = -2.5$ ,  $p < 0.0001$ ), when compared to conventionally  
 262 flooded plots. There is high variation among rates from different irrigation managements  
 263 across the season, evidenced by the significant interaction between irrigation strategies and  
 264 sampling dates ( $\chi^2 = 64.8$ ,  $p = 8.388e^{-15}$ ). No soil or water physicochemical parameter showed  
 265 significant effects on emission rates. Considering the overall seasonal emission rate mean,  
 266 MSD and AWD strategies achieved 69% and 94% decrease in CH<sub>4</sub> emission rates, respec-  
 267 tively, when compared to CON strategy (CON =  $2.2 \pm 0.3$  mg m<sup>-2</sup> h<sup>-1</sup>; MSD =  $0.7 \pm 0.1$   
 268 mg m<sup>-2</sup> h<sup>-1</sup>; AWD =  $0.1 \pm 0.003$  mg m<sup>-2</sup> h<sup>-1</sup>). Mean cumulative C-CH<sub>4</sub> emissions for the  
 269 whole growing season were 64.2 kg ha<sup>-1</sup>, 21.0 kg ha<sup>-1</sup> and 4.8 kg ha<sup>-1</sup> for plots managed  
 270 under CON, MSD and AWD strategies, respectively. Emissions from non-constant flooding  
 271 plots were reduced in 67.3% with MSD strategy and in 92.5% with AWD management, when  
 272 comparing to CON plots.

273

#### 274 3.2. Biodiversity conservation

275 A significant negative effect was recorded for AWD managed plots regarding accumulated  
 276 abundance of vertebrate and macroinvertebrate individuals across the growing season when

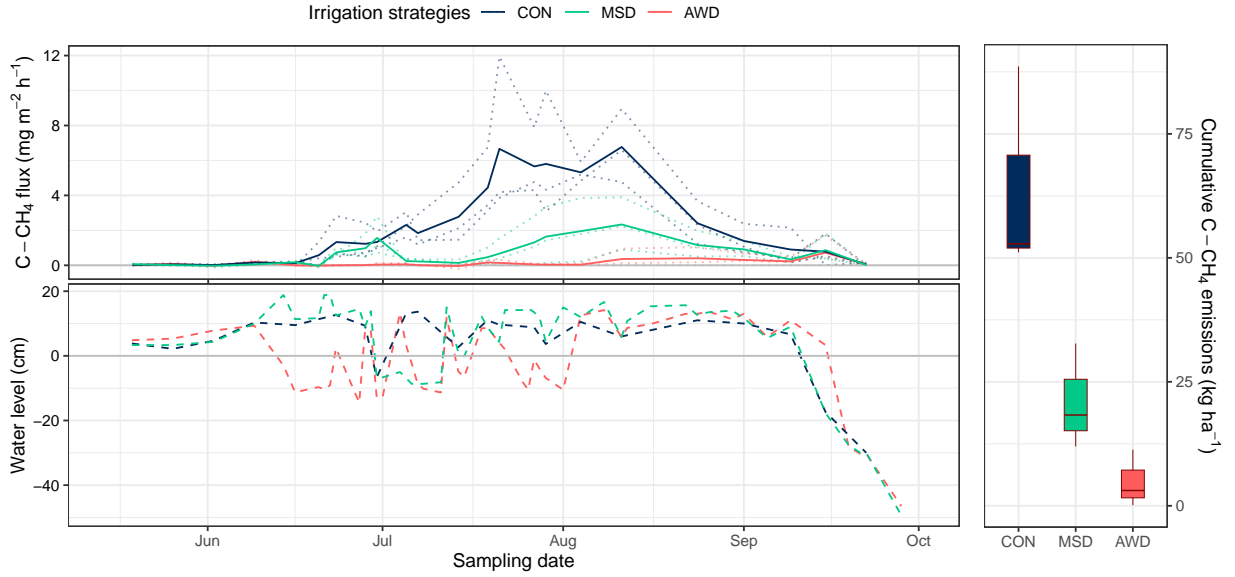


Figure 2: Methane (C-CH<sub>4</sub>) emission rates (top-left panel, in mg m<sup>-2</sup> h<sup>-1</sup>) and water level (bottom-left panel, in cm) across the rice growing season for the three water-saving irrigation strategies. Discontinuous lines in the top-left panel are mean emissions per plot, continuous lines are treatment means. Right panel shows cumulative C-CH<sub>4</sub> emissions in kg ha<sup>-1</sup> for the entire rice growing season.

277 compared to CON ( $\chi^2=8.7$ ,  $p=0.010$ ) and MSD ( $\chi^2=8.0$ ,  $p=0.024$ ) strategies. The mid-  
 278 intensity and medium water use MSD strategy achieved to maintain non-significant effects in  
 279 overall abundance in comparison to CON ( $\chi^2=0.7$ ,  $p=0.954$ ). When comparing strategies'  
 280 effect on abundance for each taxonomic group separately (Figure 3), significant differences  
 281 were observed only between AWD and CON strategies, with 65.1% and 50.8% decreases,  
 282 for Odonata ( $t=11.0$ ,  $p=0.035$ ) and Decapoda ( $t=11.7$ ,  $p=0.011$ ), respectively. No group  
 283 presented significant differences in abundance when comparing CON and MSD strategies.

284

285 Irrigation strategies did not have an overall effect on species richness of aquatic macroin-  
 286 vertebrates ( $\chi^2=3.6$ ,  $p=0.167$ ; left-hand panel in Figure 4), but significant effects were  
 287 observed for the interaction between strategies and the squared sampling dates ( $\chi^2=6.3$ ,  
 288  $p=0.043$ ). Covariates with significant effect were the squared sampling dates ( $\chi^2=10.0$ ,  
 289  $p=0.002$ ), sampling dates ( $\chi^2=6.6$ ,  $p=0.010$ ), soil electrical conductivity (EC, in  $\mu\text{S cm}^{-1}$ ;  
 290  $\chi^2=6.5$ ,  $p=0.010$ ) and oxygen concentration in the water layer ( $\text{O}_2\%$ ;  $\chi^2=5.4$ ,  $p=0.020$ ). To  
 291 further analyze this effect of sampling dates, the effect of irrigation strategies was studied for  
 292 each independent sampling date, fitting individual GLMMs per each sampling date. A signif-

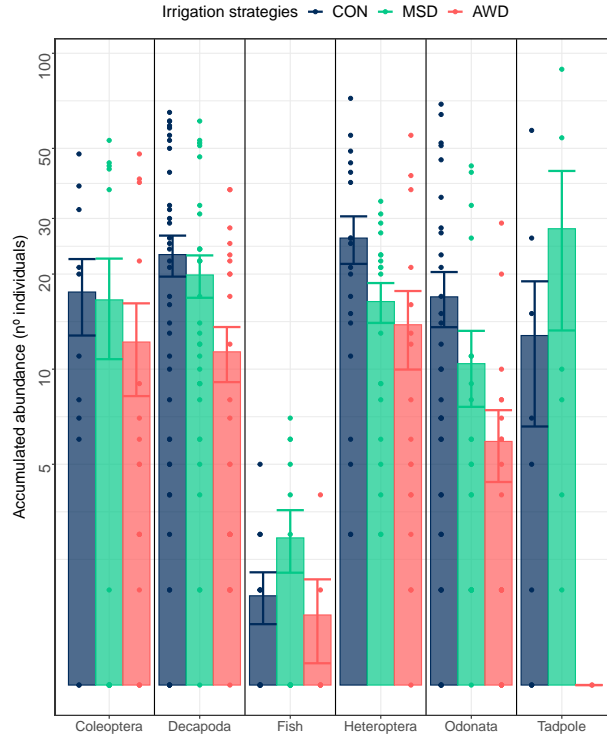


Figure 3: Accumulated abundance (number of individuals) of sampled macroinvertebrates, amphibians and fish for the three water-saving strategies across the rice growing season. Each bar shows the averaged abundance for all samplings and plots according to each strategy, points are abundance results per plot for each sampling and error bars indicate standard errors.

293 icant effect of irrigation strategy over species richness was identified for the second sampling  
 294 date ( $\chi^2=71.6$ ,  $p=2.869e^{-16}$ ), but no such effect was evident for all other sampling dates  
 295 (right-hand panel in Figure 4). Within this second sampling, both MSD ( $t=-6.7$ ,  $p=0.0005$ )  
 296 and AWD ( $t=-6.0$ ,  $p=0.0003$ ) strategies decreased species richness significantly compared  
 297 to CON. During this second sampling, MSD and AWD strategies reduced average species  
 298 richness in 53% and 55%, respectively, versus conventional flooding (CON =  $9.8 \pm 0.7$ ; MSD  
 299 =  $4.6 \pm 0.8$ ; AWD =  $4.4 \pm 0.05$ ). Soil pH showed a significant effect ( $\chi^2=11.1$ ,  $p=0.0008$ )  
 300 over species richness for sampling 2.

301

302 Shannon diversity varied significantly among different irrigation strategies ( $\chi^2=13.3$ ,  $p=0.001$ ;  
 303 Figure 5). Nevertheless, no significant effect was detected for AWD plots when comparing  
 304 to CON strategy ( $t=-0.1$ ,  $p=0.973$ ), while MSD resulted in lower diversity than both CON  
 305 ( $t=-1.9$ ,  $p=0.017$ ) and AWD ( $t=-1.7$ ,  $p=0.040$ ), contrary to the expected balancing effect.

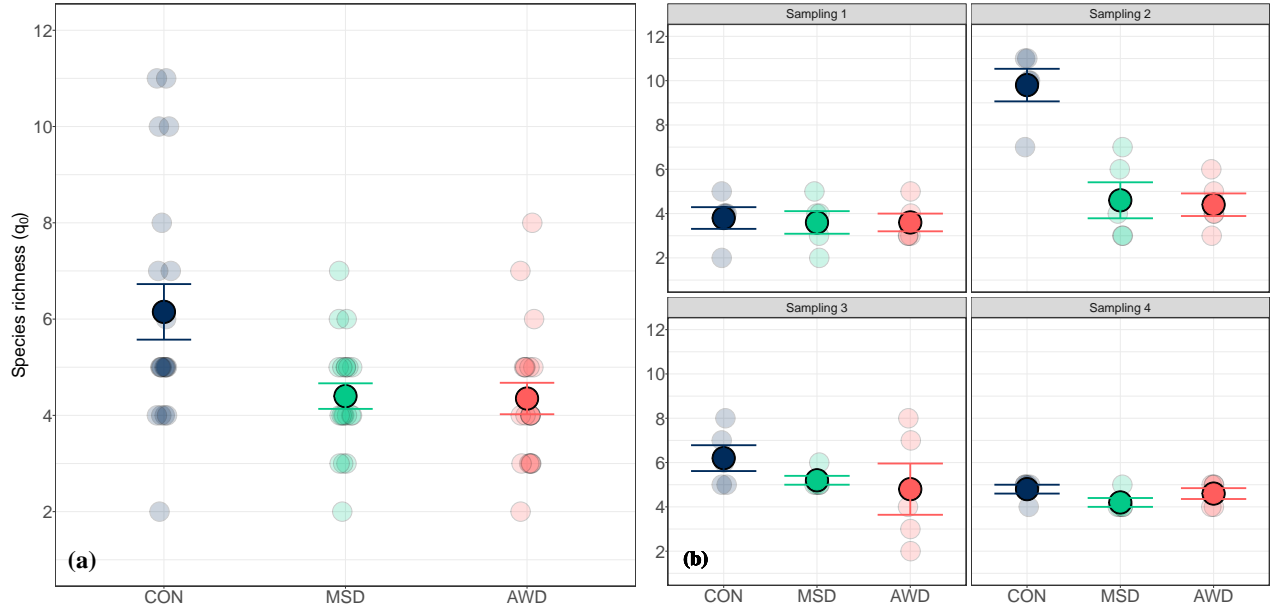


Figure 4: Macroinvertebrate species richness (number of identified species) for the three water-saving strategies. Semi-transparent points indicate plot means for each sampling date. Solid points and error bars indicate the overall means and standard errors, respectively. The left-hand panel (a) shows overall averaged results considering all four sampling dates across the rice growing season. The right-hand panels (b) show results for each sampling date separately.

Significant effects were identified for sampling date ( $\chi^2=17.6$ ,  $p=2.715e^{-5}$ ), squared sampling date ( $\chi^2=17.4$ ,  $p=3.062e^{-5}$ ), soil pH ( $\chi^2=5.5$ ,  $p=0.018$ ) and oxygen concentration ( $\chi^2=3.9$ ,  $p=0.047$ ).

### 3.3. Crop yield

Implementing an AWD, high intensity and low water-use strategy, proved to reduce significantly final rice production in comparison to both CON ( $t=-1058.6$ ,  $p=0.002$ , Figure 6) and MSD ( $t=-1117.1$ ,  $p=0.002$ ) strategies. The mid-intensity MSD strategy, on the other hand, did not result in crop production declines against a constant flooding strategy ( $t=58.5$ ,  $p=0.968$ ). Final mean yields were 8,213, 8,271 and 7,154 kg ha<sup>-1</sup>, for plots under CON, MSD and AWD irrigation, respectively. A mean yield decrease of 12.9% was observed for AWD plots when comparing to CON ones.

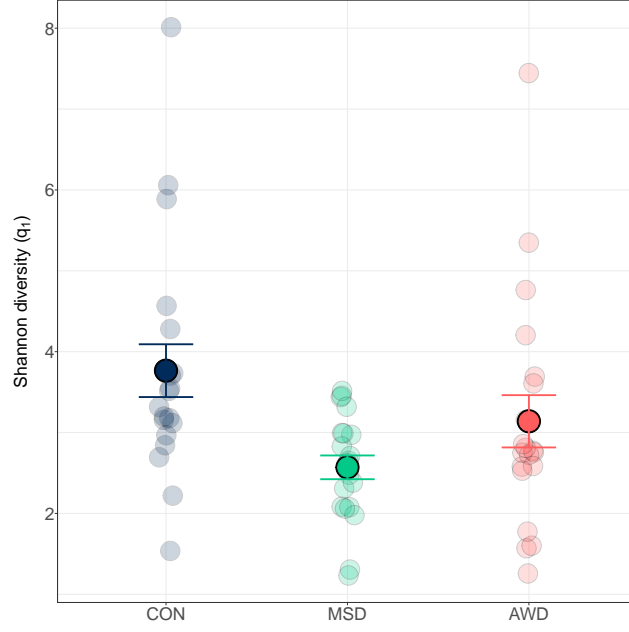


Figure 5: Macroinvertebrate Shannon diversity patterns for the three water-saving strategies. Semi-transparent points indicate plot means for each sampling date. Solid points and error bars indicate the overall means and standard errors, respectively.



Figure 6: Rice yields ( $\text{kg h}^{-1}$ ) for all three water-saving strategies. Each bar shows the averaged yield for all plots according to each strategy, error bars indicate standard errors.

## 4. Discussion

Plenty of research effort has been focused on the development of water-saving irrigation strategies in rice paddy fields to adapt to more frequent severe droughts (Bouman et al., 2007; Luo et al., 2019; Subhashini and Shankar, 2021) and to mitigate climate change impacts through a decline in CH<sub>4</sub> emission rates (Linguist et al., 2018). Potential negative effects on biodiversity conservation of those species depending on rice water layers has, nevertheless, largely been overlooked. Through this study, the scope of agricultural management assessment is extended to include possible effects on these aquatic communities.

Regarding climate change mitigation, the assessed non-constant flooding irrigation strategies resulted in positive effects, reducing CH<sub>4</sub> rates, as observed by several studies (Yagi et al., 1997; Cai et al., 2003; Li et al., 2006; LaHue et al., 2016). Emission rates from AWD managed plots remained close to zero all across the flooding-drying cycle, pattern that has already been observed (Balaine et al., 2019). This effect is attributed to aerobic oxidation by methanotrophs and reduced populations of methanogens (Kumar and Rajitha, 2019). Emissions from MSD plots increased similarly to those conventionally managed during the beginning of the season (mid- to late-June, see Figure 2), but collapsed to levels closer to AWD plots as soon as the drainage period was implemented. Right after these plots were re-flooded, emissions recovered steadily but did not return to CON plots levels. This two-peaked pattern in methane emission fluxes has been observed frequently for MSD water management in rice paddy fields (Sass et al., 1992; Yagi et al., 1997). Non-significant effects of other agronomic and environmental variables contradict previous results from a study within the Ebro Delta region by Martínez-Eixarch et al. (2018), which identified water level, plant cover and soil redox as a main drivers of methane emissions. This previous study considered only continuously-flooded fields and did not take sampling date within the growing season into account, when re-modelling current data under these conditions, rice cover ( $\chi^2=4.68$ ,  $p=0.0305$ ) and soil redox potential ( $\chi^2=4.49$ ,  $p=0.0339$ ) did prove significant. Besides re-affirming previous conclusions, this analysis indicates that under non-continuous irrigation, the main drivers of methane emission switch from agronomic and environmental to seasonality and water treatment. Emission rates and seasonal accumulated methane emis-



sions respond to the water-use gradient, with highest emissions in conventional plots and lowest in AWD plots (Islam et al., 2022; Perry et al., 2022). The MSD irrigation served, as expected, as a balancing strategy in between both gradient extremes. Results are in good agreement with those found in previous studies, which report reductions of up to 87% for AWD (LaHue et al., 2016) and 66% for MSD (Balaine et al., 2019) in seasonal cumulative emissions, when compared to continuous flooding strategies.

The effects on biodiversity of aquatic species living within water layers in rice plots is evident when analysing abundance of individuals among differently irrigated plots. There is a clear negative effect on vertebrate and macroinvertebrate biomass when comparing AWD with both CON and MSD strategies. Brief or standardized hydroperiods in rice fields might avoid the completion of active aquatic phases for many invertebrates and amphibians, causing stronger negative impacts in regional biodiversity conservation than a natural mosaic of temporary ponds (Lawler, 2001). Abundance in AWD managed plots remained low through the season, recovering slightly by the end of the season, while all lots remained flooded after heading, but not long enough to avoid significative negative effects when compared to the alternatives (see S5). Although negative effects of MSD irrigation on abundance in respect to constant flooding have been reported in previous studies (Watanabe et al., 2013), a conciliatory role of MSD as medium-intensity water-saving strategy was observed in this current study. This effect can be explained by an after-drainage flooding period long enough to allow the recovery of aquatic populations. Abundance decreased strongly in MSD plots right after the seasonal drainage (see sampling 2 in S5) but increased steadily during the remaining season in which plots were kept flooded (see samplings 3 and 4 in S5). The strong decline in Odonata individuals present in AWD plots might be attributed to the fact that such univoltine (one brood per season) predator insects are susceptible to drainage in intermittently irrigated rice fields, while multivoltine insects (e.g. mosquitoes) might withstand these dry periods (Mogi, 1993). Although only individuals within larval stages were also considered for crayfish (Decapoda), less evident physical differences between larvae and adults than in other studied groups might have caused that part of the individuals counted as larval were already in mobile stages. Drainage is a main driver of overland crayfish mobility (Ramalho

377 [and Anastácio, 2015](#)), which suggests that migration might play an important role explaining  
378 the high negative effect of intermittent drainage in AWD plots over crayfish abundance.

379

380 Even though overall species richness did not seem affected considering all four samplings  
381 across the season, there was a clear effect on the second sampling, which was carried out  
382 right after both non-continuously flooded strategies went through a drained period and were  
383 re-flooded (Figure 4). Comparing the first sampling results, in which all plots had been  
384 equally flooded and species richness was equivalent, to the second one, shows a clear increase  
385 for CON plots in contrast to stagnated MSD and AWD levels, where removing water layers  
386 might have drastically affected microinvertebrate communities. It can be hypothesized that  
387 similar number of species would have been identified during this sampling for all other plots  
388 if they had not been drained, as species richness generally increases with longer hydroperiods  
389 for temporary wetlands ([Batzler and Wissinger, 1996](#)). Plots under water-saving strategies  
390 were not able to increase the number of aquatic macroinvertebrate species present later in  
391 the season. The effects of conductivity and pH as drivers of aquatic macroinvertebrate com-  
392 munities has been previously described, conductivity is an indicator of dissolved ions and  
393 pH, interacting with ammonium and nitrate, determine nutrient availability ([Mazzoni et al.,](#)  
394 [2023](#)).

395

396 Contrary to the initial expected results, the MSD strategy did not achieve to counter-  
397 weight the negative effects of water layer removal on species richness even after keeping plots  
398 flooded for the remaining of the season. Furthermore, CON plots did not maintain higher  
399 species richness levels than those from the other two strategies for later third and fourth  
400 samplings. These patterns could be attributed to seasonal variations within the development  
401 stages of aquatic Coleoptera, Heteroptera and Odonata species. In natural wetlands, the  
402 abundance of larvae individuals of these orders tends to peak earlier in the season than that  
403 of adults, and then decreases as the season advances ([Florencio et al., 2010](#)), which coincides  
404 with the observed interaction between the effect of irrigation strategies and squared sampling  
405 dates. Non-continuous flooding strategies reset the communities to an earlier successional  
406 stage ([Lawler, 2001](#)). Draining fields in MSD and AWD managed plots just before this ex-

pected larval peak might have hindered oviposition and eclosion and, thus, resulted in the exclusion of larval stages from species that did hatch in conventionally managed plots. For Odonata species with late oviposition, rice fields might act as ecological traps if there is not enough time for emergence before drainage (Suhling et al., 2000). Besides the natural decrease in larval individuals later in the season and the effect of water management, similar low levels of species richness among all irrigation strategies during the second half of the growing season might as well have been caused by herbicides application in all plots and the dry period of 48 hours needed before re-flooding fields, in between the second and third macroinvertebrate samplings. Such applications have proven to result in high mortality of zoobenthic organisms in rice paddy fields (Baumart and Santos, 2011).

Less evident effects were identified when looking at Shannon diversity. Community structures varied through the season for all assessed strategies (see S4, S5 and S6). During the first sampling, communities from all strategies presented low diversity as they were characterized by a high relative abundance of *Hydrogliphus sp.* (Coleoptera) in comparison to other species. This initial evenness across strategies is due to no differences in irrigation regimes before this first sampling, all plots had been kept under constant flooding. While CON plots maintained homogeneous relative abundances among species through all later samplings in the season, once non-continuous irrigation treatments were implemented, MSD and AWD plots showed variation in their communities. Plots under MSD management presented lower Shannon diversity through all samplings, as they were dominated by high relative abundances of *Microvelia pygmaea* (Heteroptera) during the second and forth sampling, and *Ischnura elegans* (Odonata) during the third sampling. On the contrary, AWD managed plots resulted in more even communities (higher diversity) during mid-season samplings 2 and 3, when compared to MSD plots, and a high relative abundance of *Microvelia pygmaea* at the end of the season. Studies resulting in different trends, such as higher diversity in MSD managed plots than in CON ones (Watanabe et al., 2013), suggest that irrigation effects on biodiversity might be species and/or site specific and more research, and at longer spatial and temporal scales, should be done to identify the main drivers behind such dynamics.

Community dynamics must be understood considering both absolute abundance of individuals and diversity (or relative abundance). The high-intensity strategy AWD could be considered as less negative for aquatic communities than the mid-intensity MSD if only Shannon index was considered. When both parameters are taken into account, it is possible to notice that during the middle of the season (while intermittent flooding was implemented), communities in AWD plots were characterized by much lower absolute abundance of individuals than those in plots under MSD management. Communities in AWD plots presented higher Shannon diversity values than MSD ones due to no dominant common species during this period, but the negative impact over the amount of individuals present is considerably higher. These communities are more even but just because only a few individuals of each of the present species were able to withstand this intermittent removal of their aquatic medium.

Although final yield decreased, steeper declines as a product of intermittent irrigation, in comparison to constant flooded fields, might have been partly avoided by the implementation of a Safe-AWD practice, as opposed to experiments in which re-flooding was triggered after a fixed period from the disappearance of standing water (Tabbal et al., 2002). A mean production loss of 5.4%, and as high as 22.6%, depending on the intensity of implementation, has been observed for AWD irrigation, as alternative to CON (Carrijo et al., 2017), which is in agreement with this study's results. Nevertheless, higher water use efficiency (i.e., produced grain per water input) has been identified as a potential benefit of this practice (Wassmann et al., 2009). Bouman and Tuong (2001) pointed out that in order to increase rice production while implementing water-saving strategies, water saved in one place should be then destined to irrigate fields in other locations. Regarding MSD, drops in rice production levels were not only minimized, as expected initially with the implementation of this mid-intensity strategy, but were kept intact when compared to CON plots productivity. While such positive results might point towards the implementation of mid-season drainage as a an effective water saving strategy without negative effects on yield, attention should be put on potential long-term sustainability, as it might decrease soil fertility (Livsey et al., 2019).

## 5. Conclusions

Decisions regarding agricultural management should be assessed considering the multiplicity of its impacts. In this study, practices aimed at achieving climate change mitigation and adaptation to severe droughts, while avoiding production declines, are shown to have negative impacts on biodiversity conservation if this is not considered within initial objectives. Such trade-offs can be offset testing alternative practices that do consider multiple goals. While management focused on fewer objectives might achieve higher outcomes regarding them, alternative multi-objective strategies allow production within a positive range of results for all considered goals and avoids overseeing potential negative effects due to lack of consideration. Such more holistic approaches are to be promoted, as they lead to more sustainable production in the long term.

Rice production has been undergoing a process of innovative transformation from traditional practices, as it faces challenges regarding climate change and increasing food demand. While intermittent irrigation addresses  $\text{CH}_4$  emissions and water requirements, it might cause detrimental effects on biodiversity conservation goals if widely, and indiscriminately, implemented. Alternative, medium-intensity, irrigation practices such as mid-season drainage might hinder these negative effects and serve as conciliatory strategies. This practice accomplished to reduce  $\text{CH}_4$  emission rates in regards to constant flooding, while also avoiding production loss and drastic abundance decrease in aquatic communities when compared to alternate wetting and drying management.

This assessment and its results encourage further discussion on the optimum scope of agricultural management assessments given current and projected scenarios. Nevertheless, recognizing time and spatial scale limitations, longer term experiments under a wider range of conditions are required to identify specific management practices fitting scenarios that differ from that framing this study. Mid-season drainage is not proposed as a one-size-fit-all solution as local conditions and management objectives may vary. Long term effects should be analyzed to assess alternative, and potentially conciliatory, practices. Only through more integrative perspectives, innovations needed to tackle climate, biodiversity and production

496 challenges can be developed without compromising sustainability.

497

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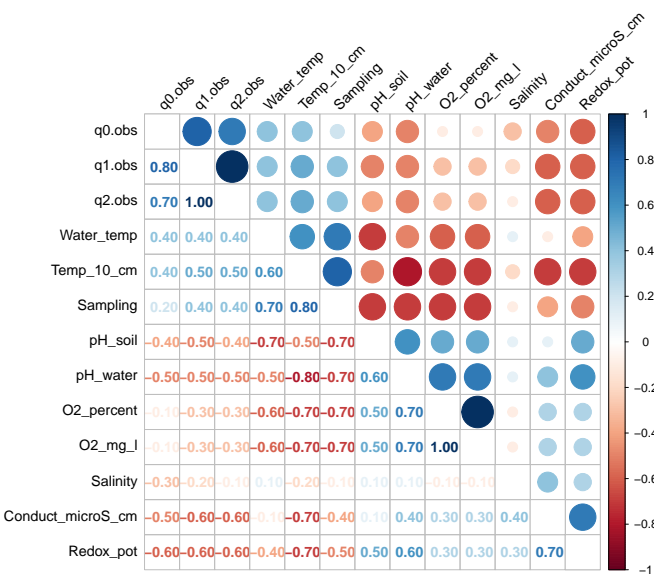


Figure S1: Spearman's rank correlation coefficient in between soil and water physicochemical covariates, and biodiversity components (q0.obs = Species richness, q1.obs = Shannon diversity, q2.obs = Simpson diversity).

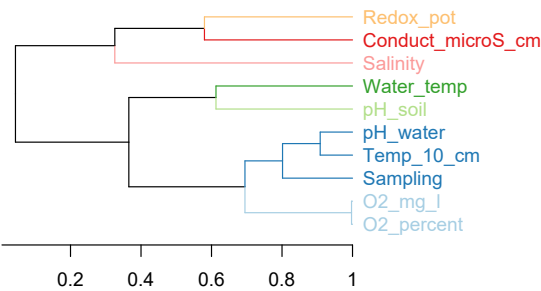


Figure S2: Dendrogram based on the Pearson correlation coefficient for soil and water physicochemical covariates.

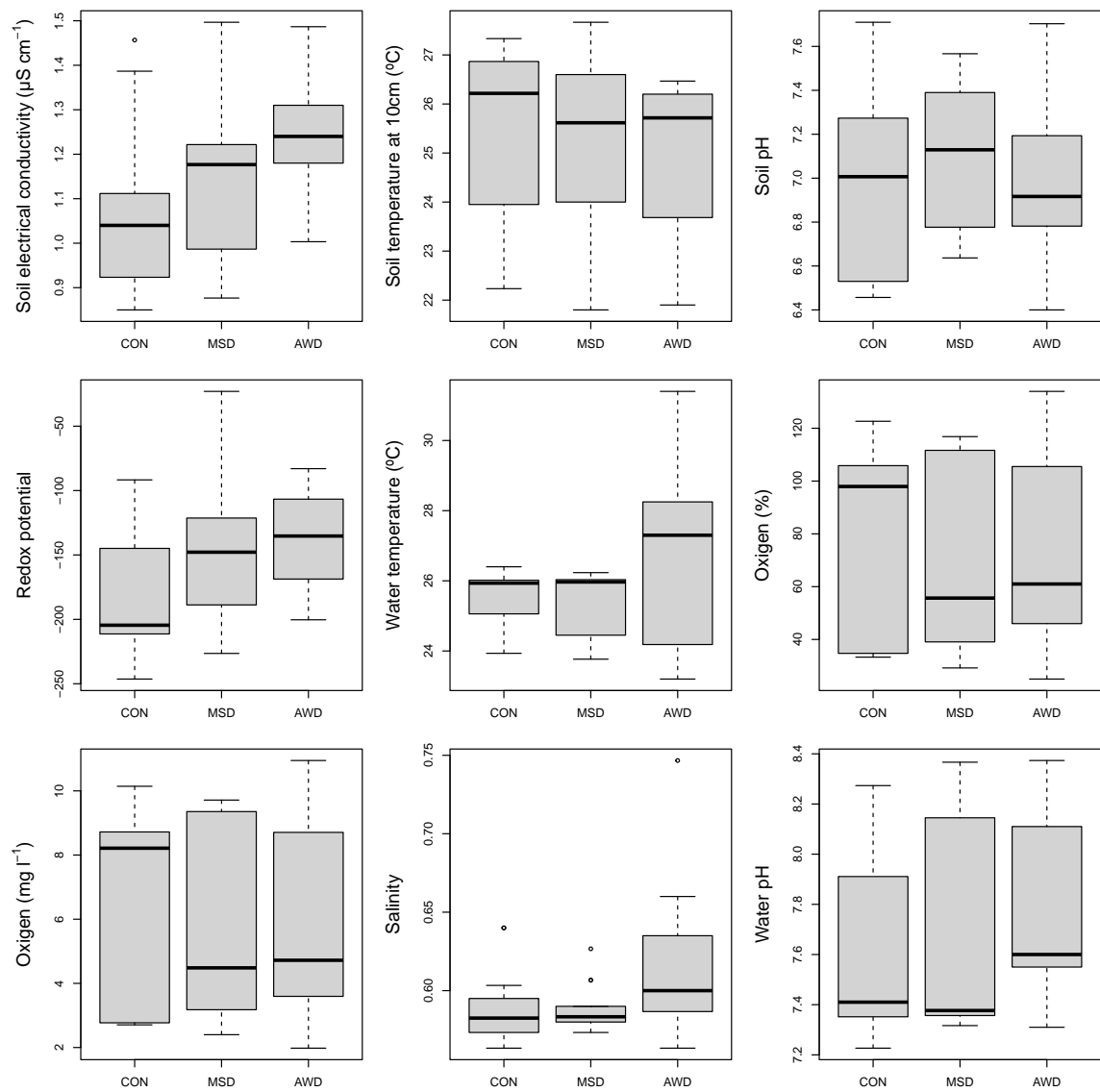


Figure S3: Soil and water physicochemical parameters for each water-saving irrigation strategy.



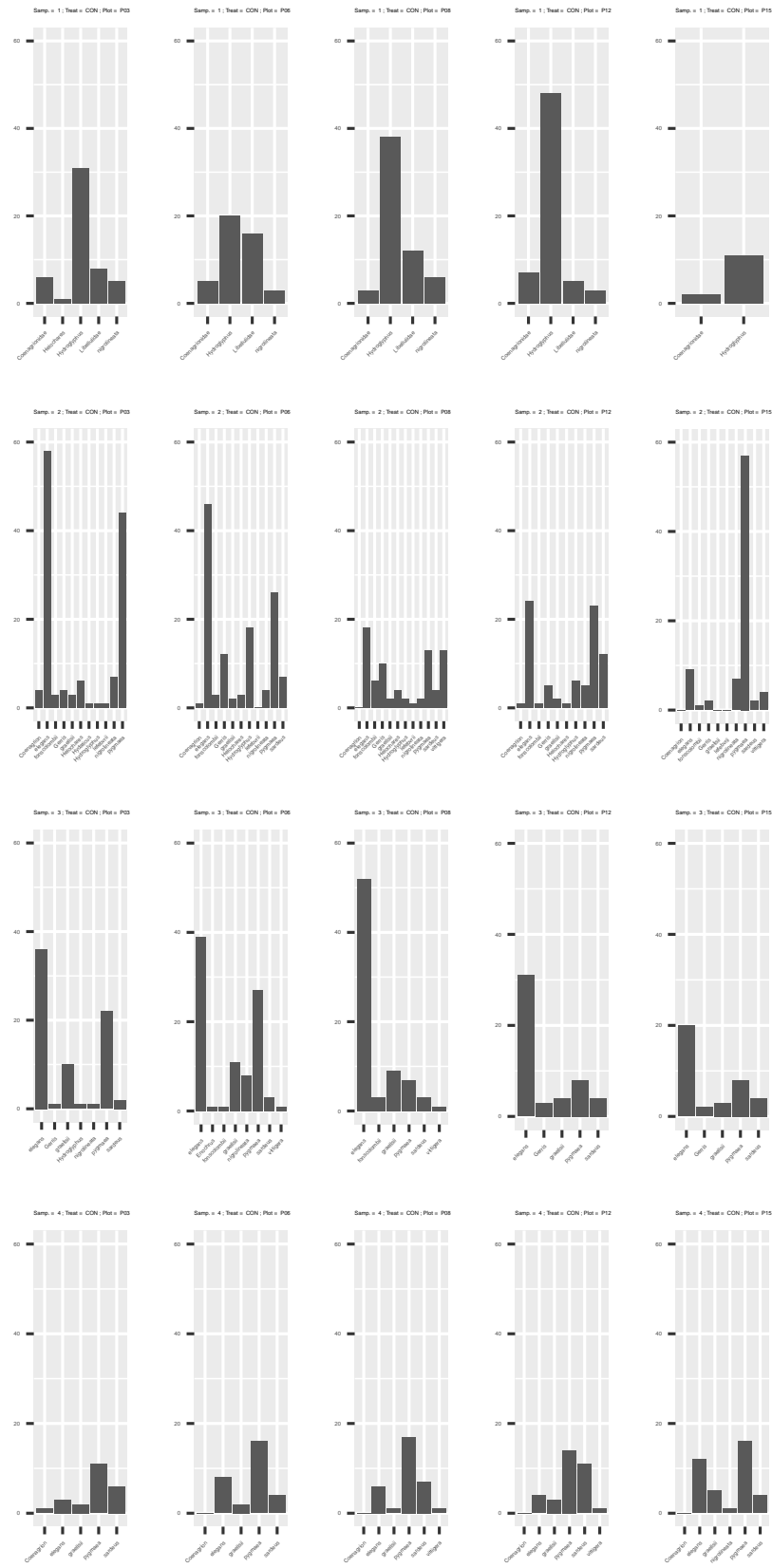


Figure S4: Abundance of individuals for all present taxa in plots managed under constant flooding regime. Grid rows are samplings and columns are individual plots.

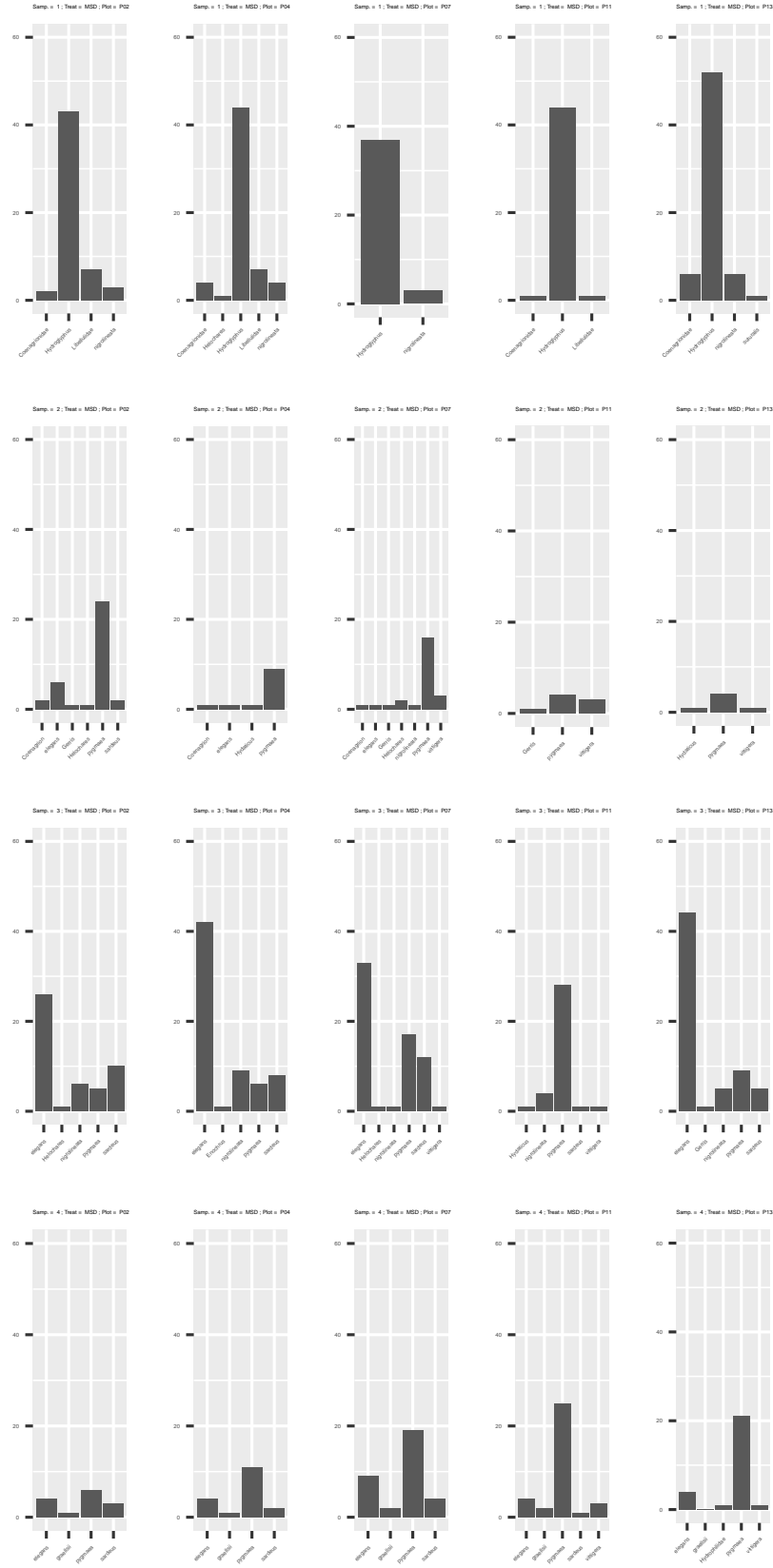


Figure S5: Abundance of individuals for all present taxa in plots managed under mid-season drainage. Grid rows are samplings and columns are individual plots.

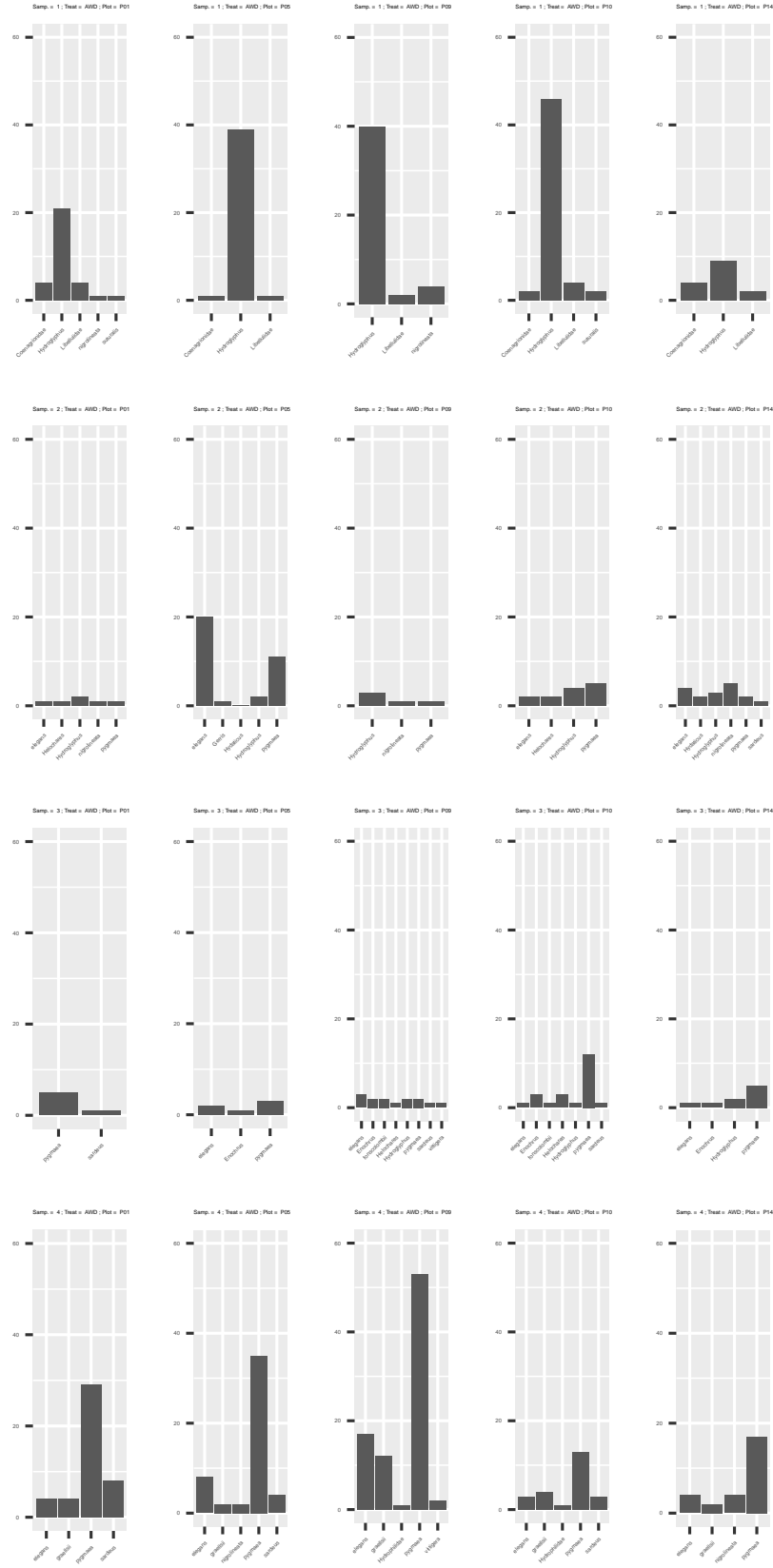


Figure S6: Abundance of individuals for all present taxa in plots managed under alternate wetting and drying. Grid rows are samplings and columns are individual plots.