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Assessing the impact of artificial summer drainage on the benthic macroinvertebrates in a freshwater wetland in northeast Italy

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1 **Assessing the impact of artificial summer drainage on the**
2 **benthic macroinvertebrates in a freshwater wetland in**
3 **northeast Italy**

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31

32

33 **Abstract**

34 Valle Mandriole is one of the two last remaining freshwater wetlands in the coastal area of Ravenna (NE
35 Italy). In 2011 a management technique that involves the complete drainage of the southern portion of Valle
36 Mandriole during summer has been undertaken. In the present study, the effects of this artificial drying on
37 the benthic macroinvertebrate fauna were assessed using a beyond before-after-control-impact (beyond
38 BACI) sampling design. Macroinvertebrates dwelling on macrophytes and in bare sediments were sampled
39 in the impact location and in four control locations, two times before and two times after the drying period.
40 Simultaneously, water samples were collected to monitor chemical properties potentially affecting the
41 studied organisms. Biological and chemical data were analysed by multivariate statistical methods.
42 The statistical analysis did not detect any significant effect of the management action on the benthic
43 macroinvertebrates or on the water chemical and physical properties. This contrasts with some previous
44 results, suggesting that the effects of a management strategy based on draining completely dry and then
45 reflooding a wetland area are site specific. However, it is necessary to consider that the present study does
46 have some limitations, in particular the differences between impact and control locations and the timing of
47 the sampling. The highest biodiversity was observed in one small and isolated control location; this
48 highlights how maintaining, protecting, restoring and even creating small ponds may play an important role
49 in biodiversity conservation.

50

51 **Keywords:** impact assessment; beyond BACI; benthic macroinvertebrates; water quality; water level
52 management; managed wetlands

53

54 **Declarations**

55

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60 **Conflicts of interest/Competing interests**

61 The Authors have no conflict of interest or competing interest to declare.

62

63 **Ethics approval**

64 Not applicable

65

66 **Consent to participate**

67 All the participants voluntarily agreed to participate in study on which the present manuscript is based.

68

69 **Consent for publication**

70 All the Authors gave their consent to publish the present manuscript in *Hydrobiologia*.

71

72 **Availability of data and material**

73 Research data are provided as electronic supplementary material to the present manuscript.

74

75 **Code availability**

76 Not applicable.

77

78

79

80 **Introduction**

81 Wetlands are extremely diverse in terms of habitats and biological communities (Moss, 2000;
82 Dudgeon et al., 2006) and are among the most productive environments all over the world (Ramsar
83 Convention Secretariat, 2013). The value of wetlands, related to the number of ecosystem services they
84 provide, has been repeatedly highlighted during the last forty years (Tiner, 1984; Postel & Carpenter, 1997;
85 Mitsch & Gosselink, 2000; Barbier, 2011; Maltby & Acreman, 2011; Sievers et al., 2018).

86 Despite their importance, over the centuries the extent of wetlands on our planet has been considerably
87 reduced as a result of human activities, *in primis*, land reclamation (Denny, 1994; Gordon et al., 2010,
88 Antonellini et al., 2015).

89 In natural alluvial landscapes, the extension of wetlands is influenced by many factors, such as the
90 hydrological cycle, the landscape morphology and the riverine regime, which can lead wetlands to evolve
91 into terrestrial environments (Ward, 1997). Under these conditions, the natural burying processes can be
92 counterbalanced by flooding of new areas. However, in inhabited regions river basin management prevents
93 these dynamics. Thus, maintaining the water level and bathymetry of wetlands present in these areas often
94 requires an active human intervention.

95 In the coastal areas of the Po Valley (northeast Italy), the alluvial wetlands generated by the Po River
96 have been reduced considerably to make room for crops (Cencini, 1998; Buscaroli et al., 2011; Antonellini
97 et al., 2015). After the reclamation process, excluding minor and hunting ponds, in the coastal area
98 surrounding the city of Ravenna only two freshwater coastal wetlands have survived to nowadays: Valle
99 Mandriole and Punte Alberete. Recognition of their important role as unique habitats that survived the
100 reclamation process has triggered actions aimed at preventing progressive burying and onset of anoxic
101 conditions, such as hydraulic intervention and vegetation clearing (Buscaroli et al., 2011).

102 A particularly drastic measure has been taken in Valle Mandriole, due to the recurrence of anoxia and
103 deterioration of vegetation. Starting from year 2011, the southern portion of Valle Mandriole has been
104 completely drained every July to allow for mineralization of sediment organic matter and to facilitate
105 vegetation clearing, then flooded again at the end of September to maintain wintering habitat for waterbirds.

106 Information on the benthic macroinvertebrates is highly relevant to the whole wetland ecosystem,
107 since they participate in the transformation of organic matter and they contribute to the cycle of energy and

108 nutrients of the ecosystem. In particular they are a primary food source for organisms at the top of the food
109 chain, i.e., fish, birds, mammals and reptiles (Covich et al., 1999, Cooper et al., 2009). Benthic
110 macroinvertebrates are often used in biomonitoring to obtain information to implement management plans
111 and improvement of environmental quality (Rosenberg & Resh, 1993). In particular, changes in the
112 hydrological regime can significantly influence the composition of the benthic macroinvertebrate
113 assemblages (Kaster & Jacobi, 1978; Furey et al., 2006; White et al., 2008; McEwen & Butler, 2010).

114 The aim of the present study was to assess if the dry period after complete drainage of the wetland
115 impacted on the benthic macroinvertebrate assemblages, by comparison of the drained location with other
116 reference locations in the same area, using a beyond before-after-control-impact (beyond BACI) sampling
117 design (Underwood, 1992, 1994). Along with the benthic macroinvertebrate assemblages, the main water
118 chemical and physical parameters have been evaluated pre and post the draining event.

119 Although it could be trivial to state that the benthos is affected in the period when the basin is
120 completely dry, the point was to assess how the imposed dry period affected the benthic assemblages when
121 the basin returned to be flooded. The drainage is supposed to improve the oxygenation conditions, of the
122 wetland, historically affected by anoxia. This improvement is hypothesized to increase the diversity of the
123 benthic assemblages and the abundance of taxa that are more sensitive to the oxygenation conditions. On
124 the other hand, this intervention annihilates the benthos for a few months, and this could result in lower
125 abundance and diversity for several months after the basin returns to be flooded (Lindegarth & Chapman,
126 2001; Bedford & Powell, 2005).

127 Although this study focuses on a specific wetland of limited extension, its scope is beyond the local
128 interest, as it can contribute to understand the implications of a management practice of simple applicability
129 and wide exportability.

130

131 **Methods**

132 *Study area*

133 The freshwater wetlands complex of Valle Mandriole (VM) and Punte Alberete (PA) is located in the
134 Po Plain, northeast Italy, 4 km from the coast of the north Adriatic Sea, 3.5 km south of the brackish
135 Comacchio lagoons, 10 km north of the city of Ravenna, covering an area of 420 hectares (Fig. 1). The two
136 wetlands are separated by the River Lamone and are the remnants of a larger complex (about 8000 hectares),

137 mostly reclaimed during the 1960s. The extension of the whole complex, as it appears today, was finally
138 established in 1972 (Lazzari, 1994; Buscaroli et al., 2011).

139

140
141

Fig. 1

142 The entire study area lies in an anthropized landscape characterized by intensive farming and by the
143 presence of a highway running along the eastern edge of both PA and VM. Moreover, the international port
144 and the industrial area of Ravenna are located just a few kilometres to the South (Fig.1).

145 The area is part of the Po Delta Regional Park, established in 1988. In 1977, both wetlands were designated
146 Ramsar sites, according to Ramsar Convention Secretariat (2013). In 1994 they were included in the Natura
147 2000 network as *site of community importance* (Council Directive 92/43/EEC) and as *special protected*
148 *areas* (European Parliament and Council Directive 2009/147/EC).

149 Nevertheless, strong pressures on these habitats driven by alien organisms such as *Procambarus*
150 *clarkii* (red swamp crayfish or Louisiana crawfish) and *Myocastor coypus* (coypu), saltwater intrusion and
151 water turbidity, have compromised over time their ecological status (Antonellini et al., 2010; Studio Silva,
152 2012; Mollema et al., 2013).

153 Valle Mandriole, extending over 240 ha, lies in the north of the complex and is mostly made up of
154 marshes with populations of *Phragmites australis*. One single sluice, located northeast, takes water in and
155 out of the River Reno through an artificial channel.

156 Until 2011, to offset the significant lowering of the water level in the summer, VM was loaded to
157 +0.80 m above mean sea level (AMSL) in September/October. This was implemented because Valle
158 Mandriole was used as a reservoir for the water supply of Ravenna. However, this practice has led to a
159 deterioration of emergent vegetation. Thus, starting from 2011 a management plan aimed at restoring the
160 functionality of the biotope has been implemented. A complete drainage of the southern half of the wetland
161 has been carried out every year during summer to foster mineralization of sediment organic matter. This
162 intervention was accompanied in 2013 by the dredging of submerged channels to increase water circulation
163 and guarantee refuge habitats for fishes.

164 Punte Alberete, extending over 186 ha, occupies the southern portion of the complex and consists
165 mostly in alluvial forests alternating with low-lying perennially submerged marshes that are connected by
166 ditches. The water level is controlled by two sluices. The first one, located in the southeast, is used to intake

167 water from River Lamone through an artificial channel. The second one, in the northeast, is used to drain
168 water to Adriatic Sea, through the Pialassa Baiona lagoon.

169 The water level of Punte Alberete is usually not actively managed. However, occasionally it is set by
170 acting on the sluices to allow for vegetation clearing. The average water level calculated on three years
171 (2010-2012) observed in the northeast sluice was +0.64 m AMSL in March and -0.18 m AMSL in August.
172 A small interdunal wetland (0.1 ha), here referred to as SV, was also included in this study (see below for
173 the sampling design). The pond is located within the San Vitale Pinewood, established on the sand dune
174 system that extends immediately east of PA and VM (Fig.1). This habitat differs from the other sampling
175 locations due to the vegetation, that includes *Thypha latifolia*, and to the higher water salinity.

176

177 *Field and laboratory methods*

178 A beyond before-after-control-impact (beyond BACI) sampling design was adopted here to assess the
179 effect on the benthic macroinvertebrate assemblages. In particular, we studied the effect of the drainage of
180 the southern part of VM, that occurred during summer 2013. In the beyond BACI design a single putatively
181 impacted location is compared with multiple control locations. All the locations are sampled at multiple
182 times before and after the impacting event has occurred.

183 Five sampling locations (one impact, four controls) were identified in the study area. VM was divided
184 into two portions (Fig. 1). The southern portion (VMS), extending approximately 120 ha, was identified as
185 the impact location, since it was completely drained and kept dry from July to September. The control
186 locations were the northern portion of Valle Mandriole (VMN), two marshes within Punte Alberete, one in
187 the northern part (PAN), one in the southern part (PAS), and the pond within the San Vitale Pinewood (SV).
188 Control locations maintained their own water level throughout the year, although minimal during summer.
189 Within each location, two sites were identified, labelled as 1 and 2 (e.g., VMS1 and VMS2). All sites were
190 located at the border between bare sediments and thickets of *Phragmites australis*, or *Typha latifolia* in the
191 case of the SV pond where *P. australis* was not present. None of the sampled sites, neither in the impact
192 location nor in the control locations, was subject to vegetation clearing during 2013. All ten sites were
193 sampled twice before summer drainage (May and June), and twice after (October and November).

194 At each site, the macroinvertebrates were sampled separately from bare sediments and from emergent
195 vegetation. For bare sediments, one sample for each site was taken using a PONAR grab with opening

196 155x175 mm (sampling surface: 0.027 m²). On vegetation, a squared-frame kick net with opening 210x210
197 mm and a 0.5 mm mesh was used by scraping the immersed portion of the stems from the base upward for
198 two minutes. Both types of samples were sieved on site with a 0.5 mm mesh conical net and then
199 immediately preserved with 10% formaldehyde. In the laboratory, all sampled specimens were identified
200 to family level and counted using a stereo microscope.

201 At each site, temperature (°C), electrical conductivity (µS/cm), pH, redox potential (mV) and
202 dissolved oxygen concentration (mg/l) of the water were measured in the field, both on the surface and at
203 the bottom, just above the sediment, using OX22 Aqualytic probe for dissolved oxygen and PCD 650
204 (EUTHECH Instruments) for the other parameters. Water alkalinity (meq/l) was also measured in the field
205 by titration using alkalinity titration test (Merck Mcolorstest™). Depth was determined using a graduated
206 pole. In addition, a 2 l sample of surface water was taken and brought to the lab where dissolved sulphate
207 (SO₄²⁻-S, mg/l), ammonium (NH₄⁺-N, mg/l), nitrite (NO₂⁺-N, mg/l), nitrate (NO₃⁺-N, mg/l) and phosphate
208 (PO₄³⁻-P, mg/l) were measured using a Hach DR/2010 spectrophotometer, and total and volatile suspended
209 solids (g/l) were determined gravimetrically using GF/F Whatman® glass microfiber filters (Eaton &
210 Franson, 2005).

211

212 *Data analysis*

213 Three different multivariate data sets were developed and then analysed separately: i) abundance of
214 taxa collected from vegetation, expressed as number of individuals per minute of sampling; ii) abundance
215 of taxa sampled from bare sediments, expressed as number of individuals per square meter; iii) physical
216 and chemical properties of the water. In addition, number of taxa, Shannon index of diversity and Pielou's
217 index of evenness were calculated for invertebrates from both vegetation and bare sediments.

218 To assess the impact of the drainage of VMN and the effects of the other factors, each data set was
219 analysed by PERMANOVA (Anderson, 2001; Anderson et al., 2008). PERMANOVA is a non-parametric
220 analysis analogue to the analysis of variance (ANOVA) that allows for multivariate (and univariate) data
221 to be analysed and tested based on any resemblance measure. Analysis of complex designs, involving
222 several orthogonal and nested factors is possible. All tests of hypothesis, including those regarding
223 interactions between orthogonal factors, are performed using permutation techniques; 9999 permutations
224 were used for all the analyses presented here.

225 The Bray-Curtis distance was used as the resemblance measure for taxa abundances, after square root
226 transformation of the raw data. The Euclidean distance was used as the resemblance measure for chemical
227 and physical properties, after standardization of the raw data. The Euclidean distance was used also for the
228 diversity indices.

229 PERMANOVA was applied in accordance with the principles of Beyond BACI, following the
230 indications of Anderson et al. (2008) for the analysis of asymmetrical designs.

231 The factors included in the analysis were:

- 232 – control vs. impact (I, fixed, two levels: control and impact);
- 233 – location (L, random, nested in the factor control vs. impact);
- 234 – site (S, random, nested in the factor location, two sites for each location);
- 235 – period (P, fixed, two levels: before and after the drainage of VMS);
- 236 – time (T, random, nested in the factor period, two times before and two times after the drainage
237 of VMS).

238 The design is asymmetrical, since only one impact location was available (VMS), as opposed to four
239 control locations (VMN, PAN, PAS and SV). For each combination of site and time, only one sample was
240 collected for the benthos on vegetation, one for the benthos in bare sediments and one for the water.
241 Replicated sites were sampled within each location, but there were no replicates within each site; as a
242 consequence, the lowest-level interaction, i.e., site×time, had to be used as the residual source of variation
243 and could not be tested.

244 According to the beyond BACI rationale, an effect of the drainage of VMS would cause the temporal pattern
245 of benthic abundances or of chemical properties in the impacted location to differ from the temporal patterns
246 in the control locations. Thus, an impact would be detected if the I×P interaction or the I×T interaction were
247 significant.

248 To complement PERMANOVA, two additional multivariate methods, based on resemblance
249 measures, were applied. Non-metric multidimensional scaling (MDS) was used to graphically represent the
250 relationships among samples. Similarity percentage (SIMPER) was used to quantify the contribution of
251 each taxon to the similarity within and to the dissimilarity between groups of samples (Clarke, and
252 Warwick, 2001). Ordination of samples based on water chemistry variables was performed by discriminant
253 function analysis, using the location as the grouping criterion (Legendre & Legendre, 2012).

254 The software StatSoft Statistica was used to carry out the discriminant function analysis. All the other
 255 calculations were performed using the software PRIMER 6 with the PERMANOVA+ add-on.

256

257 **Results**

258 *Macroinvertebrates on vegetation*

259 It was not possible to demonstrate any impact of the drainage of VMS on the abundances of benthic
 260 macroinvertebrate taxa sampled from vegetation, since the PERMANOVA analysis did not detect a
 261 significant I×P or I×T interaction (Table 1).

262

263 **Table 1** Results of the PERMANOVA test for the benthic macroinvertebrates sampled from vegetation.

264 *: significant ($P < 0.05$); **: highly significant ($P < 0.01$)

Source of variation	Degrees of freedom	P value			
		Abundances	N of taxa	H'	J'
I: impact vs. control	1	0.771	0.934	0.980	0.522
P: period, before vs. after	1	0.522	0.262	0.948	0.988
L: location (nested in I)	3	0.005**	0.003**	0.006**	0.001**
T: time (nested in P)	2	0.025*	0.880	0.034*	0.013**
I×P	1	0.636	0.516	0.272	0.254
S: site (nested in L)	5	<0.001**	0.192	0.236	0.834
I×T	2	0.181	0.140	0.411	0.567
L×P	3	0.433	0.008**	0.206	0.334
L×T	6	<0.001**	0.445	0.781	0.776
S×P	5	0.042*	0.960	0.584	0.580
Residual	10	–	–	–	–
Total	39	–	–	–	–

265

266 The L×T interaction was significant, denoting that the temporal patterns of benthic abundances
 267 differed among locations; however, these differences could not be ascribed to an effect of the drainage of
 268 VMS, since they were present also among control locations.

269 The factor location was significant also as a main effect, indicating that the abundances of benthic
 270 organisms differed among locations not only for their temporal pattern of variation but also for their mean
 271 values over the considered time span.

272 In fact, The MDS plot (Fig. 2) shows that samples from different locations are clearly separated on
273 the ordination plane; more precisely the samples from the pond in San Vitale Pinewood (SV) are grouped
274 on the higher part of the diagram, the samples from both locations of Punte Alberete (PA) are grouped on
275 the lower right and the samples from both locations in Valle Mandriole (VM) are grouped on the lower left
276 side of the plane.

277

278
279

Fig. 2

280 According to the SIMPER analysis, the taxa that most contributed to the distance between the samples
281 from SV and those from PA are Physidae, Baetidae, Chironomidae, which were more abundant in SV
282 samples, and Naididae, more abundant in PA samples (Tables S1, S2 in the Supplementary Data). The same
283 taxa gave the highest contribution to the distance between SV and VM, all being more abundant in SV
284 samples (Tables S1, S3 in the Supplementary Data). The pinewood pond was characterized by the highest
285 abundance of all taxa, excluding Naididae and Cambaridae, and by the presence of several families of
286 Diptera, Coleoptera, Hetroptera and Odonata, absent in the other areas. Naididae is the taxon that by far
287 gave the highest contribution to the distance between PA and VM, due to its high abundances in PA (Tables
288 S1, S4 in the Supplementary Data). In general, VM was characterized by the highest abundances of
289 Cambaridae and low abundances of other taxa. However, Baetidae were more abundant than in PA.

290 The horizontal sorting of the points in the MDS plot is mostly determined by the abundance of
291 Naididae, which increases from left to right (Fig. S1a in the Supplementary Data). The vertical sorting is
292 determined by the abundances of several taxa (notably Chironomidae, Baetidae, Physidae) that increase
293 from bottom to top (Fig. S1b–d).

294 As for the temporal variation, in SV, Chironomidae, Baetidae and Physidae decreased in abundance
295 over time, while Naididae increased in abundance. Coenagrionidae were sampled only in June (second time
296 of the before period). In both locations of PA the abundance of Naididae increased from May to June and
297 then decreased to the lowest values in the after period. Corixidae were relatively abundant in May and June
298 and almost absent in October and November. One difference between PAS and PAN is the relatively high
299 abundance of Gammaridae in the former in May and June. It is difficult to identify clear temporal patterns
300 for VM due to the general low abundance of macrobenthic organisms. However, Cambaridae were

301 generally more abundant in the after period, particularly in October. The highest abundance of Baetidae
 302 was recorded in June for VMS and in October for VMN.

303 The I×P and I×T interactions were not significant also for the diversity indices, while the main factor
 304 location was highly significant for all of them (Table 1). SV had the highest richness (14-20 taxa), VM
 305 constantly had a low richness (4-6 taxa) and PA had a decreasing richness over time from 9-11 taxa to 1-4
 306 taxa (Fig. S2a in the Supplementary Data). The highest values of Shannon's index of diversity H' were
 307 recorded in SV, the lowest in PA, were they decreased over time alongside with the reduction of the number
 308 of taxa (Fig. S2b). Despite the low number of taxa, H' values in VM were higher than in PA, since all taxa
 309 had low abundance, and none were dominant. In fact, the highest values of Pielou's index of evenness J'
 310 were recorded in VM, the lowest in PA, where Naididae were highly dominant (Fig. S2c).

311

312 *Macroinvertebrates in bare sediments*

313 It was not possible to demonstrate any impact of the drainage of VMS on the abundances of benthic
 314 macroinvertebrate taxa sampled from bare sediments, since the PERMANOVA analysis did not detect a
 315 significant I×P or I×T interaction (Table 2).

316

317 **Table 2** Results of the PERMANOVA test for the benthic macroinvertebrates sampled from bare
 318 sediments. *: significant (P < 0.05); **: highly significant (P < 0.01)

Source of variation	Degrees of freedom	P value			
		Abundances	N of taxa	H'	J'
I: impact vs. control	1	0.962	0.848	0.979	0.994
P: period, before vs. after	1	0.021*	0.993	0.736	0.384
L: location (nested in I)	3	0.028*	0.009**	0.003**	0.013*
T: time (nested in P)	2	0.893	0.735	0.488	0.393
I×P	1	0.513	0.949	0.984	0.980
S: site (nested in L)	5	0.041*	0.409	0.330	0.310
I×T	2	0.878	0.599	0.158	0.091
L×P	3	0.507	0.022*	<0.001**	0.079
L×T	6	0.016*	0.410	0.208	0.223
S×P	5	0.156	0.108	0.191	0.399
Residual	10	–	–	–	–
Total	39	–	–	–	–

319

320 Similarly to the vegetation samples, both the L×T interaction and the location main factor were
321 significant, even if at higher P values, indicating that the mean values and the temporal patterns of the
322 abundances of benthic taxa were different among locations, but that these differences could not be ascribed
323 to an impact of the drainage.

324 The MDS plot (Fig. 3) shows that the points that represent the samples from VM and PA are clustered
325 in the lower part of the diagram and largely overlap, indicating that the benthic assemblages in the two
326 areas were rather similar in bare sediments, while on vegetation they were quite distinct. The samples from
327 SV are scattered in the upper part of the plot, fairly spaced from each other and mostly clearly separated
328 from those from VM and PA, indicating that the benthic assemblages of bare sediments of SV were not
329 only different from those of the other areas but were also more heterogeneous.

330

331

332

Fig. 3

333 According to the SIMPER analysis, the samples from SV were characterized by the lowest mean
334 abundance of Naididae and by the significant abundance of other taxa, in particular Chironomidae,
335 Cerataopognidae and Chaoboridae (Tables S5-S7 in the Supplementary Data). On the other hand, the
336 macrobenthos of bare sediments in VM and PA consisted almost exclusively of Naididae and the main
337 difference between the two locations was that these organisms were on average more abundant in PA
338 (Tables S5, S8 in the Supplementary Data).

339 The sorting of the points in the MDS plot is mostly determined by the abundance of Naididae, which
340 increases moving to the lower right corner of the plot (Fig. S3a in the Supplementary Data) and by the
341 abundance of Chironomidae, which increases moving in the opposite direction (Fig. S3b).

342 The abundance of Naididae decreased over time in all the locations, although to a different extent in
343 each of them.

344 Again, the I×P and I×T interactions were not significant for the diversity indices, while the location
345 factor was significant as main effect for all the indices and the L×P interaction was significant for H' (Table
346 2). The highest values were observed in SV, where they further increased in the after period (Fig. S4 in the
347 Supplementary Data).

348 In summary, the temporal pattern of variation of the benthic assemblages at the impact location was
349 within the range defined by the other wetlands in the area, both on vegetation and in bare sediments. The
350 structure of the benthic assemblages and their temporal patterns were related to the wetland where each
351 location was situated (SV, PA or VM) and not to the classification of the location as impact or control.
352 Consequently, it was not possible to detect any statistically significant impact of the drainage of VMS on
353 the macrobenthic fauna.

354

355 *Water chemistry*

356 Fig 4. shows the range of variation of chemical and physical properties at the five locations and at
357 three sites on the main rivers and channels in the surrounding area. Dissolved oxygen below 1 mg/l was
358 measured only once (site PAS1, October); PAN was the location with lower values on average. The
359 dissolved oxygen recorded at VMS and VMN was always relatively high, with no indications of anoxia;
360 however, the months from July to September in which anoxic events are more frequent were excluded from
361 the sampling.

362

363

364 **Fig. 4**

365

366 It was not possible to demonstrate any impact of the drainage of VMS on the water chemistry
367 variables, since the PERMANOVA analysis did not detect a significant I×P or I×T interaction (Table 3).

367

368 **Table 3** Results of the PERMANOVA test for the chemical properties of the water. *: significant (P <
 369 0.05); **: highly significant (P < 0.01)

Source of variation	Degrees of freedom	P value
I: impact vs. control	1	0.957
P: period, before vs. after	1	0.369
L: location (nested in I)	3	0.007**
T: time (nested in P)	2	0.057
I×P	1	0.643
S: site (nested in L)	5	0.010*
I×T	2	0.690
L×P	3	0.079
L×T	6	<0.001**
S×P	5	0.289
Residual	10	–
Total	39	–

370

371 The L×T interaction and the location main factor were significant, as for the macrobenthos on
 372 vegetation and in bare sediments, indicating that the mean values and the temporal patterns of the
 373 considered chemical variables were different among locations, but that these differences could not be
 374 ascribed to an impact of the draining.

375 Fig. 5 shows the ordination plot based on the discriminant function analysis, using the location as the
 376 grouping criterion. The analysis identified electrical conductivity, sulphates, volatile suspended solids and
 377 phosphates as the variables giving a significant contribution to the separation between locations. The first
 378 discriminant axis has a strong negative correlation with electrical conductivity and clearly separates SV
 379 (average conductivity around 4 mS/cm) from the other locations, characterized by conductivity below 1
 380 mS/cm. The second discriminant axis is positively correlated with volatile suspended solids, on average
 381 higher in the PA locations, and is negatively correlated with sulphates, higher in the VM locations. The
 382 samples from the two locations within Valle Mandriole, the impact location (VMS) and the control location
 383 (VMN), largely overlap. The two locations from PA, that are actually two separate ponds, are close to each
 384 other on the ordination plane, but still clearly distinct.

385
 386

387
 388

Fig. 5

389 In summary, similarly to what observed for the benthic assemblages, the values of the water chemistry
390 and their temporal patterns were related to the area where the location was situated and not to the
391 classification of the location as impact or control.

392

393 **Discussion**

394 The preservation and protection of wetlands is a goal that has been repeatedly emphasized in recent
395 years (e.g., Mitsch & Gosselink, 2000; Sievers et al., 2018). In many cases this goal is pursued with specific
396 management actions, of which the summer drainage of the southern part of Valle Mandriole is an example.
397 Assessing the consequences of these actions is fundamental to identify the most effective practices.

398 The present study did not detect any adverse effect of the drainage on the benthic macroinvertebrates,
399 suggesting that this practice is acceptable, at least as regards the investigated assemblages. On the other
400 hand, the results also suggested that the drainage did not produce any benefit on the macrobenthic fauna, at
401 least in the short term.

402 To adequately evaluate this result, it is necessary to take into consideration the characteristics of the
403 studied wetlands and of their benthic assemblages. In addition, it is important to be aware of the limitations
404 of the present study and, in general, of the problems involved in assessing the environmental impact of a
405 specific event.

406

407 *The studied wetlands and their benthic macroinvertebrate assemblages*

408 In the present study benthic macroinvertebrates were identified at family level. We acknowledge that
409 a more detailed taxonomical resolution would have been preferable and that, working at family level,
410 relevant information is lost, in particular regarding the assessment of biodiversity. This was considered an
411 acceptable compromise based on Bowman & Bailey (1997) and Mueller et al. (2013). These studies
412 analysed several independent benthic macroinvertebrate datasets collected in freshwater ecosystems, both
413 lotic and lentic. Both studies acknowledge the importance of identification to species or genus level in some
414 circumstances. However, both studies concluded that multivariate characterizations of community
415 composition do not seem to be sensitive to taxonomic resolution, at least to family level, if quantitative data
416 (abundances) are considered. In fact, in many ecological studies on the benthic macroinvertebrates of

417 shallow lentic freshwater habitats organisms are identified at the family level, including those cited in the
418 next section.

419 Even if based only on identification at family level, the present study evidenced that the
420 macroinvertebrate assemblages dwelling on vegetation and in bare sediments of Valle Mandriole (VM) and
421 Punte Alberete (PA) had low abundance and diversity in comparison to the pond in the San Vitale Pinewood
422 (SV). In particular, the macrobenthic fauna of bare sediments was almost entirely composed by Naididae.

423 A comparison with the results of Zanni (1998) shows that the taxa richness radically decreased over
424 the last decades. In particular, several families of Coleoptera (Dytiscidae, Haliplidae, Dryopidae), Odonata
425 (Lestidae, Libellulidae and Aeshnidae), and Heteroptera (Pleidae, Mesovelidae) once present in both VM
426 and PA were not sampled during the present study.

427 The low water quality in the two wetlands and in the rivers that supply them water could have played
428 a role in the decreased diversity of the benthic fauna. In this regard, both the Lamone River, which supplies
429 Punte Alberete, and the Reno River, which supplies Valle Mandriole, failed to achieve good chemical
430 status, under the European Water Framework directive in the period 2010–2012 (ARPAE, 2015).

431 In addition, the annual mean concentrations of ammoniacal nitrogen in the Reno River in the period
432 2010–2013 were in the range 0.43–0.64 mg/l, placing the river at the fifth level (worst quality class) for
433 this indicator, under the Italian regulations (Fig. 4). Even higher concentrations of ammoniacal nitrogen
434 were recorded in the Destra Reno channel. This artificial channel does not supply water to VM or PA;
435 however, since it drains the farmland area surrounding VM and PA, its water properties give some
436 indications on the surface runoff entering the two wetlands. Indeed, high concentrations of ammoniacal
437 nitrogen (up to 1.1 mg/l) were measured in several water samples collected from PA.

438 A feature of PA is the recurrence of suspended mucilage, often observed in the water also during the
439 present study, consistently with the high values of volatile suspended solids, generating strong water
440 turbidity (Fig. 4).

441 A second possible reason for the impoverishment of the benthic assemblages is the presence the
442 invasive alien crayfish *Procambarus clarkii*. This species was not recorded by Zanni (1998); on the
443 contrary, it was frequently sampled during the present study. In Europe, the first introductions of *P. clarkii*
444 from North America occurred in Spain in 1973 for aquaculture. The species was introduced in Italy in 1989,
445 for the same purpose. After escaping into freshwater bodies, it has since steadily spread across Europe due

446 to its ecological plasticity, high fecundity, rapid life cycle and dispersal capacities. *P.clarkii* has been
447 documented to heavily affect abundance and diversity of benthic invertebrate assemblages both by direct
448 predation and by modifying habitat composition, through consumption of living macrophytes and litter
449 (Souty-Grosset et al., 2016).

450 Thirty-one families were identified in the macrobenthic fauna of the pond in the San Vitale Pinewood (SV),
451 including several recorded by Zanni (1998) in PA and VM and not sampled in these wetlands during the
452 present study (Culicidae, Tipulidae, Aeshnidae, Lestidae, Libellulidae, Mesovelidae, Dytiscidae,
453 Haliplidae). Among the features of SV that might concur in determining the higher taxonomic diversity is
454 the origin of its water, which comes mainly from the local underneath aquifer. The San Vitale Pinewood is
455 established on a paleodune system constituted by 20 m thick coarse sand deposits (Amorosi et al., 1999 and
456 Greggio et al., 2018) where the coastal aquifer is phreatic and rainwater infiltrate and freshen the underneath
457 saline groundwater (Antonellini et al., 2008). If the budget between precipitation and evapotranspiration is
458 positive, freshwater lenses settle down on top of saline groundwater; where the shallow water table meets
459 low topographic surface, interdunal wetlands appear (Cozzolino et al., 2017). So, SV is not affected by the
460 sources of pollution occurring to PA and VM where water is supplied by the final stretch of Lamone and
461 Reno rivers running through a heavily anthropized lowland area. Accordingly, most of the chemical
462 properties of the water of SV such as nutrients and suspended solids, are indicative of a good state (Fig. 4).
463 On the other hand, the electrical conductivity of the water is rather high (about 4 mS/cm) compared to VM
464 and PA (less than 1 mS/cm), even if it can be considered relatively fresh for Ravenna coastal aquifer
465 (Mollema et al., 2013; Greggio et al., 2020). However, this does not seem to hinder the development of a
466 well differentiated, typically freshwater, biological community. Based on the comparison with SV, it can
467 be inferred that the richness of the benthic fauna in VM and PA was clearly below the potential of the area.
468

469 *The effect of drainage on benthic macroinvertebrates*

470 The impoverished fauna of VM could be a reason why no effects of the drainage were detected in the
471 present study: there was little potential for change due to the presence of few taxa and low abundances. The
472 benthic assemblages of both VM and PA testify the low habitat quality of the whole wetland system. The
473 draining of VMS was apparently inadequate to increase abundance and diversity of the benthic
474 macroinvertebrate assemblages, possibly because the starting point was so disadvantaged. In addition, even

475 if the environmental conditions had improved in VMS, there would have been little possibility to develop
476 a richer fauna by colonization from nearby areas. On the other hand, it could be argued that it was
477 impossible to further worsen an already compromised situation.

478 However, the absence of lasting effects on the benthic assemblages in cases of drying events has been
479 documented also in previous studies. Vander Vorste et al. (2016) applied a multisite before-after-control-
480 impact (BACI) design to quantify the effects of drying events of different durations on benthic invertebrates
481 in gravel-bed, braided rivers in southeast France and concluded that taxonomic composition, functional
482 diversity, trait richness, and trait composition showed no effects of drying events.

483 On the other hand, effects of dry periods on the macrobenthic fauna, lasting long after the water level
484 had recovered, were documented, both in cases of management actions and in cases of drought. Lindegarth
485 & Chapman (2001) tested the effect on benthic invertebrates of controlled drainage and subsequent refilling
486 in a small managed marsh in Australia. The goal of the water level manipulation was to improve the habitat
487 for water birds and an increase in abundances of benthic invertebrates was expected. Contrary to the
488 expectations, a decrease in abundance and taxa richness of benthic invertebrates, chironomids in particular,
489 was evidenced.

490 Bedford & Powell (2005) monitored the invertebrate fauna associated with the litter of *Phragmites*
491 *australis* in a managed reedbed in northwest England by bi-monthly sampling over 6 years. Management
492 involved an annual cycle of summer draw-down and winter reflooding. Based on comparisons with data
493 from previous studies on permanently flooded reedbeds, these Authors concluded that the manipulation of
494 water levels caused low invertebrate abundances and prevented the development of diverse communities.

495 Bertoncin et al. (2019) assessed the effect of a prolonged drought on the benthic invertebrates of a
496 small (60×15 m) pond located within an island of the upper Paraná River, Brazil. The benthic assemblages
497 became more homogeneous after the drought, in the sense that the differences between the three sites within
498 the pond decreased. However, both diversity and abundance increased within each site, in contrast to the
499 results of Lindegarth & Chapman (2001).

500 Unambiguously measuring the impact of a specific event in a natural ecosystem is challenging (see
501 next section) and the above cited studies, including ours, have limitations. However, taken together, they
502 indicate that the effects of a management strategy based on completely drying and then reflooding a wetland
503 area are site specific. As for benthic invertebrates both an increase and a decrease in abundance and diversity

504 is possible, as well as it is possible that no substantial changes occur. In our view, this depends on the
505 balance between the actual improvement of the oxygenation conditions and the disturbance caused by the
506 temporary cancellation of the aquatic habitat. The relative importance of these two drivers is influenced by
507 climate, morphology, hydrology and water quality, along with the ecological community present at the site
508 and the availability of refuges or nearby habitats from which recolonization can occur.

509

510 *Impact assessment*

511 Evaluating the outcomes of management actions is an essential but often neglected aspect of
512 environmental management (Walsh et al., 2012). The effect of a specific action can only be evaluated within
513 an experimental framework that should be planned before the action takes place (Lindgarth & Chapman,
514 2001). Even when this condition is met, achieving a quantitative and unambiguous assessment is
515 challenging.

516 The major problem in assessing the impact of a specific event in a natural ecosystem is that there is
517 usually only one potentially affected location (Stewart-Oaten et al., 1986; Underwood, 1992). This lack of
518 replication makes it difficult to separate the anthropogenic effects from natural variability in space and time.

519 In an attempt to overcome this problem, the before-after-control-impact (BACI) sampling design was
520 proposed. The BACI approach dictates that a single impact location, putatively affected by the event of
521 interest, is compared with a single control location, surely unaffected by the same event. Both the impact
522 and the control locations are sampled at multiple times both before and after the event has occurred
523 (Bernstein & Zalinsky, 1983; Stewart-Oaten et al., 1986).

524 The shortcomings of this approach were highlighted by Underwood (1992, 1994), that proposed the
525 beyond before-after-control-impact (beyond BACI) sampling design, where the single impact location is
526 compared with multiple control locations, the same design we adopted in the present study. While the
527 approach was originally devised to deal with human interventions expected to cause adverse effects on the
528 ecosystems, it is equally suited to assess the effectiveness of actions aimed at some improvement.

529 Some authors (notably Stewart-Oaten & Bence, 2001; Stewart-Oaten, 2008; Paul, 2011), strongly
530 criticized the Beyond BACI and other “design based” approaches, which are founded on sampling design
531 and ANOVA or related statistical methods. In open contrast with Underwood (1992, 1994), they maintain
532 that multiple control locations are not indispensable to properly measure the ecological effects of an event.

533 These authors advocate “model based” approaches, i.e., the use of predictive models, parametrized using
534 data collected from the putatively impacted location before the event of interest occurs. The ecological
535 impact should then be measured as the difference between the predictions of the model and the actual
536 observations made after the event has occurred.

537 One of the fundamental tenets stated by Underwood (1992, 1996) is that impact assessment studies
538 should be treated as experiments. This is the most criticized aspect of the approach. As Stewart-Oaten &
539 Bence (2001) and Stewart-Oaten (2008) pointed out, statistical inferences in experiment are based on the
540 assumption that the experimental units are randomly chosen from the same large population or, at least,
541 randomly assigned to the treatments. Conversely, in an impact assessment study, the impact location is not
542 chosen at random, either from a population or from the sites used in the study.

543 We acknowledge that the theoretical framework of the beyond BACI approach may have weaknesses,
544 if considered from a formal statistical standpoint. However, we adopted this approach in the present study,
545 because it is our opinion that its fundamental rationale is sound: an ecological effect is detected if the
546 temporal pattern of variation observed at the impact location is outside the range defined by a set of control
547 locations, which are unaffected by the event of interest but otherwise comparable with the impact location.
548 The use of more than one control location takes into account that different unaffected locations may exhibit
549 different temporal patterns. If only one control location is sampled, differences that could exist even if the
550 impact location were actually unaffected could be interpreted as an ecological effect of the event of interest.

551 On the other hand, a model based impact assessment requires a validated model that can predict how
552 the value of the response variables would change over time at the impact location if this were unaffected
553 by the planned intervention. Complete confidence in the model is required since any deviation from its
554 predictions would be interpreted as an actual impact. A long data series, collected before the planned
555 disturbance, is essential to develop and validate the model. We did not follow this approach as it appeared
556 unrealistic to develop a sufficiently reliable model for the abundances of benthic invertebrates and the
557 values of the chemical properties of Valle Mandriole.

558 While we think that, under the circumstances, there were no better alternatives to evaluate the effect
559 of the summer drainage of VM on the benthic invertebrate fauna, the specific application of the Beyond
560 BACI approach carried out in the present study does have some important limitations.

561 The real situation hardly fitted to the reference model of a population of locations from which the
562 impact locations and the control locations were independently and randomly selected. While all the sampled
563 locations are freshwater wetlands, the benthic assemblages and the chemical properties of Valle Mandriole
564 (impact location and one control location), of Punte Alberete, (two control locations) and of the pine wood
565 pond were quite distinct.

566 According to Underwood (1994) in the application of the beyond BACI approach, there is no need to
567 attempt to choose places with identical characteristics or abundances of the investigated populations. The
568 set of locations chosen to serve as controls must simply represent the range of habitats like the one that
569 might be affected (the Impact location). Obviously, the control locations must be a representative sample
570 of places of the same general habitat as the impact location.

571 However, what can be considered the same general habitat, is rather subjective. Our strategy was to
572 sample the highest possible number of locations and to represent the whole range of freshwater wetlands in
573 the area. Since the control locations define the norm against which the temporal pattern observed in the
574 impact location is evaluated, our idea was that the norm should have some degree of generality. In our
575 opinion this is consistent with the rationale of the Beyond BACI approach.

576 The opposite strategy would have been to favour the homogeneity between the impact and the control
577 locations. It could be argued that the differences between SV and the other locations are too large to
578 represent the same general habitat. Admittedly, the benthic assemblages of SV are clearly distinct from
579 those of VM and PA. However, regarding the temporal patterns (how the structure of the benthic
580 assemblages changes over time) the difference between SV and the other locations do not seem to be larger
581 than the differences among the other locations, at least for the fauna sampled from vegetation (Fig 2, Fig.
582 S2 in the Supplementary Data). In any case, omitting the SV from the statistical analyses does not cause
583 major changes in the results and the interaction terms relevant to the detection of the impact are still not
584 significant (data not shown).

585 In fact, while PA and VM both had an impoverished benthic fauna, their benthic assemblages and the
586 properties of their water were still clearly distinct. Adopting homogeneity with the impact location as the
587 sole criterion to select the control locations, would imply that VMN is the only legitimate control location
588 for VMS, and would make it impossible to apply a beyond BACI approach.

589 Arguably, in the present study, the range of temporal patterns observed at the control locations, which
590 defined the norm, was so wide that possibly prevented the detection of any deviation of the impact location
591 from the norm itself, except for extreme deviations.

592 Indeed, the most important shortcoming of the present application of the Beyond BACI approach is
593 probably that all the samples were collected during one single year (2013) and “before” and “after” are
594 referred to a single drainage of VMN. Since the drainage was performed on summer every year, starting
595 from 2011, sampling over a longer time span, including a period before the first occurrence of the summer
596 drainage would have been more relevant to the problem considered. Unfortunately, the local authority that
597 planned and funded the intervention, apparently did not deem important to plan a study to properly assess
598 its effectiveness.

599

600 **Conclusions**

601 The present study, even taking account of its limitations, did not detect any statistically significant
602 effect on the benthic macroinvertebrates, suggesting that this practice is acceptable, at least in regard to the
603 investigated assemblages. On the other hand, the results also suggest that the drainage does not produce
604 any benefit on the macrozoobenthic fauna, at least in the short term. The effects of a management strategy
605 based on draining completely dry and then reflooding a wetland area appear to be site specific.

606 Our study, by comparison to previous data, evidenced a severe impoverishment of the benthic fauna
607 of Valle Mandriole and Punte Alberete, as can be inferred by comparison to the San Vitale pinewood pond
608 which, despite being of lesser extension, sustains a much more diverse fauna. This also highlights how
609 small water bodies can preserve the diversity of aquatic species of an area, even when larger water bodies
610 are degraded, and that they are potentially important pools of species that could recolonize larger habitats,
611 once the environmental quality of the latter is restored. Maintaining, protecting, restoring and even creating
612 small ponds may play an important role in nature conservation.

613

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764

765 **Captions for figures**

766

767 **Fig.1** Study area and sampling sites

768

769 **Fig. 2** Non-metric multidimensional scaling (MDS) plot of 10 sites sampled four times, two times before
770 (May, June) and two after (October, November) the drainage of the southern part of Valle Mandriole
771 (VMS). The MDS is based on square root transformed abundances of families of benthic
772 macroinvertebrates sampled from vegetation and Bray-Curtis distance

773

774 **Fig. 3** Non-metric multidimensional scaling (MDS) plot of 10 sites sampled in four times, two times before
775 (May, June) and two after (October, November) the drainage of the southern part of Valle Mandriole
776 (VMS). The MDS is based on square root transformed abundances of families of benthic
777 macroinvertebrates sampled from bare sediments and Bray-Curtis distance

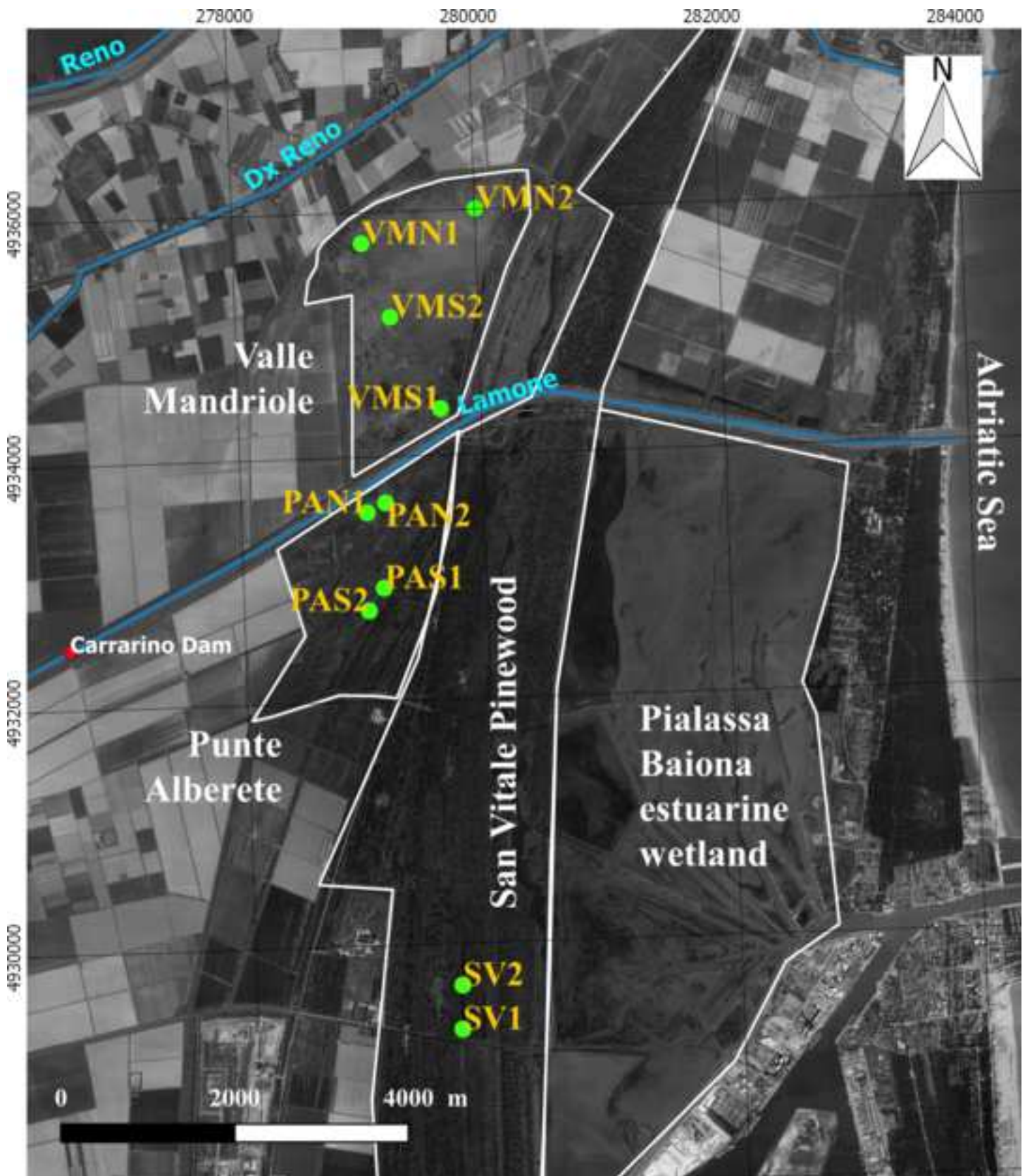
778

779 **Fig. 4** Range of variation of water column properties in the five wetland locations and three related
780 riverine locations in year 2013. The wetland data are from the present study, the riverine data were
781 collected by the regional environmental agency (available at:
782 https://www.arpae.it/dettaglio_documento.asp?id=6312&idlivello=2020). VMS: Valle Mandriole South
783 (impact location); VMN: VMN: Valle Mandriole North; PAN, PAS: Punte Alberete north and south; SV:
784 San Vitale. Riverine locations: Lamone, Destra Reno, Reno. Boxes: 2nd and 3rd quartile; the whiskers
785 represent the whole range without outliers. Outliers are defined as value outside 1.5 times the interquartile
786 range above the 3rd quartile and below the 2nd quartile

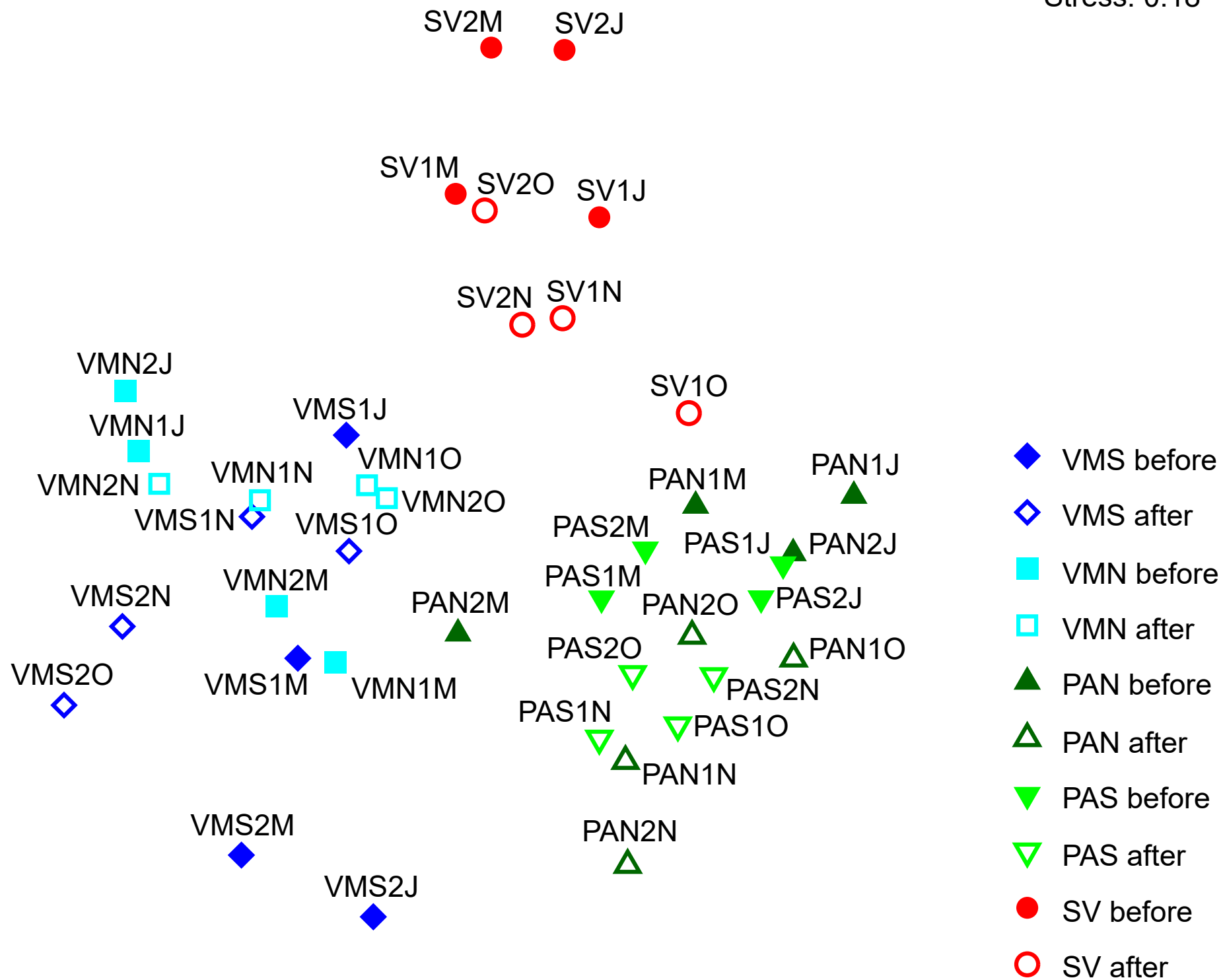
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788 **Fig. 5** Discriminant function analysis ordination plot using the location as the grouping criterion and the
789 water chemistry variables as predictors

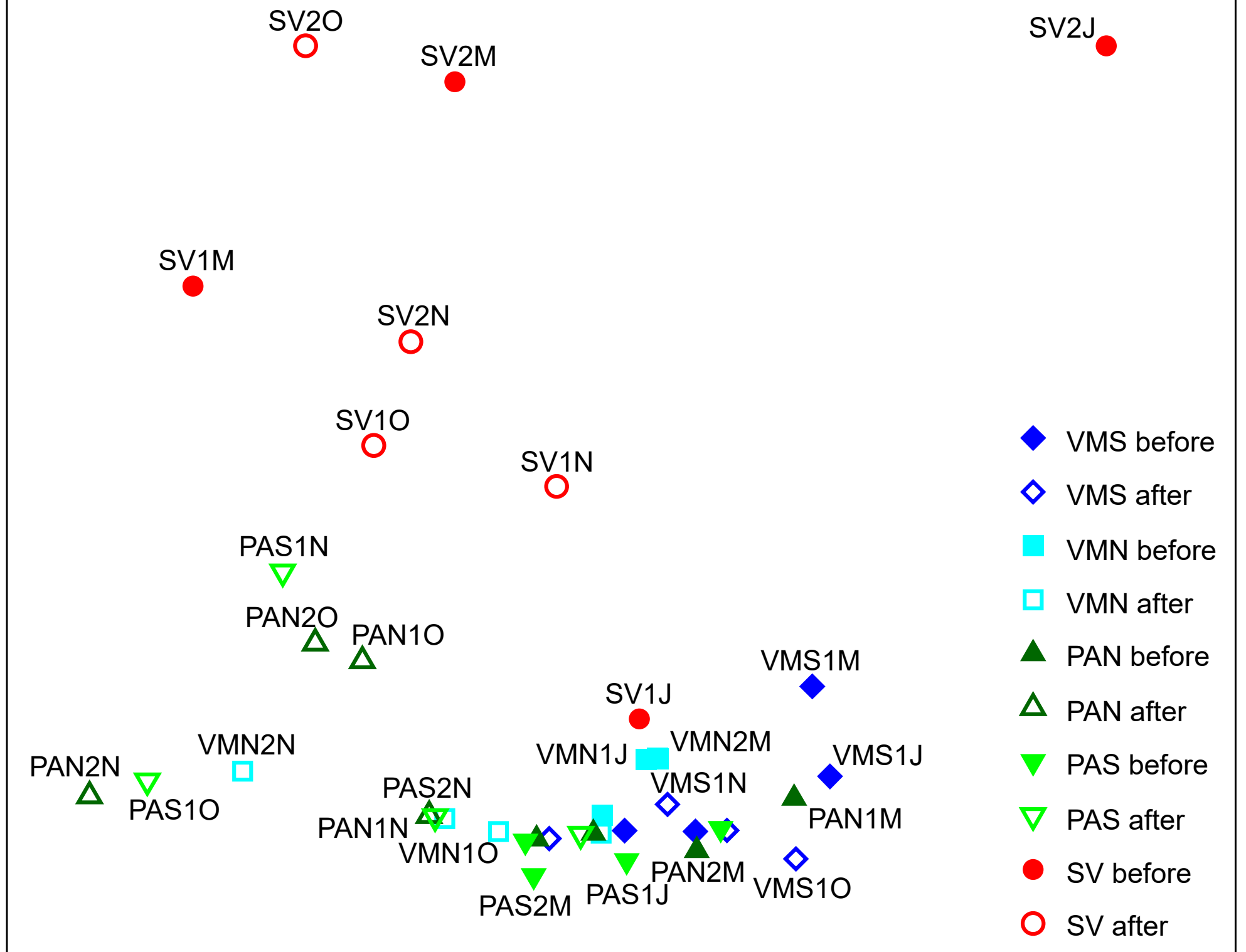
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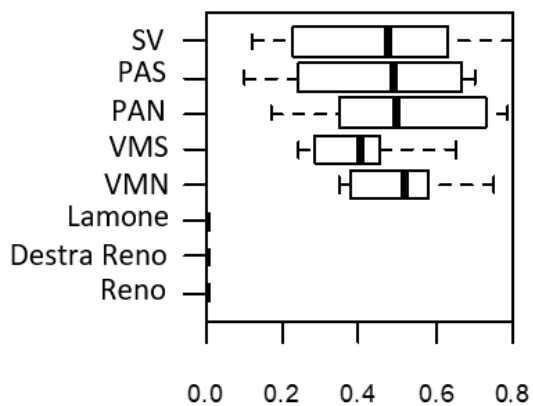
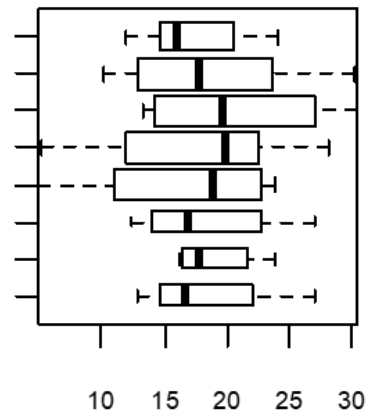
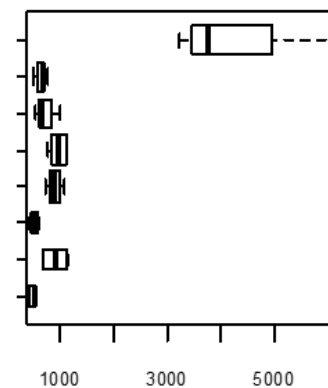
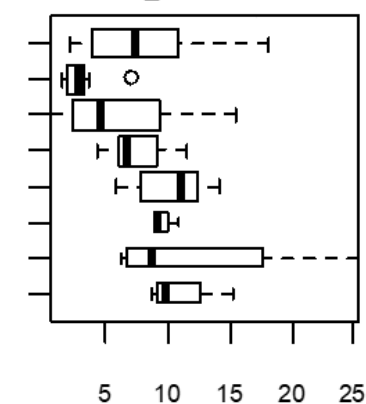
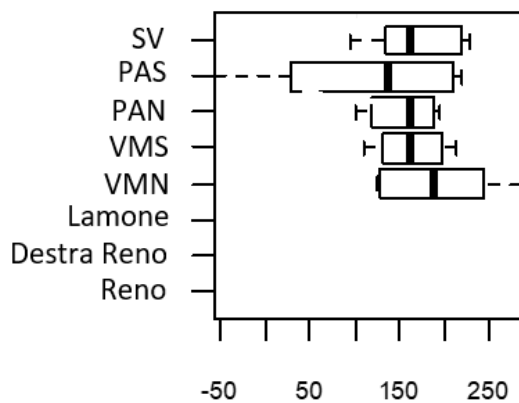
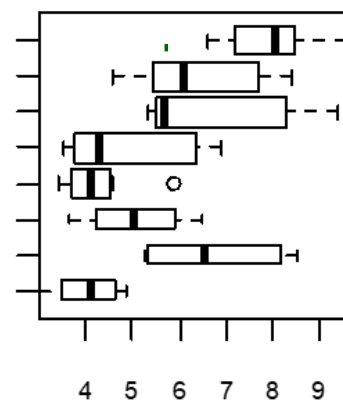
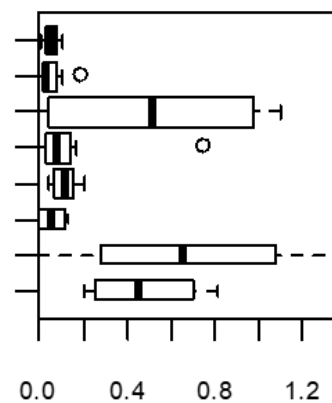
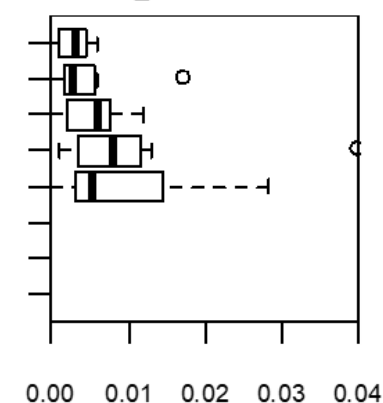
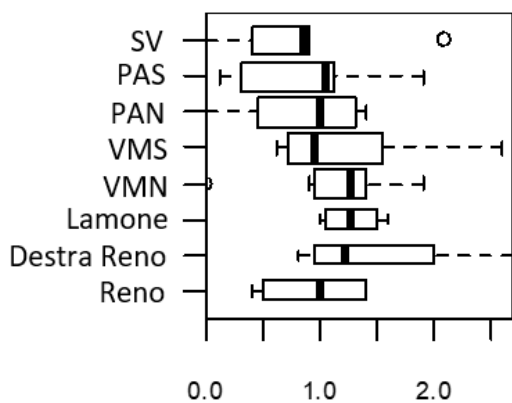
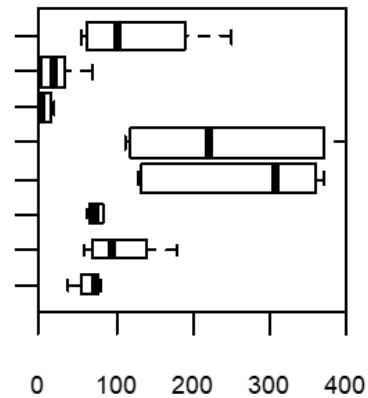
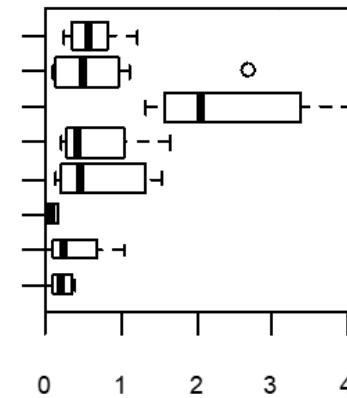


Stress: 0.18



Stress: 0.06



Water depth [m]**T [°C]****Elect. cond. [$\mu\text{S}/\text{cm}$]****O₂ [mg/l]****Eh [mV]****Alkalinity [meq/l]****NH₄⁺-N [mg/l]****NO₂⁻-N [mg/l]****NO₃⁻-N [mg/l]****SO₄²⁻-S [mg/l]****PO₄³⁻-P [mg/l]****Tot. suspended solids [g/l]**