

1 **Title:** Magnetic microparticles as a new tool for lake restoration: a microcosm experi-
2 ment for evaluating the impact on Phosphorus fluxes and sedimentary Phosphorus pools

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20 restoration

21 **Abstract**

22 In the last decades, magnetic particles (MPs) as adsorbents have gained special attention
23 due to their high adsorption capacity and the possibility of recovering them by applying
24 a magnetic separation gradient. For the first time MPs have been tested as P adsorbents
25 in a microcosm experiment in a context of lake restoration. MPs were added to sediment
26 cores from a hypertrophic lake, at MPs:P_{Mobile} molar ratio of 285:1 and 560:1 under
27 both, oxic and anoxic conditions. We have found that, under unfavorable conditions
28 (anoxic), MPs are able to completely reduce P release rate from the sediment to the
29 overlying water and even to reduce sedimentary P_{Mobile} concentration (a 22-25% reduc-
30 tion within 0-4 cm depth compared to controls). Under oxic conditions, the addition of
31 MPs cause no effect on P fluxes across the sediment and water interface since the lake
32 sediment is naturally rich in iron oxides; however a reduction in sedimentary P_{Mobile}
33 concentration (12-16 % reduction in 0-10 cm depth) have been measured , thus contrib-
34 uting to a reduction in long-term P efflux.

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43 **1. Introduction**

44 Eutrophication is subject of concern to managers since it is the main problem that im-
45 pairs water quality of 30-40 % of water resources of all over the world (Hupfer and Hilt,
46 2008; Søndergaard et al., 2007). The EU Water Framework Directive (Ec, 2000) estab-
47 lishes that all Member States must implement measures to achieve a “good ecological
48 and chemical status” in water bodies by 2015. Since phosphorus (P) is the nutrient that
49 often limits primary production in freshwater, it is a basic requirement to reduce its con-
50 centration in order to combat eutrophication and meet the requirements set by law
51 (Schindler et al., 2008; Carpenter and Lathrop, 2008). Therefore, the first measure to
52 consider in lake restoration should be the reduction of P external loading (Cooke et al.,
53 2005; Reitzel et al., 2003). However, a successful reduction of external P loading is not
54 always practical or economically feasible due to the difficulty in controlling external P
55 inputs dominated by non point sources and due to the drastic reduction in external P
56 loading required to accomplish the desired outcome (Marsden, 1989; Istvanovics and
57 Somlyódy, 2001; Deppe and Benndorf, 2002). Moreover, biological resilience and P
58 release from lake sediments (P internal loading) under certain conditions usually delay
59 ecosystem recovery after a reduction in external P loading (Jeppensen et al., 1991;
60 Søndergaard et al., 2003). In such situations, it is recommended, apart from reducing P
61 external loading, the application of in-lake techniques to decrease internal P loading and
62 accelerate lake water improvement (Lürling and Oosterhout, 2013); Cooke et al., 2005).
63 Some of these techniques are based on preventing P release from sediment by
64 hypolimnetic aeration, nitrate (NO_3^-) addition or sediment capping (Hupfer and Hilt,
65 2008). Some other in-lake techniques are focused on enhancing P adsorption capacity of
66 sediment by adding in-lake P- binding alum (Al), iron (Fe) or Calcium (Ca) salts, solid
67 phase P-sorbing products (PSPs) coming from industrial waste and also Phoslock® that

68 aims to remove P from water column by P inactivation in the sediment (Cooke et al.,
69 2005; Deppe and Benndorf, 2002; Lewandowski et al., 2003; Meis et al., 2012; Spears
70 et al., 2013b; Yamada et al., 2012). In spite of its widespread application and success
71 achieved, the main drawback about salt addition is that, although inactivated, P remains
72 in sediment and may be released to water column under changing physicochemical and
73 biological conditions such as temperature, pH, redox potential, biological activity or
74 resuspension (Jensen and Andersen, 1992; Søndergaard et al., 1992; Rydin and Welch,
75 1998; Egemose et al., 2009). Although, in general, Al is preferred to Fe (Boers et al.,
76 1992), since it keeps invulnerable to shifts in redox potential at sediment-water inter-
77 face, Al application has also some limitations. For instance, Al addition is limited to a
78 pH range between 5.5 and 8 to assure the absence of toxicity and the efficiency in P
79 removal (Lewandowski et al., 2003; Reitzel et al., 2013). As a result, Al treatment is not
80 recommended in low alkaline lakes where the limited buffering capacity is not enough
81 to prevent a decline in pH, and in these cases is necessary to add an alkaline buffer
82 along with the acidic Al salt (Reitzel et al., 2013). Moreover, Al-rich sediment layers
83 reduce sediment consolidation degree, which hinders the establishment of macrophytes
84 and favors resuspension events and temporal P release (Egemose et al., 2010). Even
85 more, it is important to consider that the effectiveness of these methods is also time-
86 limited since P adsorption capacity of Al floc is notably reduced by aging (Berkowitz et
87 al., 2006; de Vicente et al., 2008a). In particular, P maximum adsorption capacity is
88 reduced up to 76% when aging Al floc for 3 months in the absence of PO_4^{3-} compared to
89 fresh Al floc (de Vicente et al., 2008a). This fact gives rise to a continuous demand for
90 salts addition. Despite of the well known dependence of Fe salts on redox conditions,
91 recent studies have suggested that the relevance of vivianite formation
92 $[\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}]$ for preventing anaerobic P release is higher than previously believed

93 (Kleeberg et al., 2013; Rothe et al., 2014). In this sense, Rothe et al. (2014) found that
94 vivianite formation continues to be triggered, in Lake Groß –Glienicke, by the artificial
95 Fe amendment more than 20 years ago, significantly contributing to P retention in sur-
96 face sediments. To counteract the limitations of using Fe and Al salts, in the mid-1990s
97 an alternative adsorbent, a lanthanum modified bentonite clay, Phoslock®, was invented
98 for trapping P in aqueous solutions (Robb et al., 2003; Ross et al., 2008; Spears et al.,
99 2013a). However, Phoslock® has some drawbacks since P binding capacity declines
100 when pH is above 8.1 and when increasing alkalinity, having some limitations in
101 hardwater systems (Ross et al., 2008; Reitzel et al., 2013). Moreover, chemical interfer-
102 ences with humic substances which lastly affect P removal efficiency have been found
103 (Lürling and Faassen, 2012; Reitzel et al., 2013; Lürling et al., 2014). In addition, the
104 high Phoslock®: P mass ratio necessary for retaining P, which has been identified as
105 higher than 100:1 (Reitzel et al., 2013), and its high price make this technique specially
106 unviable from an economic point of view in some circumstances.

107 As a result, new methods are required for lake restoration in order to overcome draw-
108 backs of current practices. A large background of laboratory experiments has shown
109 that magnetic particles (MPs) are good and cost-effective pollutant adsorbents (Tang
110 and Lo, 2013). Among the main advantages for using these particles are their high sur-
111 face area, which is responsible for a high adsorption capacity; a fast adsorption kinetic
112 and their magnetism properties (Huber, 2005; Tang and Lo, 2013). The last characteris-
113 tic of MPs implies that contaminant-loaded MPs can be efficiently removed from solu-
114 tion by applying a magnetic separation gradient, minimizing alteration in water quality
115 and enabling their reuse in subsequent adsorption processes (de Vicente et al., 2010b;
116 Funes et al., 2014). Although nano zero valent Fe particles (nZVI) have been applied in
117 permeable reactive barriers as contaminant reducing agents at *in situ* remediation pro-

118 jects, up to date, no full-scale application of MPs to remove pollutants from water bo-
119 dies by applying magnetic separation techniques has been reported (Tang and Lo,
120 2013).

121 In the case of P removal from aqueous solutions, it has been previously tested the con-
122 venience of using MPs as P adsorbents in laboratory scale under both batch mode (de
123 Vicente et al., 2010b) and flow conditions (Merino-Martos et al., 2011). Apart from
124 being considered as efficient P adsorbents (P uptake efficiency is always higher than
125 80% whatever pH value from 6 to 9), MPs present a fast adsorption kinetic (less than 1
126 hour) and keep a high P removal efficiency when reusing them in subsequent removal
127 processes (de Vicente et al., 2010b; Merino Martos et al., 2014).

128 In this context, the aim of this study was to assess the effect of adding MPs on P fluxes
129 across the sediment-water interface and on the sedimentary mobile P concentration. To
130 get these purposes, a microcosm experiment using sediment cores and lake water col-
131 lected from a hypereutrophic wetland (Honda lake, Southeast of Spain) was run under
132 both oxic and anoxic conditions. Similarly to Reitzel et al. (2003), we proposed to add
133 MPs relative to sediment mobile P pool (P_{Mobile}). Specifically, we considered the con-
134 trols (no MPs addition) and treatments with two different MPs: P_{Mobile} molar ratios
135 (285:1 and 560:1). Microcosm experiments tries to mimic more realistic conditions than
136 the previously performed laboratory experiments (de Vicente et al., 2010b) and alt-
137 hough this type of design obviously lacks the complexity of the whole ecosystem
138 (Schindler, 1998), it may constitute a previous and necessary step for a future whole-lake
139 application.

140 **2. Materials and Methods**

141 ***2.1. Study site***

142 Honda lake is a shallow (surface area = 9 ha, mean depth = 1.3m and maximum depth =
143 3.2 m), hardwater (alkalinity 3.17–6.21 meq L⁻¹), brackish water (3.09 mS cm⁻¹) and hy-
144 pertrophic coastal wetland located in Albuferas of Adra lacustrine complex (southeast
145 of Spain). Despite of being designated as Natural Reserve (1989) and Ramsar Site
146 (1994), among others protection status, its high external and internal P loads have im-
147 paired water quality as a result of eutrophication processes (de Vicente et al., 2003). The
148 external P loading (1.73 g P m⁻² year⁻¹), mostly constituted by diffuse inputs (about
149 90%) that enter to the wetland by intermittent episodes of run-off, is enhanced by nutri-
150 ents coming from fertilizers used in intensive agricultural practices of greenhouses,
151 which are surrounding the limits of the wetland (de Vicente and Cruz-Pizarro, 2003; de
152 Vicente et al., 2003). In relation to the relevance of internal P load in this lake, its shal-
153 lowness and the presence of intense winds cause frequent resuspension events which are
154 lastly responsible for bringing back sediment associated particulate and dissolved nutri-
155 ents into the water column where then can be released (de Vicente et al., 2010a). There-
156 fore, the high water turbidity characterizing this ecosystem avoids the establishment of
157 aquatic submerged macrophytes, leading to sediment destabilization and dominance of
158 phytoplankton community (de Vicente et al., 2010a). Apart from sediment resuspension,
159 the release of nutrients across sediment-water interface may be favored by anoxic condi-
160 tions at hypolimnion during the weak thermocline established during summer time peri-
161 od (de Vicente et al., 2003). As a result of the inherent wetland characteristics (shallow-
162 ness, frequent resuspension events and hypolimnetic anoxia) the use of P binding salts
163 commonly used for in-lake restoration techniques (Al or Fe salts) may be not recom-
164 mended since P is likely to be released during resuspension or under shifting physico-
165 chemical conditions. Instead, new techniques intended to restore shallow and polymictic
166 lakes may be focused on increasing P export from the system. In this context, the use of

167 MPs as P adsorbents and its subsequent removal from the ecosystem seems to be a suit-
168 able restoration option.

169 **2.2 Materials**

170 Micronized Fe MPs were obtained from BASF (Germany) and used as P adsorbent.
171 According to the manufacturer, MPs are chemically characterized as follows: 97.5% Fe,
172 0.9% carbon, 0.5% oxygen, and 0.9% nitrogen. Previous characterization has shown
173 that MPs are spherical in shape and relatively polydisperse in size with a mean particle
174 diameter of 800 ± 10 nm (de Vicente et al., 2010b). This adsorbent is defined as a soft
175 magnetic material since it has a negligible coercitive field and remnant magnetization;
176 in other words, magnetization becomes zero when removing magnetic field (Funes et
177 al., 2014). MPs present a thin oxidized surface layer with amphoteric functional groups
178 which determines surface charge as a function of pH (Hunter, 1993). Previous data have
179 revealed that MPs surface is negatively charge in the studied pH range (6-9) and the
180 magnitude of charge increases as pH value increases (de Vicente et al., 2010b). These
181 authors found that there is a slight decrease in P removal efficiency with increasing pH,
182 from pH 5 to pH 9, which could be qualitatively explained through electrophoretic mo-
183 bility curves. These results suggest that the adsorption mechanism is not purely electro-
184 static, but also specific adsorption is involved since negatively charged MPs still do
185 adsorb a very significant amount of P.

186 **2.3 Sequential extraction procedure of sedimentary P pools**

187 In July 2013, 3 large cores ($\Theta = 5.4$ cm; $h = 50$ cm) were collected at 2 m depth in Hon-
188 da lake. In situ, sediment cores were sectioned at intervals of 2 cm up to 10 cm obtain-
189 ing five different sediment slices. The three samples of each depth were pooled and ho-
190 mogenized before analysis. A sequential chemical extraction procedure according to
191 (Paludan and Jensen, 1995) was used in order to quantify the concentration of P_{Mobile}

192 pool in the upper 10 cm of sediment as well as the relative importance of the other P
193 pools in the sediment. This sequential extraction scheme discriminates sediment P into
194 seven P fractions. The first step of extraction consisted of determining pore water inor-
195 ganic P (o-P) and loosely adsorbed o-P ($P_{\rightarrow\text{Water}}$) by shaking ≈ 1.2 grams of wet sedi-
196 ment with distilled water. Secondly, a bicarbonate dithionite solution (BD reagent: 0.11
197 M NaHCO_3 , 0.11 M $\text{Na}_2\text{S}_2\text{O}_4$) was added to the sediment in order to extract o-P bound
198 to reducible metals such as Fe and Mn($P_{\rightarrow\text{BD}}$). In step 3, a 0.1 M NaOH solution was
199 applied to release o-P adsorbed to Al oxides, clay minerals and humic acids ($P_{\rightarrow\text{NaOH}}$).
200 In this step, P bound to humic acids ($P_{\rightarrow\text{NaOH, Humic}}$) was precipitated and separated from
201 solution by adding 1.5 ml of 2 M H_2SO_4 . Magnesium and calcium bound o-P was ex-
202 tracted by shaking the sediment with 0.5 M HCl ($P_{\rightarrow\text{HCl}}$). Finally, the remaining sedi-
203 ment was combusted at 520 °C for 5.5 h in order to later obtain refractory organic P by
204 acid hot digestion at 120 °C with 1 M HCl ($P_{\rightarrow\text{HCl, Res}}$). Additionally, a wet oxidation
205 process with 0.18 M $\text{K}_2\text{S}_2\text{O}_8$ was applied to the 3 first steps ($P_{\rightarrow\text{Water}}$, $P_{\rightarrow\text{BD}}$ and $P_{\rightarrow\text{NaOH}}$)
206 in order to obtain Total P concentrations (Tot-P) in each fraction. Non-reactive P (NRP)
207 was calculated as the difference between Tot-P and o-P in each of these fractions. As a
208 result, organic P labile ($\text{Org-P}_{\text{Labile}}$) was calculated as the sum of NRP of the three first
209 fractions and represents more labile organic P compounds (Hansen et al., 2003).
210 Among all P pools that define total P in sediment, only P_{Mobile} is expected to contribute
211 to internal P loading (Boström et al., 1988; Rydin, 2000). P_{Mobile} was estimated as the
212 sum of $P_{\rightarrow\text{Water}}$, $P_{\rightarrow\text{BD}}$ and $\text{Org-P}_{\text{Labile}}$ in the upper 10 cm of sediment (Rydin, 2000; Han-
213 sen et al., 2003; Reitzel et al., 2003). Total sediment P ($\text{Tot-P}_{\text{sed}}$) and total sediment Fe
214 ($\text{Tot-Fe}_{\text{sed}}$) were determined on parallel by ashing the sediment samples (520°C, 5.5 h)
215 followed by an acid hot digestion with 1 M HCl (120 °C, 1 h). In order to minimize the

216 intrinsic error of extraction process, P pools data were normalized according to Tot-P_{sed},
217 which is supposed to be more accurate.

218 Soluble reactive P (SRP) was determined spectrophotometrically using the molybdenum
219 blue method proposed by Murphy and Riley (1962). In the same extractants for P meas-
220 urements, Fe was determined following the spectrophotometric ferrozine method pro-
221 posed by Gibbs (1979). In particular, the next fractions were distinguished: Fe_{→Water},
222 Fe_{→BD}, Fe_{→NaOH}, and Fe_{→HCl}.

223 Finally, sediment was also analyzed for dry weight (DW, %) at 105°C for 24 h and loss
224 on ignition (LOI, %) at 520°C for 5.5 h.

225 ***2.4 Microcosms experiment with sediment cores***

226 In July 2013, 18 small cores (inner diameter = 5.4 cm, height = 25 cm) were taken at 2
227 m depth in Honda lake. Sediment height was adjusted to give 10 cm of the overlying
228 water in all cores. Before running the experiment, and similarly to Rydin and Welch
229 (1999) and Reitzel et al. (2003), we first measured sedimentary P_{Mobile} in three large
230 cores and the mass of adsorbent required was estimated as a function of P_{Mobile} pool.
231 Considering that the maximum P adsorption capacity by MPs (under batch conditions)
232 was 18.83 mg P g⁻¹ Fe (de Vicente et al., 2010b) and that the mass of sedimentary
233 P_{Mobile} in the upper 10 cm of the sediment was 19.51 mg, we obtained that 1.04 g MPs
234 were needed to be added to the cores. As a result, we decided to consider two different
235 treatments, one adding 10 g (T₁) and another adding 20 g (T₂) to check the performance
236 of MPs in excess in order to counteract possible chemical interferences as previous
237 studies have found (de Vicente et al., 2011; Merino-Martos et al., 2015), giving a final
238 MPs: P_{Mobile} molar ratio of 285:1 and 560:1, respectively.

239 During the experiment, cores were kept in darkness and at room temperature (23 ± 2°C)
240 for 24 h. In total, six different treatments, which were run in triplicates, were consid-

241 ered. On the one hand, three treatments were bubbled with oxygen (O₂) (oxic treat-
242 ments): one of them was not amended with MPs, being considered as control (O-
243 Control), while the other two treatments were enriched with 10 g (O-T₁) and 20 g (O-
244 T₂) of MPs. On the other hand, the other three treatments were incubated under anoxic
245 conditions and the same procedure as mentioned before was applied: no addition of
246 MPs (A-Control), addition of 10 g (A-T₁) and addition of 20 g (A-T₂) of MPs. Sediment
247 cores incubated under oxic conditions were continuously bubbled with O₂ during the
248 whole experiment. Anoxic conditions were promoted by adding, at the beginning of the
249 experiment, BD reagent to reach a final concentration of 4 mg L⁻¹, which was previous-
250 ly tested as the right concentration for assuring anoxic conditions. Thus, O₂ concentra-
251 tion was always higher than 4 mg L⁻¹ in all oxic cores and lower than 1 mg L⁻¹ in all
252 anoxic ones during the whole experiment. When MPs were added (T₁ and T₂), they
253 were homogeneously distributed over the entire surface of sediment cores. After 24 h of
254 incubation, MPs were removed from sediment cores by applying a magnetic field gradi-
255 ent exerted by a permanent magnet (volume = 25.6 cm³; NB032, Aiman GZ, Spain).
256 Removal process of MPs was carried out by immersing the magnet twice for 15 seconds
257 in the cores. Water displaced by magnet immersion was considered in calculations. Ini-
258 tial and final (both before and after removing MPs) P concentrations were determined
259 following the above mentioned method (Murphy and Riley, 1962). P efflux from sedi-
260 ment to water column was finally measured as the change in P concentration in overly-
261 ing water during 24h of incubation time. Initial and final (after removing MPs) Fe con-
262 centrations were determined for the same period of incubation in order to see the effect
263 of MPs on Fe concentrations in water column. In addition, pH and O₂ concentration
264 (mg L⁻¹) were measured during the experiment using a VWR Symphony
265 multiparametric sensor (VWR International Eurolab S.L.; Spain).

266 Finally, and in order to assess the effect of adding MPs on changes in sedimentary Fe
267 and P pools (specifically on P_{Mobile} concentration) a sequential extraction procedure de-
268 scribed in section 2.3 was carried out in sediment cores from all treatments at the end of
269 the incubation time. In addition, P retention capacity of the sediment in was estimated
270 by the molar ratio between $\text{Fe}_{\rightarrow\text{BD}}$ and $\text{P}_{\rightarrow\text{BD}}$ in the upper 10 cm in the initial fractiona-
271 tion (Jensen et al., 1992).

272 ***2.5 Statistical analysis***

273 All data reported in this work have been obtained in triplicates. In order to test signifi-
274 cant differences in P fluxes and Fe concentrations in water column as a result of MPs
275 addition, a comparison between control and treatments was carried out using one-way
276 ANOVA and then Least Significant Difference (LSD) post-hoc test. Differences in ini-
277 tial and final Fe concentrations of each treatment and control were tested by applying
278 paired t-test. Significant differences were accepted when $p < 0.05$.

279 Shapiro-Wilk test and Levene's test were performed to check normality and homogenei-
280 ty of variances, respectively. In order to satisfy normality assumptions, data were log-
281 transformed before performing statistical analysis. Statistical analysis was done using
282 SPSS software.

283 **3. Results**

284 ***3.1 Effect of MPs addition on P fluxes across the sediment - water interface***

285 P exchange rates across the sediment-water interface were estimated for two different
286 situations after finishing the incubation period: a) when MPs were still in the sediment;
287 b) after removing MPs from the cores by using magnetic separation. P fluxes across the
288 sediment and water interface are shown in Fig.1a and Fig.1b, respectively. When MPs
289 were still in sediment, all treatments amended with MPs evidenced a net P uptake by the
290 sediment in both, oxic and anoxic conditions (Fig. 1a). In particular, P exchanges rates

291 were -1.66 ± 0.34 and $-1.95 \pm 0.19 \mu\text{g cm}^{-2} \text{day}^{-1}$ in O-T₁ and O-T₂ respectively; and -
292 0.21 ± 0.58 and $-1.80 \pm 0.34 \mu\text{g cm}^{-2} \text{day}^{-1}$ in A-T₁ and A-T₂, respectively.

293 After removing MPs from sediment cores, control and treatments under oxic conditions
294 kept exhibiting a net P uptake by the sediment and no significant differences among
295 neither of them were noticed (Fig. 1b). In anoxic conditions, the highest P release rate
296 ($1.96 \pm 2.52 \mu\text{g P cm}^{-2} \text{day}^{-1}$) was observed when no MPs were added (A-Control)
297 compared to treatments (A-T₁ and A-T₂). P efflux registered in A-T₂ ($-1.27 \pm 0.28 \mu\text{g P}$
298 $\text{cm}^{-2} \text{day}^{-1}$) was significantly lower than that found in A-T₁ ($p= 0.043$) and in A-Control
299 ($p= 0.011$). By contrast, no significant differences were found between A-Control and
300 A-T₁.

301 It is important to consider that P fluxes were measured before and after removing MPs
302 from the cores in order to take into account possible P release during resuspension trig-
303 gered by MPs removal with the magnet. However, statistical analyses have shown that P
304 exchange rates were not significantly different between these two stages, neither in oxic
305 nor in anoxic conditions in any treatment.

306 ***3.2. Effect of MPs addition on Fe concentrations in water column***

307 In order to assess the effect of MPs addition on Fe concentrations in lake water, Fe con-
308 centrations were measured in controls and treatments as initial Fe concentration (before
309 adding MPs) and as final Fe concentration (at the end of the incubation period). While
310 in oxic conditions (both in control and treatments) Fe concentration in the overlying
311 water was always below the detection limit ($1 \mu\text{g Fe l}^{-1}$), in anoxic cores the trend was
312 very different (Fig.2). Fe concentration did not significantly change in A-Control during
313 the experiment whereas in A-T₁ and A-T₂ treatments there was a significant increase in
314 Fe concentrations from 1.15 ± 0.40 to $8.63 \pm 1.23 \text{ mg L}^{-1}$ in the case of A-T₁ and from
315 0.88 ± 0.60 to $12.74 \pm 3.43 \text{ mg L}^{-1}$ in the case of A-T₂. As a consequence, at the end of

316 the experiment, Fe concentration in A-T₁ was 10 times higher than in A-Control
317 ($p < 0.001$) whereas in A-T₂ was 15 times higher than in A-Control ($p < 0.001$).

318 **3.3 Sediment characterization**

319 *3.3.1 Initial sedimentary P pools*

320 As it can be seen in Fig.3, Tot-P_{sed} concentration decreased from 1022.26 $\mu\text{g P gDW}^{-1}$ at
321 sediment surface to 847.37 $\mu\text{g P gDW}^{-1}$ at 10 cm depth. As an average of the first 10
322 cm, the percentage of each P fraction with respect to Tot-P_{sed} was: 1.6 (P_{→Water}), 16.0
323 (P_{→BD}), 5.7 (P_{→NaOH}), 0.5 (P_{→NaOH, Humic}), 54.2 (P_{→HCl}), 9.3 (Org-P_{Labile}) and 12.8 %
324 (P_{→HCl, Res}). Org-P_{Labile} concentration reduced with depth. P_{→NaOH, Humic} and P_{→HCl} in-
325 creased slightly with depth whereas P_{→BD}, P_{→NaOH} and P_{→HCl, Res} fractions kept practical-
326 ly constant at all depths.

327 *3.3.2 Changes in sediment composition induced by MPs addition*

328 The percentage distribution of the seven P pools with respect to Tot-P_{sed} was rather
329 similar in the initial fractionation and the final fractionation in all treatments and con-
330 trols. This similarity was in accordance to that obtained by Hansen et al. (2003) when
331 applying different treatments (addition of Fe, Al and O₂ application) to sediment cores.
332 However, some quantitative differences in sedimentary P pools concentrations emerged
333 when comparing treatments and controls in final fractionation. As a way of illustration,
334 Fig.4 shows P_{Mobile} concentrations at five depth intervals in oxic (Fig.4a) and anoxic
335 (Fig.4b) conditions. Controls, both in oxic and anoxic conditions showed higher P_{Mobile}
336 concentrations than treatments (T₁ and T₂) at all depths. Considering the 10 cm of the
337 upper sediment, MPs addition caused a reduction in sediment P_{Mobile} concentration,
338 ranging from 12 % in O-T₁ to 16 % in O-T₂ with respect to O-Control, and 16 % in A-T₁
339 and the 17 % in A-T₂ compared to A-Control. By dividing the sediment in just two
340 depths, the uppermost (0-4cm) and the deepest layer (4-10cm), we can see that contrary

341 to what it might be expected, the highest reduction in P_{Mobile} concentration occurred in
342 the deepest sediment layer in oxic conditions (18 % both in O-T₁ and O-T₂). By con-
343 trast, an inverse tendency was observed for anoxic conditions where the highest reduc-
344 tion in P_{Mobile} concentration was measured in the upper sediment layer, which reached
345 the 22% and 25% in A-T₁ and A-T₂, respectively.

346 It is worth to remark that changes in P_{Mobile} pool between treatment and controls were
347 mostly due to changes in Org- P_{Labile} fraction, since no differences were noticed for
348 $P_{\rightarrow\text{Water}}$ and $P_{\rightarrow\text{BD}}$ after MPs addition. Because of that, next we focus on Org- P_{Labile} frac-
349 tion. Specifically, considering the upper 10 cm, Org- P_{Labile} reduction was 34 % in O-
350 T₁ and 44% in O-T₂ compared to O-Control and 32 % in A-T₁ and 45 % in A-T₂ com-
351 pared to A-Control. Similarly to what occurred with P_{Mobile} under oxic conditions the
352 higher reduction in Org- P_{Labile} took place within the deepest layer accounting for 45% in
353 O-T₁ and 53% O-T₂ compared to O-Control. In anoxic conditions, Org- P_{Labile} concentra-
354 tion was reduced in a higher proportion in the upper sediment layer than in the deepest
355 sediment, reaching 37 % of Org- P_{Labile} reduction in A-T₁ and 54 % of reduction in A-
356 T₂. The rest of P fractions does not seem to have suffered any significant change when
357 comparing control and treatments.

358 Based on these sediment data, an estimation of the removed mass of P_{Mobile} by the dif-
359 ferent treatments compared to controls was calculated for the whole lake: O-T₁, O-T₂,
360 A-T₁ and A-T₂ treatments would lead to a reduction of 130, 195, 62 and 141 Kg P, re-
361 spectively.

362 Changes in $\text{Fe}_{\rightarrow\text{BD}}$ and Tot- Fe_{sed} pools as a consequence of MPs performance can be
363 seen in Table 1. By analyzing $\text{Fe}_{\rightarrow\text{BD}}$ and Tot- Fe_{sed} , no remarkable differences in Fe
364 concentration were observed between controls and treatments, neither in oxic nor in
365 anoxic conditions over the depth intervals. The same trend was found for the other dif-

366 ferent Fe fractions (data not shown). Table 1 also shows that similar LOI contents were
367 present at the end of incubation period in treatments and controls.

368 **4. Discussion**

369 *4.1. Effect of anoxic conditions on P exchange across the sediment and water inter-* 370 *face*

371 Comparison of controls (A-Control and O-Control) in which the only different factor
372 was redox potential, highlights the importance of the classical model proposed by
373 Einsele (1936) and Mortimer (1941) which pointed out the close coupling between re-
374 dox-sensitive Fe compounds and P exchange between sediment-water interface.

375 Under oxic conditions, sediment of Honda lake exhibited a net P uptake. It is very well
376 known that in these conditions, there exists an oxidized microzone in the uppermost
377 sediment layer in which precipitated Fe naturally present in sediment is able to retain P
378 (Mortimer, 1971). It is important to remark that under oxic conditions, sediment P ad-
379 sorption capacity and thus, P transport across sediment-water interface is mainly con-
380 trolled by $Fe_{\rightarrow BD} : P_{\rightarrow BD}$ molar ratio (Jensen, 1992). In Honda lake, this molar ratio was
381 15:1 which is considerably higher than the molar ratio (8:1) required for trapping P in
382 the sediment (Jensen, 1992).

383 By contrast, in anoxic conditions, P exchange rate across the sediment and water inter-
384 face was $1.96 \mu\text{g P cm}^{-2} \text{ day}^{-1}$ (A-Control) which is in the range of a similar study car-
385 ried out in the hypertrophic Danish shallow lake Sonderby ($3.14 \mu\text{g cm}^{-2} \text{ day}^{-1}$; Reitzel et
386 al., 2003). This positive P efflux and the increase of Fe concentration in water column
387 along the incubation period evidenced the existence of Fe reduction and the subsequent
388 P mobilization from lake sediment to the overlying water (Marsden, 1989; Hupfer and
389 Lewandowski, 2008). In such conditions, P transport from sediment to water column is
390 mediated by the concentration gradient between dissolved P in pore water sediment and

391 overlying water (Eckert et al., 1997; Sondergaard et al., 2001). Similarly, Hansen et al.
392 (2003) found a higher P flux in sediment cores incubated under anoxic conditions (1.32
393 $\mu\text{g P cm}^{-2} \text{ day}^{-1}$) than cores which were bubbled with O_2 ($0.21 \mu\text{g P cm}^{-2} \text{ day}^{-1}$) with no
394 salts addition for a period of 35 days of incubation.

395 As a way of illustration of the relevant effect of internal P loading on the P concentra-
396 tion of the water column, next we present some calculations. Since the lake presents an
397 average of 204 anoxic days per year (de Vicente et al., 2003) and in this study we have
398 measured a P release flux of $1.96 \mu\text{g P cm}^{-2} \text{ day}^{-1}$ under anoxic condition, the internal P
399 loading is calculated to be responsible for 376 kg P yr^{-1} . However under oxic conditions,
400 natural Fe oxides present in this sediment would retain 360 kg P during this period (161
401 days yr^{-1}). The net result of both processes would give rise to a P concentration in the
402 water column of $135.6 \mu\text{g L}^{-1}$, contributing to almost half of the annual mean TP con-
403 centration ($312 \mu\text{g L}^{-1}$; de Vicente et al., 2003).

404 ***4.2. Effect of adding MPs on P exchange fluxes across the sediment-water interface*** 405 ***and on Fe concentrations in water column.***

406 *Oxic treatments*

407 On the one hand, sediment from Honda lake experienced a net P uptake from the over-
408 lying water after MPs amendment (O-T₁ and O-T₂), both before and after removing
409 them from the cores. This behavior highlights the scarce relevance of P release due to
410 the resuspension when removing MPs under oxic conditions. On the other hand, the
411 rather similar P fluxes values in the control and treatments evidenced the negligible ef-
412 fect of adding MPs on P efflux. The reason behind this finding is that in the sediment of
413 the study lake, as it has been already stated, Fe oxides concentration was high enough to
414 trap P under oxic conditions. Regarding Fe concentrations in water column, it was ob-
415 served that initial and final Fe concentrations kept similar in control and treatments.

416 These data along with the fact that Tot-Fe_{sed} was not significantly increased in treatments
417 compared to control indicates that MPs do not release Fe in oxic conditions.

418 *Anoxic treatments*

419 In anoxic conditions, the addition of MPs caused an important reduction in P fluxes
420 compared to the control (A-Control). While in the case of A-Control P was released
421 from the sediment to the overlying water, in the case of A-T₂ treatment, P mobilized by
422 the reduction of Fe oxides did not go to the water column but was adsorbed on MPs, as
423 it is reflected by a net P uptake by the sediment. Hence, our results have shown that the
424 addition of MPs in a molar ratio of 560:1 respect to P_{Mobile} concentration was enough to
425 inactivate P internal loading. A similar P efflux reduction (100 % reduction compared to
426 control) under anoxic conditions have been reported by some authors such as Hansen et
427 al. (2003) (P efflux = -0.025 $\mu\text{g P cm}^{-2} \text{ day}^{-1}$) when using Al: P_{Mobile} molar ratio of 10:1;
428 Reitzel et al. (2003) (P efflux = -0.097 $\mu\text{g P cm}^{-2} \text{ day}^{-1}$) when using Al: P_{Mobile} molar
429 ratio of 4:1 and Gibbs et al. (2011) (-0.7 $\mu\text{g P cm}^{-2} \text{ day}^{-1}$ and -0.8 $\mu\text{g P cm}^{-2} \text{ day}^{-1}$) when
430 adding Phoslock® at a 50:1 and 100:1 Phoslock®: P weight ratio, respectively. Please,
431 note that P uptake rate by sediment was a little bit lower in these studies compared to
432 our value. By contrast, P efflux after removing MPs reflected that the 285:1 MPs: P_{Mobile}
433 molar ratio was not enough to suppress short-term P efflux (95 % of reduction). It is
434 important to remark that P concentration in water column was measured right after re-
435 moving MPs, and thus higher concentration of P due to resuspension could be meas-
436 ured. Likewise, the 10:1 Fe:P molar ratio proposed by Hansen et al. (2003) as well as
437 the 8:1 Al: P_{Mobile} molar ratio proposed by Reitzel et al. (2003) were not enough to re-
438 tain P by the sediment (P efflux = 0.053 $\mu\text{g P cm}^{-2} \text{ day}^{-1}$ and 0.16 $\mu\text{g P cm}^{-2} \text{ day}^{-1}$, re-
439 spectively) giving a 95 % of reduction in both cases.

440 Final Fe concentrations in water column were remarkably higher in treatments (A-T₁
441 and A-T₂) compared to A-Control, reflecting that under anoxic conditions MPs were
442 releasing Fe to the water column, probably due to the dissolution of MPs in contact to
443 BD reagent used to guarantee anoxic conditions. Hunt et al. (1995) and Fine et al.
444 (1992) reported that fine grain magnetite (with a similar surface of our MPs) undergo
445 dissolution in the presence of Citrate-BD solution. Fe concentration in freshwater is
446 naturally less than 1 mg L⁻¹, whereas in anaerobic groundwater where the Fe⁺² specie
447 predominates it usually ranges 0.5-10 mg L⁻¹ (World Health Organization, 2003; Xing
448 and Liu, 2011). According to some studies, there is no evidence of negative effects of
449 increasing Fe concentrations on the maximum achievable ecological quality for fish,
450 macrophyte and diatom communities although it has been reported a decrease of
451 macroinvertebrate populations in contact with high total Fe concentrations (WFD-
452 UKTAG, 2012). Therefore, some thresholds have been proposed to guarantee the good
453 state of these communities such as 1 mg L⁻¹ total Fe (USEPA, 1976), and 0.21 mg L⁻¹ or
454 1.74 mg L⁻¹ for no changes or slight to moderate changes in community structure, re-
455 spectively (Linton et al., 2007). At this point, it is of great importance to consider that
456 the use of BD in this study for achieving anoxic conditions could have increased Fe
457 concentrations more than natural anoxic conditions occurring in lakes, so lower Fe con-
458 centrations are likely to be measured when adding MPs in natural anoxic conditions. In
459 addition, it is essential to work in synthesizing new MPs that do not release Fe in anaer-
460 obic conditions in order to reduce undesirable effects on biota.

461 ***4.3 Effect of adding MPs on sedimentary P pools***

462 On the one hand, from initial sediment fractionation analysis we can conclude that
463 P_{→HCl} pool which is not expected to contribute to internal P loading, is by far the most
464 important P form in our sediment. In ecosystems with high alkalinity (as in our case) P

465 can be immobilized in sediment by coprecipitation or adsorption with carbonates at high
466 pH values contributing to the burial of P in sediment (Otsuki and Wetzel, 1972; Eckert
467 et al., 1997). Since in these wetlands the amount of P bound to carbonates is remarka-
468 ble, potential internal P loading is likely to be closely related to reductions in pH values
469 (Rydin and Welch, 1998). P_{Mobile} pool, which accounted for 8.52 g m^{-2} within the first
470 10 cm, has an outstanding importance as it can be used for predicting future internal P
471 loading. The three P pools contributing to P_{Mobile} fraction ($P_{\rightarrow\text{Water}}$, $P_{\rightarrow\text{BD}}$, $\text{Org-P}_{\text{Labile}}$)
472 consist of 37 % of $\text{Tot-P}_{\text{sed}}$ at surface sediment and decrease with depth up to 21% at 10
473 cm depth due to the well-known upward transport of P to upper sediment layers (Rydin,
474 2000), suggesting that these three pools do not contribute to burial flux of P in sedi-
475 ments (Reitzel et al., 2003).

476 On the other hand, the addition of MPs has caused a reduction in P_{Mobile} concentration
477 both in oxic and anoxic condition even in the deeper sediment layer (10 cm). In fact, the
478 presence of MPs is not restricted to surface sediment as they are characterized by a
479 much higher density (7.8 g cm^{-3}) than sediment (1.1 g cm^{-3}). Moreover, the high ionic
480 strength present in the lake (3.09 mS cm^{-1} , de Vicente, 2004) implies a reduction in the
481 zeta potential value which is involved in electrostatic repulsion interactions between
482 MPs, leading to their aggregation and rapid sedimentation (de Vicente et al., 2011;
483 Gunnars et al., 2002). The high density of MPs compared to the low density of other P-
484 capping agents as Al salts (Lewandowski et al., 2003) entails a different behavior in
485 sediment, causing P inactivation in deeper sediment layers. P adsorption mechanism
486 onto MPs has been previously reported to take place by ligand exchange, in which hy-
487 droxyl groups are replaced by P species forming inner-sphere surface complexes at wa-
488 ter-oxide interface (de Vicente et al., 2011; Daou et al., 2007). Despite of being nega-
489 tively charge under these experimental conditions of pH (pH=8), MPs are able to adsorb

490 considerable amounts of P due to specific adsorption (de Vicente et al., 2011; Daou et
491 al., 2007). Org-P_{Labile} was the only P sedimentary pool comprising P_{Mobile} which experi-
492 enced a reduction in treatments compared to controls both in oxic and anoxic condi-
493 tions. However, no changes in LOI % content between control and treatments have been
494 observed which could be explained by the lower sensibility of the ignition method. The
495 higher affinity of MPs for Org-P_{Labile} is likely to be the result of the well-known strong
496 affinity of organic compounds by Fe oxides. In particular, Illés and Tombácz, (2003)
497 reported a notable adsorption of organic substances such as fulvic and humic acids (HA)
498 by magnetite at low pH (4.5) but even at higher pH similar to those registered in our
499 experiments (anoxic conditions, pH 6.2 ± 0.18 ; oxic conditions, pH = 8.15 ± 0.08). Simi-
500 larly, Merino-Martos et al. (2015) reported a remarkable decrease in P adsorption effi-
501 ciency, when using the same carbonyl Fe particles as in this experiment, in the presence
502 of high HA concentrations (from 94% when HA = 17 mg C L⁻¹ to 12 % when HA = 35.5
503 mg C L⁻¹).

504 ***4.4 Some considerations for lake restoration***

505 In the framework of the whole available methods for lake restoration, MPs addition
506 could be considered as a method focused on increasing P export for reducing lake P
507 concentration. The use of MPs has several advantages compared with other drastic
508 techniques that aim at increasing P export, such as sediment dredging. High turbidity
509 due to resuspension of finely divided sediment, liberation of nutrients and toxic metals
510 to water column, destruction of benthic fauna and high costs are some of the negative
511 aspects about using sediment dredging (Petersen, 1982). In this sense, MPs minimizes
512 impacts of resuspension and water column since they are more selective with sediment
513 (they have more affinity with sedimentary P forms). Next, we will summarize some
514 advantages of using MPs. First, P can be recovered from the aquatic ecosystem, which

515 allows the later potential use of P as P-containing fertilizers. In this sense, it is nowa-
516 days consider that they are a non-renewable resource and could be exhausted in the next
517 50-100 years (Cordell et al., 2009). This situation has become alarming due to the in-
518 creasing population density, above all in developing and emerging countries, which
519 demands a high food production. So, it is required new challenges to find solutions
520 where the equilibrium P-cycle is reached. Second, MPs can be recovered from the solu-
521 tion reducing both economic costs and toxic effect on the biota. In relation to the eco-
522 nomic cost, it is essential to consider that before making any whole lake application it is
523 essential to gain also some knowledge about the economic balance. For making a eco-
524 nomic balance, we have compared some crucial characteristics (price and P removal
525 efficiency) of MPs and some of the most commonly used P adsorbent for lake restora-
526 tion such as $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (i.e. Rydin, 2000; Reitzel et al., 2005; Egemose et al., 2011)
527 and Phoslock® (i.e. Robb et al., 2003; Lürling and Tolman, 2010). In brief, we can con-
528 clude that when re-using MPs more than 2 times it is economically worth to use this
529 adsorbent instead of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ or Phoslock®. In relation to the toxic effect on the
530 biota, recent studies have found that MPs concentration have to be added in high con-
531 centration to cause negative effect on planktonic and benthonic organisms. In particular,
532 Álvarez-Manzaneda et al. (unpublished results) have measured that the concentration of
533 MPs that produces the immobilization of 50% of the individuals during exposition peri-
534 od (CE_{50} ; OCDE 2004) was 0.9 and 0.4 g L^{-1} for *Daphnia magna* and *Chironomus* sp.,
535 respectively. At this point, it is relevant to consider that, considering the MPs: P ratio
536 reported in previous studies (de Vicente et al., 2010; Merino-Martos et al., 2011), the
537 addition of 0.4 g L^{-1} would not be required as it corresponds to extremely high P concen-
538 trations. As a result, it is expected that in a whole lake application, MPs will not cause
539 any toxic effect on the biota.

540 **4.5 Conclusions**

541 Despite the broad use of P-binding compounds for restoring eutrophicated waters, up to
542 date, there is not a universal adsorbent for applying in whatever physico-chemical con-
543 ditions (i.e. pH, redox potential) and therefore more research in new adsorbents is re-
544 quired. This study has evidenced that internal P loading during the anoxic period con-
545 tributes to half of the annual mean P concentration in the water column of the study
546 lake. In this sense, the use of MPs seems to be a suitable technique to apply since it is
547 able of completely suppressing the short-term P efflux under the most undesirable con-
548 ditions (anoxic) at the 560:1 MPs: P_{Mobile} ratio. However, when Fe oxides naturally pre-
549 sent in sediment are enough to uptake P from water column, MPs do not show any ef-
550 fect on P fluxes. Apart from the effect of MPs on P efflux under anoxic conditions, MPs
551 also reduces sedimentary P_{Mobile} concentration in both oxic and anoxic conditions, lead-
552 ing to a reduction in long-term P efflux. The best results have been found when adding
553 MPs at the MPs: P_{Mobile} molar ratio of 560:1, which conducted to the removal of 141 kg
554 of sedimentary P in anoxic and to 191 Kg P under oxic conditions for the whole lake
555 area.

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803 **Tables**

804

805 **Table 1.** Fe_{BD} fraction, Total sediment Fe (Tot-Fe_{sed}) and LOI(%) in treatments and
806 controls within 0-10 cm depth at the end of the incubation period. Standard deviation
807 (SD).

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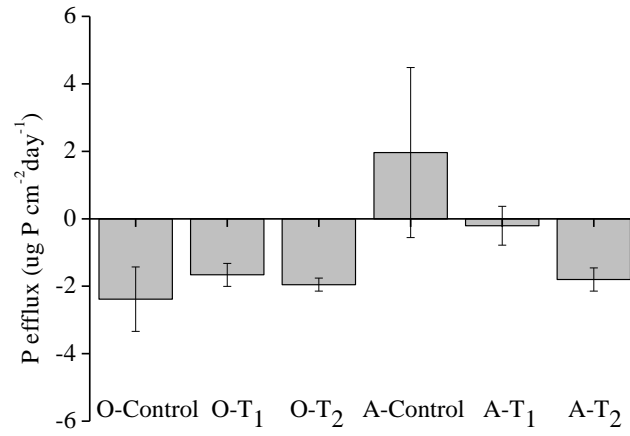
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	O-Control		O-T ₁		O-T ₂		A-Control		A-T ₁		A-T ₂		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Fe_{→BD}	0-2	4.43	0.24	6.89	0.16	5.99	0.25	4.33	0.13	5.51	0.14	4.54	0.08
	2-4	5.42	0.08	6.62	0.28	5.93	0.10	4.52	0.21	4.74	0.08	4.26	0.31
	4-6	4.66	0.01	5.40	0.08	5.26	0.09	3.99	0.03	4.82	0.16	3.84	0.43
	6-8	4.72	0.14	5.04	0.06	5.19	0.09	3.88	0.10	4.26	0.11	5.27	0.10
	8-10	4.17	0.17	5.03	0.08	4.68	0.18	3.70	0.13	4.47	0.05	3.51	0.11
	Total 0-10 cm	23.40	0.34	28.98	0.35	27.05	0.35	20.42	0.30	23.80	0.26	21.42	0.56
Tot-Fe_{sed}	0-2	15.25	1.05	15.09	0.29	16.07	0.75	15.63	1.16	15.31	1.24	15.45	0.24
	2-4	16.22	0.69	14.63	1.28	17.35	0.98	14.09	4.84	16.60	1.19	16.72	1.20
	4-6	17.32	0.32	15.41	0.54	17.95	1.18	17.01	0.24	16.32	0.59	16.47	0.20
	6-8	16.99	0.57	15.64	0.35	16.94	0.46	17.16	0.65	15.91	0.52	17.30	0.62
	8-10	17.02	0.11	15.26	1.39	17.19	0.50	17.36	0.51	16.90	0.12	17.41	0.09
	Total 0-10 cm	82.80	1.42	76.03	2.02	85.50	1.84	81.25	5.05	81.04	1.89	83.35	1.39
LOI %	0-2	11.55	0.45	10.79	0.38	10.81	0.33	11.17	0.57	10.55	0.21	10.98	1.06
	2-4	11.08	0.63	10.42	0.11	10.64	0.32	11.41	0.36	10.80	0.46	9.90	0.27
	4-6	11.22	0.56	9.18	0.07	7.46	6.47	11.03	0.77	10.92	0.69	10.03	0.64
	6-8	10.64	1.51	8.84	0.22	10.40	0.34	8.38	0.38	10.50	1.65	8.42	0.55
	8-10	9.10	1.11	8.75	0.10	8.39	0.05	5.91	0.24	8.58	0.31	7.23	0.65
	Total 0-10 cm	53.59	2.10	47.98	0.47	47.70	6.50	47.90	1.12	51.35	1.88	46.56	1.53

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Fig.1

a



b

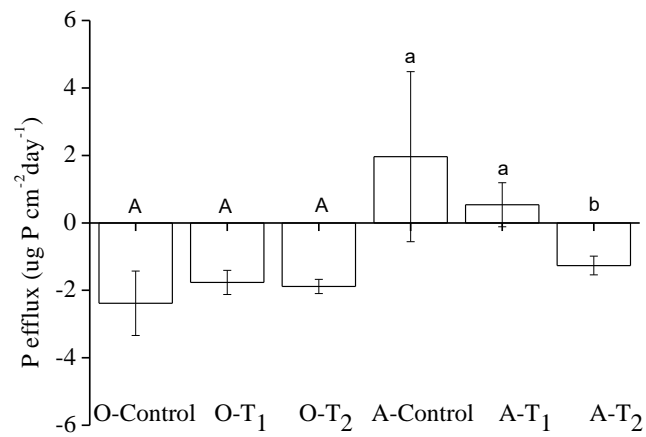


Fig. 1. Sediment P fluxes before (1a) and after removing MPs (1b). Significant differences ($p < 0.05$) between treatments are indicated with letters. Standard deviation is represented by vertical bars.

Fig. 2

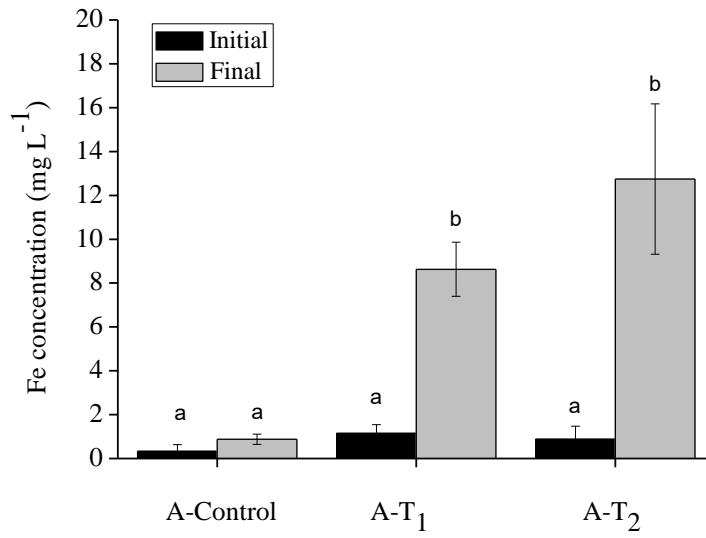


Fig. 2. Fe concentration in water column in control and treatments under anoxic conditions. Standard deviation is represented by vertical bars. Significant differences between initial and final concentrations are written with letters.

Fig.3

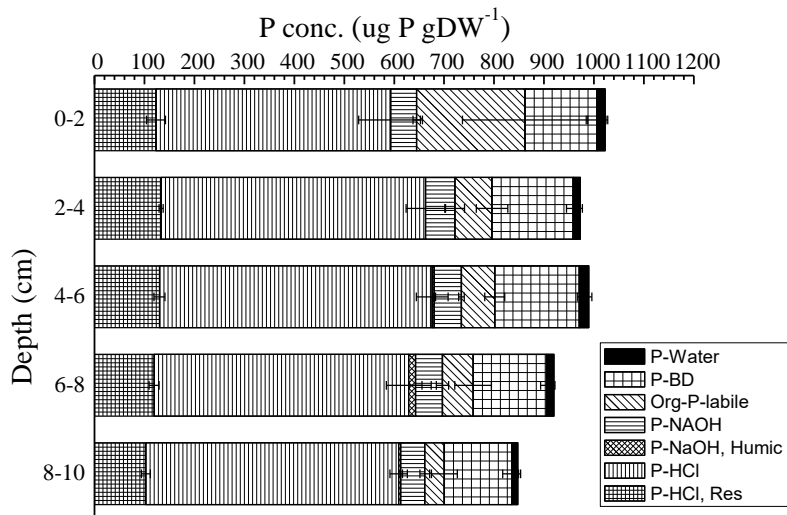
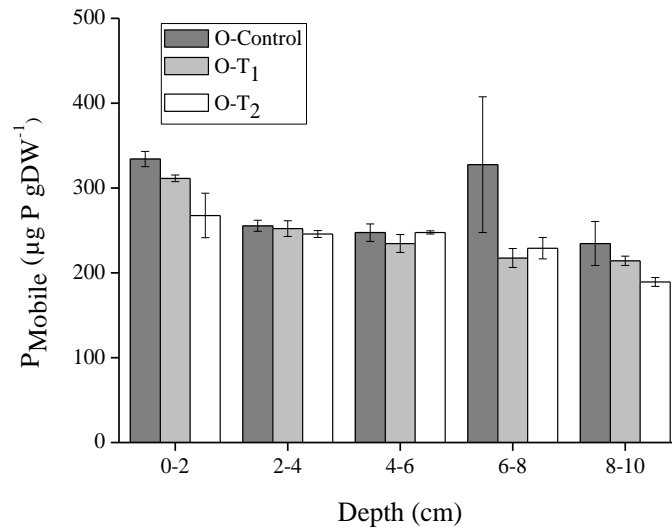


Fig.3. Initial sedimentary P fractionation of untreated sediment. Standard deviation of each fraction is represented by horizontal bars.

Fig.4

a



b

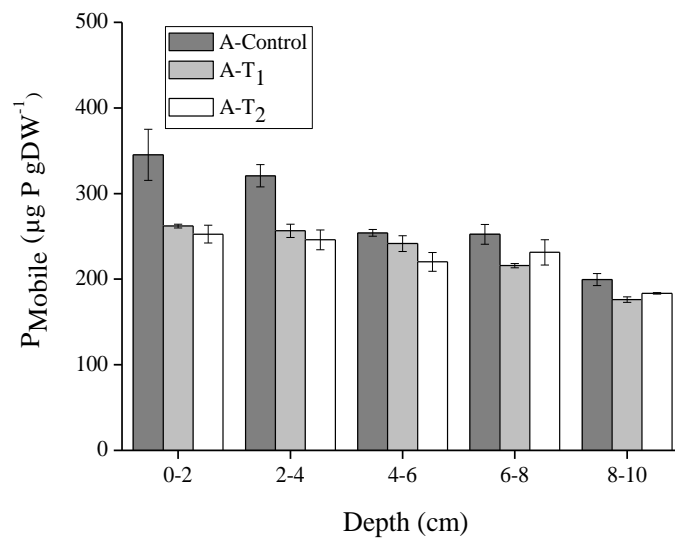


Fig.4. Changes in P_{Mobile} concentration with depth after removing MPs. Fig. 4a corresponds to oxic treatments and Fig. 4b refers to anoxic treatments. Significant differences ($p < 0.05$) between treatments by are indicated with letters. Standard deviation is represented by vertical bars.

