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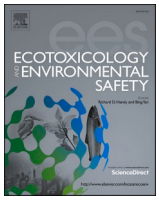

Assessing the toxic effects of magnetic particles used for lake restoration on phytoplankton: A community-based approach

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ABSTRACT

Inactivation by adding different phosphorus (P) adsorbents is one of the most frequently used methods for combating inland water eutrophication. The aim of this work was to assess the toxic effects of novel P adsorbents (magnetic particles, MPs) on the phytoplankton community. An outdoor microcosm experiment, containing lake water and surface sediment from a hypertrophic Mediterranean lake, was carried out following a factorial design (n = 5) with three different treatments: control (C), where no MPs were added; Treatment-Water (T-W) and Treatment-Sediment (T-S). In T-W and T-S treatments, MPs were added on the surface water layer and on the sediment, respectively, to obtain a final concentration of 1.4 g MP L^{-1}. This concentration was based on both the sedimentary mobile P concentration of the study site and the maximum P adsorption capacity of the MPs, obtained from the literature. After 24 h of contact time, the MPs were removed using a magnetic rake. Physicochemical measurements and biological samples were taken after 24 h of exposure to the MPs and at different time points after such exposure (day 2, 7, 21, 35 and 70). Changes in phytoplankton community such as abundance (biovolume and *Chla*), species composition and taxonomic groups were assessed, as well as changes in the Shannon-Wiener diversity index. Additionally, the eutrophic metric Algae Group Index (AGI), one of the metrics proposed in the Water Framework Directive, was also calculated. Our results indicate that there is no strong evidence to infer that MPs caused an effect on the phytoplankton community, since no significant differences (GLM test; $p > 0.05$) were found between controls and treatments in any of the studied variables (phytoplankton taxonomic groups, AGI, *Chla* concentration, biovolume, diversity and community responses). Accordingly, MPs did not cause any toxic effects on the phytoplankton community of the lake, encouraging the use of MPs in a future whole-lake restoration strategy. However, if the final goal of the restoration plan is to combat nuisance cyanobacteria blooms, higher initial MPs doses or repeated MPs applications are required to achieve a reduction in P concentrations below biological thresholds in order to prevent algal blooms.

1. Introduction

Eutrophication remains a worldwide threat to water quality of inland and marine waters (Paerl et al., 2010; Smith and Schindler, 2009; Vitousek et al., 1997). It is clearly generating major disruptions to aquatic ecosystems and has impacts on related goods and services (Le Moal et al., 2019). Overall, eutrophication is responsible for not only a drastic impairment of ecosystem structure and function (e.g. blooms of blue-green algae) but also for economic losses, causing annual costs of approximately $2.2 billion in the U.S. alone (Dodds et al., 2009). Eutrophication phenomena started to be recognized in the early 20th century and, in the context of the present climate change, it is alarming that it will amplify its symptoms (Woznicki et al., 2016; Paerl et al., 2014; Moss et al., 2011). Thus, it is imperative to develop new strategies to combat the eutrophication of aquatic ecosystems.

Concerning phytoplankton changes coupled to extreme eutrophication, biomass increase, shift to bloom-forming algal species and decrease of species diversity are frequently noted (Schindler, 2006). In fact, the number of harmful cyanobacteria blooms (O'Neil et al., 2012) and cyanobacteria dominance within the phytoplankton community (Taranu et al., 2015) have increased worldwide in north temperate lakes (Nürnberg, 2017). Increased temperatures, salinity and anthropogenic

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activities have resulted in cyanobacteria gaining greater advantage over other phytoplankton groups in freshwaters (Paerl and Huisman, 2009). For example, in Europe, Asia and America, more than 40% of lakes and reservoirs are now eutrophic and offer favorable conditions for cyanobacterial mass development (Bartram et al., 1999). A special concern is that it has been estimated that 25–75% of cyanobacterial blooms are toxic (Bláhová et al., 2008, 2007; Chorus, 2001).

To face the worldwide eutrophication challenge, successful restoration strategies may generally combine both the decrease of phosphorus (P) loads from the catchment and the increase of P retention in lake sediments (Carpenter, 2008; Schindler, 2006; Jeppensen et al., 1991). Therefore, methods for controlling P inputs (external and internal) must be an essential part of any restoration program. The most common restoration strategies are dredging, oxygenation and the addition of different chemical adsorbents/flocculants for P inactivation (Tu et al., 2015; Yin and Kong, 2015). Regarding P inactivation, the most frequently used P adsorbents are calcium, aluminum and Phoslock© (Yin and Kong, 2015; Spears et al., 2013; Egemose et al., 2011; Reitzel et al., 2005). Among them, Phoslock© has been extensively and successfully used in lakes in recent studies to trap P from the water column and the lake sediment (Copetti et al., 2016; van Oosterhout and Lürling, 2013; Meis et al., 2012). However, the high cost of a whole-lake restoration project (Spears et al., 2013) and the potential long-term effects of a permanent allochthonous layer lead to support new research for more cost-effective methods where the absorbent could be potentially recovered.

In this context, magnetic particles (MPs) arise as an innovative P adsorbent. Among MPs, carbonyl iron (Fe) particles have been recently proposed for combating eutrophication, as they combine both (i) high maximum P adsorption capacity and (ii) low economic cost, since they can be firstly recovered by magnetic devices from the aqueous solution and later both P and MPs can be reused (Merino-Martos et al., 2011; de Vicente et al., 2010). Briefly, carbonyl Fe particles can rapidly adsorb P under batch and flow conditions (Merino-Martos et al., 2011; de Vicente et al., 2010) and under aerobic and anoxic conditions (Funes et al., 2016). Then, P can be desorbed from P-loaded MPs under basic conditions, after which it can be used as a potential fertilizer. Additionally, MPs can be reused for a further P adsorption cycle, as they still exhibit a high maximum P adsorption capacity (de Vicente et al., 2010). The recovery of P from eutrophicated aquatic ecosystems is of special relevance, since P is a non-renewable resource and the current agricultural and industrial demands may lead to its depletion in 50–100 years (Cordell et al., 2009; Smil, 2000). Despite all the mentioned advantages of using MPs, their addition may also raise toxicity concerns that must be addressed before accomplishing any whole-lake application. Indeed, the addition of MPs could cause direct and/or indirect effects on aquatic biota. Direct effects could be attributed to the physical or chemical toxicity of MPs, while indirect effects could be linked to the potential release of Fe from MPs or to other indirect detrimental effects (e.g., nutrient limitations). Specifically, the potential release of Fe from MPs is a key issue, since, although it is a limiting nutrient for primary production when it is present at low concentrations, it can cause toxic effects on lake biota at high concentrations.

Although several recent studies have focused on assessing the toxic effects of MPs on phytoplankton using single-species toxicity tests (Álvarez-Manzaneda et al., 2019a; Álvarez-Manzaneda and de Vicente, 2017), up to date there are no studies testing both direct and indirect effects of MPs addition on a real whole-lake phytoplankton community. In this context, it is important to consider that single-species toxicity tests achieved under laboratory conditions have several limitations (Álvarez-Manzaneda et al., 2019b; del Arco et al., 2017) and, therefore, it is recommended to use outdoor microcosm experiments to evaluate the fate and effects of chemicals at many different levels of organization through appropriate endpoints (EFSA, 2013).

The general goal of this study was to assess the toxic effect of MPs addition on the phytoplankton community of a hypertrophic Mediterranean lake using large microcosms containing lake water and lake sediment. In particular, we hypothesized that the phytoplankton community is negatively affected by MPs used for lake restoration. The specific objectives were to assess the phytoplankton community response to MPs addition by monitoring changes in phytoplankton abundance (*Chla* and biovolume), phytoplankton species composition and taxonomic groups, as well as changes of some selected ecological indices. Lastly, it is worth mentioning that this study was framed within a broader project focused on determining the consequences of MPs application on water quality and sediment P pools (Funes et al., 2017), as well as on the zooplankton community (Álvarez-Manzaneda et al., 2019b).

2. Material and methods

2.1. Study site

The Adra wetland is located in a semiarid region in Southeast Spain (Almería). It consists of two adjacent shallow lakes: Honda lake (9 ha and mean depth of 1.3 m) and Nueva lake (26 ha and mean depth of 2.28 m; de Vicente et al., 2003; Moreno-Ostos et al., 2007). The Andalusian Government declared it as a Natural Reserve (1989) and it was later included as a protected area in the Ramsar Convention (1994). Despite of its local, national and international protection, the wetland is seriously affected by anthropogenic activities such as intensive agricultural activity. Due to all these threats, both lakes are classified as hypertrophic (Honda) and eutrophic (Nueva; de Vicente et al., 2003). Regarding phytoplankton, previous studies have reported a richness of 60 species over a 3-year study period (1999–2001) in Honda lake (Cruz-Pizarro et al., 2003). The relative abundance of the identified taxonomic groups was: 49% Chrysophyceae, 24% Chlorophyceae, 17% Cryptophyceae, 8% Cyanobacteria, 1% Dinoflagellata and 1% Euglenaceae. Cyanobacteria blooms have been previously observed in late summer by Moreno-Ostos et al. (2007).

2.2. Microcosms set-up and magnetic particles

On July 2015, surface lake water was collected using a peristaltic pump, while surface sediment (100 dm^3) was sampled with an Ekman dredge at the deepest site of the lake. Once in the laboratory, 15 microcosms (PVC black containers; Ø = 38 cm; h = 58 cm), filled with homogenized surface sediment and lake water, were randomly placed in an outdoor roofed area. The roofed area was open on the sides, allowing natural light exposition along the experiment (mean light intensity: 159.40 μmol photons m^{-2} s^{-1}). Later, 25 L of lake water with concentrated zooplankton (obtained from vertical and horizontal hauls with a plankton net of 30 μm) were homogenized and distributed in the microcosms to obtain a final volume of 40 L of lake water. During the experiment (70 days), all microcosms were kept oxygenated using an aeration pump and they were covered with a mesh to prevent the aerial colonization of flying insects or falling spores.

After a one-week stabilization period, the experiment was initiated with three different treatments (n = 5): a control (C) and two enriched MPs treatments (Treatment-Water, T-W, and Treatment-Sediment, T-S). These treatments simulated two different restoration scenarios: a top-MPs addition over the water (T-W) and a bottom-MPs addition close to the sediment surface (T-S). Briefly, MPs were added in aqueous dispersion (120 g MP L^{-1}) using a peristaltic pump to obtain a final concentration of 1.4 g MP L^{-1} in each microcosm. As Funes et al. (2017) explained, we selected this MPs concentration, which corresponds to a MPs: sedimentary mobile P molar ratio of 85:1, in order to counteract any possible chemical interferences, which frequently occur in natural waters (de Vicente et al., 2011).

The MPs used were carbonyl Fe particles, supplied by BASF (Germany), with a chemical composition of 97.5% Fe, 0.9% C, 0.5% O and 0.9% N and an average diameter of 800 nm (Merino-Martos et al., 2011;

de Vicente et al., 2010). The use of these particles was based on the fact they are characterized by a large magnetization for a given external magnetic field compared to Fe oxides, which lastly increase their later removal by using magnetophoresis (de Vicente et al., 2010).

On the first day (day 0), a sample was collected to record baseline physicochemical and biological conditions before adding the MPs. Later, the MPs were added and, after 24 h of contact time (day 1), they were recovered by fully immersing an especially designed magnetic rake in the microcosms down to the surface sediment (Álvarez-Manzaneda et al., 2019b). The efficiency of MPs removal by the magnetic rake was 91% and 32% for T-W and T-S, respectively (Funes et al., 2017).

2.3. Monitoring of the microcosms

Physicochemical measurements and biological samples were taken after 24 h of MPs addition and at different time points after MPs removal (day 2, 7, 21, 35 and 70). Specifically, temperature (T, °C), pH, dissolved oxygen concentration (O_2; mg L^{-1}), conductivity (Cond.; mS cm^{-1}) and total dissolved solids concentration (TDS, mg L^{-1}) were measured using a multiparametric probe (Hanna Instrument, HI 9829). Each sampling day, a water sample (1.5 L) was taken from each microcosm to analyze physicochemical parameters. Among them, dissolved inorganic P (DIP, Murphy and Riley, 1962) and total dissolved Fe (Tot-Fe_{dis}, Gibbs, 1979) concentrations were measured. More details about these chemical analyses can be found in Funes et al. (2017). The water removed by sampling and evaporation was replaced weekly with previously filtered lake water (30 μm).

To analyze phytoplankton community abundance and structure, subsamples (100 mL) were immediately fixed in acetic Lugol's solution (4% final concentration). Phytoplankton was identified (aliquots volume: 3 mL) and counted under an inverted microscope by Utermöhl (1958) technique. Larger and smaller taxa were counted at 400x and 1000x magnification, respectively. A minimum of 100 cells or setting units (colonies, filaments) of the most frequent species were identified. Counting was extended until no more new species were encountered for 10 microscope fields to obtain a reliable abundance and diversity data. Identification was carried out at the species level. In particular, phytoplankton abundance, estimated as biovolume, % of taxonomic groups (% abundance based on biovolume) and several ecological indices were calculated for each sampling time. These indices were: (i) Shannon-Wiener diversity index [H′ (log_2) - Shannon, 1948]; and (ii) Algae Group Index (AGI) (IGA Catalán and Ventura, 2003). Phytoplankton biovolume (μm^3 L^{-1}) was estimated by multiplying the abundance of each species by the mean cell volume (Hillebrand et al., 1999), based on measurements of at least 30 individuals, when possible (Rojo and Rodríguez, 1994). Biovolume was calculated for all quantified species. It is worth mentioning that all taxa were used to calculate the Shannon-Wienner diversity index. Additionally, the AGI was based on the biovolume proportions of the phytoplankton groups in terms of units (colony-*vs*. non-colony-forming taxa) in the samples with respect to the total sample biovolume of all species. The AGI formula is $GI = (1 + 0.1 * Cr + Cc + 2 + (Dc + Chc) + 3 * Vc + 4 * Cia)/(1 + 2 * (D + Cnc) - + Chnc + Dnc)$, where Cr, Cc, Dc, Chc, Vc, Cia, D, Chnc and Dnc abbreviations stand for the biovolume of the following taxa groups: cryptophytes, colonial chrysophytes, colonial diatoms, colonial Chlorococcales, colonial Volvocales, cyanobacteria, dinoflagellates, non-colonial chrysophytes, non-colonial Chlorococcales and non-colonial diatoms, respectively. This index was included for its relevance to classify the study lake within national categories for future assessments. The species composition change was analyzed through Principal Response Curves (PRC), considering a sub-set of all quantified species accounting for more than 0.1% of the total abundance over the experimental period.

Finally, subsamples were taken to determine *Chla* concentration. To this end, 50 mL of sample water were filtered using filter paper (Whatman GF/F, 0.7 μm pore size); the latter were subsequently placed in a glass vial with 5 mL of 90% acetone at 4 °C in the dark for 24 h. The extract was then filtered and measured using a spectrophotometer (Biochrom-Libra S50) at 630, 645, 665, and 750 nm. *Chla* concentration was lastly estimated according to Jeffrey and Humphrey (1975).

2.4. Statistical analysis

The effect of MPs addition on environmental variables (e.g., T, O_2), nutrient availability (P-DIP) and biological variables (e.g., *Chla* concentration, biovolume) was assessed using *General Linear Models* (*GLM*; MASS package in R software). The GLM models considered the interaction of treatments and time using as link function the quasi-Poisson distribution (Zuur et al., 2009). In addition, differences in phytoplankton species composition (in terms of total biovolume of each species) among treatments were tested using Principal Response Curves analysis (PRC; vegan package in R software, based on van den Brink and ter Braak (1999)). The curves represent the most dominant community response (y axis) to the treatments on each sampling day (x axis). It is complemented by a diagram showing the species weights on such community response. The results were considered statistically significant for $p \leq 0.05$, and moderately significant when $0.05 < p < 0.10$. For the multivariate analysis, we considered a sub-set of those species accounting for more than 0.1% of the total abundance over the experimental period, due to the very low abundance of some of them, introducing noise for further community response analyses. PRC is especially useful for micro and mesocosm experiments when evaluating changes generated over time in the structure of a community, being a very useful tool for ecotoxicological experiments (Brock et al., 2000; Moser et al., 2007; Zafar et al., 2012).

3. Results

3.1. Effects of magnetic particles addition on environmental variables

Table 1 shows the mean values of some selected environmental variables monitored during the experiment (more information can be found in Funes et al., 2017; Álvarez-Manzaneda et al., 2019b). In Table 2, the associated GLM results are shown. No significant differences among treatments were found for any of the physicochemical parameters. By contrast, and as expected, the addition of MPs caused a significant decrease in P-DIP concentrations (see Table 1). Regarding the potential release of Fe from MPs, it is important to note that Tot-Fe_{dis} concentration was, in all cases (treatments and time points), below the detection limit (<10 μg L^{-1}; Table 1).

3.2. Effects of magnetic particles addition on phytoplankton community abundance and structure

On average over the experimental period for both the controls and the treatments, the selected species were clustered as taxonomic groups representing Cyanobacteria (95.2%), Chlorophyta (2.7%),

Table 1
Average values and standard deviations of the physicochemical and chemical parameters measured in the water column during the experimental period (modified from Funes et al., 2016). BDL and AGI stand for Below Detection Limit and Algae Group Index.

	Control	T-W	T-S
T (°C)	21.2 ± 3.9	21.2 ± 4.0	21.2 ± 3.9
pH	9.0 ± 0.3	9.0 ± 0.3	9.0 ± 0.3
O_2 (mg L^{-1})	7.5 ± 0.8	7.5 ± 0.8	7.4 ± 0.9
Cond. (μS cm^{-1})	6625 ± 468	6516 ± 422	6437 ± 392
TDS (mg L^{-1})	3369 ± 357	3306 ± 276	3226 ± 194
P-DIP (μg L^{-1})	288 ± 87	106 ± 71	105 ± 71
Tot-Fe_{dis} (mg L^{-1})	BDL	BDL	BDL
AGI	193 ± 37	212 ± 31	185 ± 49

Table 2
Results of the General Linear Models for both physicochemical parameters (taken from Funes et al., 2017) and phytoplankton community metrics. df = degrees of freedom; * (p ˂ 0.01); ** (p ˂ 0.001); *** (p ˂ 0.0001). D and NS stand for Deviance and Not Significant.

	TREATMENT			TIME			TREATMENT x TIME		
	df1	D	*p value*	df1	D	*p value*	df1	D	*p value*
T (°C)	–	–	–	–	–	–	–	–	–
pH	2	12	NS	1.3	15.9	–	–	–	–
O_2 (mg L^{-1})	–	–	–	–	–	–	–	–	–
Cond. (µS cm^{-1})	2	10	NS	1.4	13.9	***	2.8	13.9	NS
TDS (mg L^{-1})	2	12	NS	2.2	26.7	*	4.4	26.7	NS
P-DIP (µg L^{-1})	2	12	***	3.5	42.3	***	7.0	42.3	***
Chla	2	4183	NS	1	16811	**	2	2669	NS
Biovolume	2	2200588	NS	1	304370459	***	2	1087457	NS
Shannon-Wiener	2	0.45	NS	1	0.01	NS	2	0.01	NS
AGI	2	69	NS	1	162	NS	2	53	NS
PRC (biovolume)	–	–	–	–	–	–	1	4.64	NS

Bacillariophyta (1.5%), Cryptophyta (0.2%) and Ochrophyta (0.3%; Fig. 1). Accordingly, the community was clearly dominated by cyanobacteria, being the filamentous *Shaerospermopsis aphanizomenoides* the most abundant species in biovolume. This species has been renamed and distinguished from *Anabaena reniformis* and *Aphanizomenon aphanizomenoides* (Zapomělová et al., 2009).

Apart from *S. aphanizomenoides,* a total number of 64 phytoplankton species were present in the microcosms over the experimental period (Table 3). Among them, 15 species were the most abundant based on the 0.1% selection criteria (ordered from higher to lower biovolume): *S. aphanizomenoides, Jaaginema* cf. *subtilissimim*, *Geitlerinema amphibium, Planktolyngbya limnetica*, *Microcystis aeruginosa*, *Raphidiopsis mediterranea, Crucigenia tetrapedia, Merismopedia punctata*, *Planktothrix* cf. *agardhii*, *Cyclotella meneghiniana*, *Coelastrum* sp., *Oocystis* sp., *Cryptomonas* sp., *Ochromonas* sp., and *Koliella* sp.

In order to complement the information about the community abundance, considering the biovolume, the 21 species which represented at least 0.1% of the total biovolume over the experiment (ordered from higher to lower values) were: *S. aphanizomenoides*, *Leponcinclis texta*, *Microcystis* sp., *Cryptomonas* sp., *C. tetrapedia*, *R. mediterranea*, *Chaetoceros* sp., *Coelastrum* sp., *Jaaginema* cf. *subtilissimum*, *C. meneghiniana*, *P. limnetica, Oocystis* sp., *G. amphibium, Koliella* sp., *Siderocystopsis fusca, Planktothrix* cf. *agardhii*, *L. subsalsa*, *Chlorella* sp., *Ochromonas* sp., *Gymnodinium* sp. and *Prymnesium* sp.

With respect to the assessment of the effect of MPs addition on phytoplankton composition, Table 3 shows that 14 species were considered as common (present 100-80% of the experimental time) both in the control and treatments. Interestingly, all common species in the control (no MPs addition) were also considered as common in the treatments (except for *Planktothrix* cf. *agardhii*), evidencing that MPs addition did not cause any negative species-specific effect.

Our results also evidenced the absence of significant differences in *Chla* concentration, algal biovolume, diversity index (H′) and AGI both between treatments and in the time × treatment interaction, although significant differences were found over time for biovolume and *Chla*, resulting in a decrease of both indicators (Fig. 2; Table 2). Regarding the AGI index, it did not show any significant change neither between treatments ($p = 0.402$, Table 2) nor in the time × treatment interaction ($p = 0.513$, Table 2).

Finally, PRC revealed no significant effects of treatment on phytoplankton biovolume along the experimental period ($p = 0.897$; Table 2). In Fig. 3, the control treatment is represented by a straight central line and the other two curves show the development of the community under the T-W and T-S treatments along time. The species weight listed on the right axis of the diagram can be considered as the affinity of each species with the PRC response. The display of species within the PRC graph reinforced the observed dominance of *S. aphanizomenoides* over the rest of species grouping closer to each other and far from *S. aphanizomenoides*, regardless of the treatments.

4. Discussion

Our results indicate that there is no strong evidence to infer that MPs caused an effect on phytoplankton community, since no significant differences were found between controls and treatments in any of the studied variables and index. The absence of any significant toxic effect on the phytoplankton community agrees with previous results obtained, in the same experiment, for the zooplankton community (mainly composed of rotifers, Álvarez-Manzaneda et al., 2019b). Specifically, Álvarez-Manzaneda et al. (2019b) found no significant differences (RM-ANOVA test; $p > 0.05$) in total abundance, species richness or species diversity of the zooplankton community among treatments. Accordingly, top-down control on phytoplankton was not significantly affected as a result of MPs addition. With respect to physicochemical and chemical drivers of phytoplankton, MPs addition did not change any of them, except P-DIP concentration. As Funes et al. (2017) recognized, MPs addition reduced P concentrations in lake water and sediment, with both treatments producing a mean reduction of 68 ± 6% P-DIP. At this point, and considering the bottom-up control mediated by nutrient availability, it is crucial to note that, although MPs caused a significant reduction in P-DIP concentration, P-DIP and N-nitrate (NO_3^-) absolute concentrations were still too high for limiting primary production (Funes et al., 2017). Consequently, no changes in the phytoplankton community were plausible, since the still high nutrient concentrations were not limiting for any algae group. In fact, P-DIP and N–NO_3^- concentrations were above the minimum concentration needed for phytoplankton growth, which were proposed by Reynolds (1992, 1999) as 3 µg P-DIP L^{-1} and 80 µg N-Dissolved Inorganic N L^{-1}. In this sense, the presence of the non-N_2 fixing cyanobacteria *Microcystis* sp. in all control and treatments, may confirm that both N and P concentrations are far above their biological requirements. In this scenario, it is outstanding to consider that, in order to cause phytoplankton community changes and promote a notable reduction in water turbidity, TP must be reduced below 0.05–0.1 mg P L^{-1} (Jeppesen et al., 2000). Accordingly to the gained knowledge of our set of studies, a higher initial MPs dose or an additional MPs treatment would be required to reduce TP concentration to a level that promotes the change from the actual hypertrophic cyanobacteria-dominated state towards a mesotrophic community. In this sense, Lürling et al. (2016) noted that, although repeated interventions are not a favorite subject for water managers, in many cases it is unlikely that a single intervention will represent a permanent solution.

Apart from the direct top-down and bottom-up effect of MPs on the phytoplankton community, an additional concern is the indirect effect caused by the potential release of Tot-Fe_{dis} from MPs. In fact, Fe is considered as an essential micronutrient for metabolic and

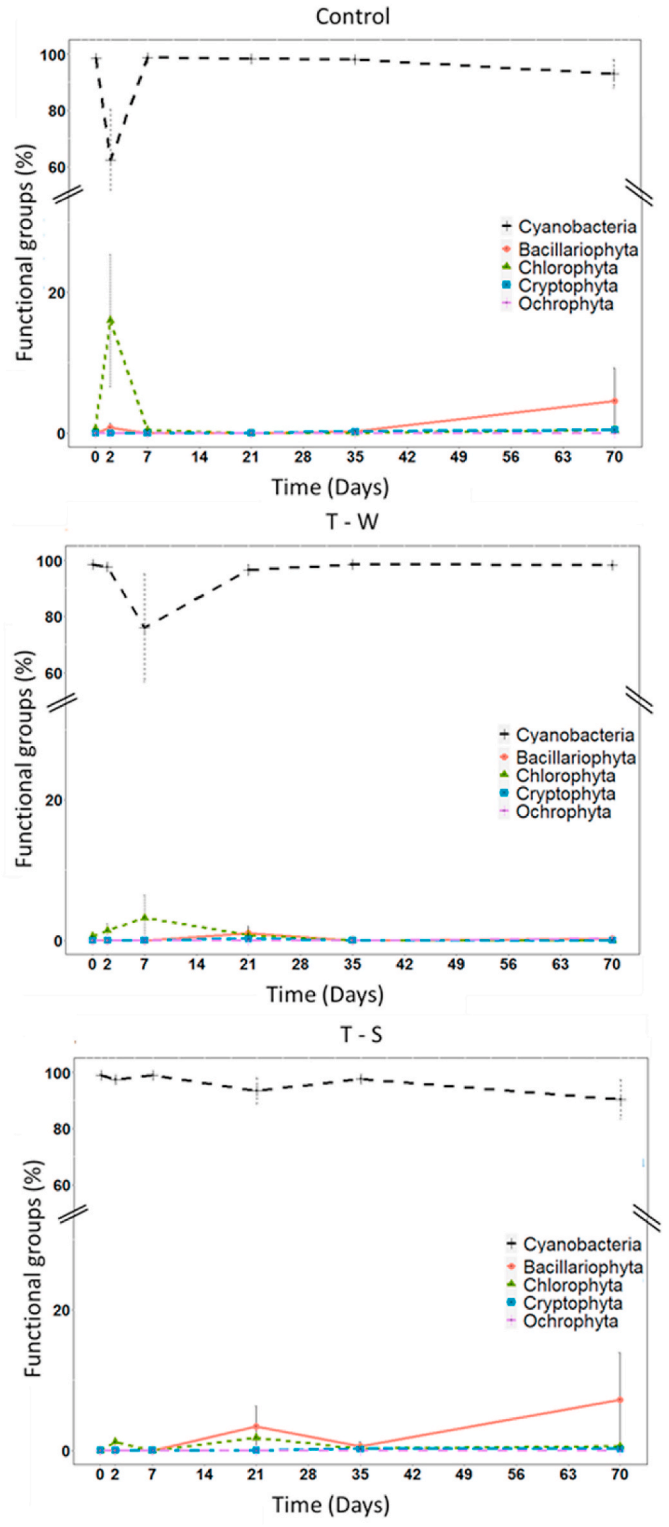

Fig. 1. Temporal dynamics of phytoplankton taxonomic groups (in biovolume) in the control and treatments (T-W and T-S). Error bars represent the standard deviation (mean ± SD, $n = 5$).

photosynthetic functions, nitrate and nitrite reductions, N_2 fixation and detoxification processes (Sunda and Huntsman, 1997). However, at concentrations above its metabolic requirements, Fe is toxic. In this sense, Jagadeesh et al. (2015) found an increase in algal growth of the fresh water algae *Mougeotia* sp. at lower concentrations of Fe oxide nanoparticles (0.1 and 1 mg L^{-1}), while observing toxic effects that

Table 3
List of phytoplankton species present along the experiment. C: Common species (present 100-80% of the experimental time); F: Frequent species (present 80-60% of the experimental time); O: Occasional species (present 60-40% of the experimental time); and R: Rare species (present ≤ 40% of the experimental time).

Taxa	C	T-W	T-S
Amphidinium sp.	R	R	R
Chaetoceros sp.	C	C	C
Chlorella sp.	R	R	R
Chroococcus vacuolatus	R	R	R
Coelastrum sp.	C	C	C
Crucigenia tetrapedia	C	C	C
Cryptomonas sp.	O	F	C
Cyclotella meneghiniana	C	C	C
Cymbella pusilla	R	R	R
Desmodesmus communis	F	O	O
Dictyosphaerium sp.	F	O	R
Dinoflagelate sp.1	R	R	R
Dinoflagelate sp.2	R	R	R
Encyonopsis sp.	R	R	R
Euglena cf. *agilis*	C	C	C
Euglena cf. *oxyuris*	R	R	O
Flagelate sp. 1	R	R	R
Flagelate sp. 2	R	R	R
Flagelate sp. 3	R	R	R
Fragilaria crotonensis	R	R	R
Franceia sp.	R	R	R
Geitlerinema amphibium	C	C	C
Golenkiniopsis parvula	R	R	R
Gymnodinium sp. 1	F	C	O
Gymnodinium sp. 2	R	R	R
Halamphora coffeaeformis	R	R	R
Jaaginema cf. *subtilissimum*	C	C	C
Kirchneriella sp.	R	R	R
Koliella sp.	C	C	C
Lagerheimia subsalsa	F	F	F
Lepocinclis cf. *fusiformis*	R	R	R
Lepocinclis texta	F	O	F

Taxa	C	T-W	T-S
Merismopedia punctata	C	C	C
Merismopedia tenuissima	R	R	R
Microcystis sp.	C	C	C
Monomorphina pyrum	F	R	C
Monoraphidium contortum	R	R	R
Monoraphidium griffithii	R	R	R
Monoraphidium minutum	F	C	C
Navicula cryptocephala	R	R	R
Navicula sp. 1	R	R	R
Navicula tripunctata	R	R	R
Navicula veneta	R	R	R
Nitzschia elegantula	R	R	R
Nitzschia fonticola	R	R	R
Nitzschia palea	O	R	R
Nitzschia sp.	R	R	R
Ochromonas sp.	F	F	O
Oocystis sp.	C	C	C
Phacus orbicularis	R	O	F
Plagioselmis sp.	F	O	F
Planktolyngbya limnetica	C	C	C
Planktothrix cf. *agardhii*	C	C	O
Prymnesium sp.	O	R	F
Pseudanabaena catenata	R	R	R
Raphidiopsis mediterranea	C	C	C
Scenedesmus obliquus	R	R	R
Siderocelis ornata	R	R	O
Siderocystopsis fusca	R	R	R
Sphaerospermopsisaphanizomenoides	C	C	C
Tetraedron minimum	O	O	F
Tetrastrum staurogeniaeforme	R	R	R
Trachelomonas sp.	R	R	O
Tryblionella gracilis	R	R	R

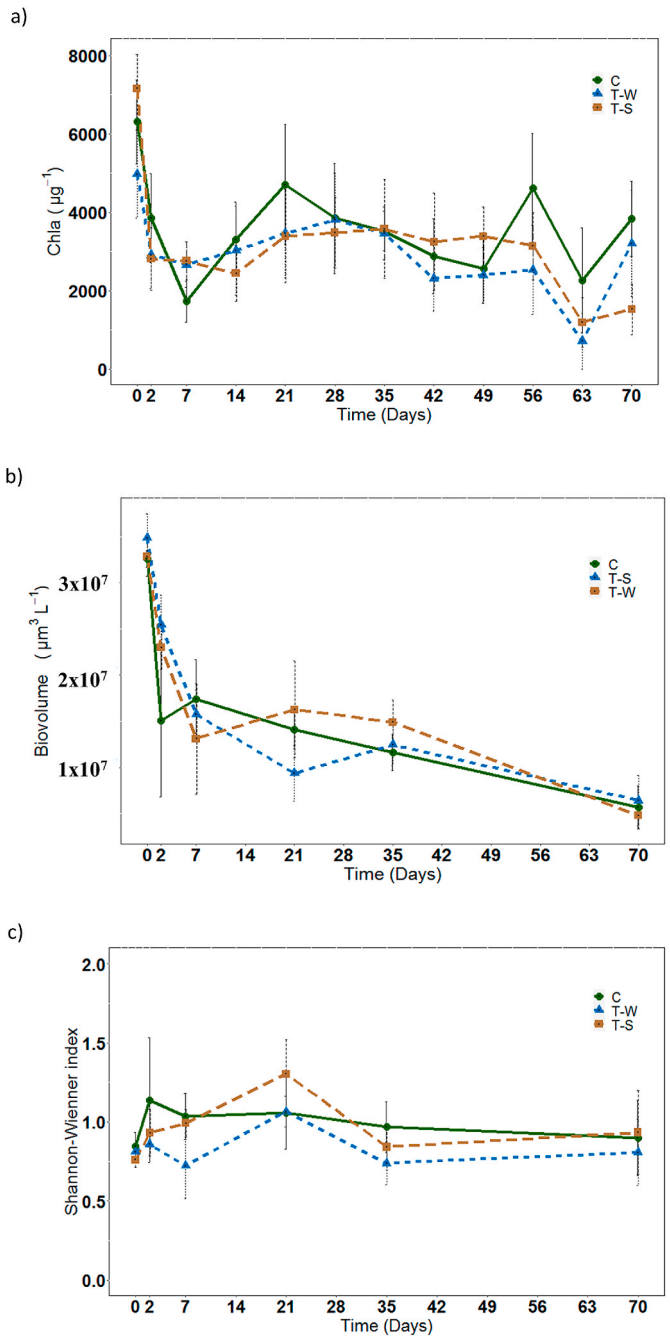

Fig. 2. Temporal dynamics of *Chla* (a); phytoplankton biovolume of all species identified (b) and Shannon-Wiener phytoplankton diversity index (H′) (c) over the experimental period. Error bars represent the standard deviation (mean ± SD, $n = 5$).

caused a decrease of algal growth for 5, 10 and 25 mg L^{-1}. Similarly, Keller et al. (2012) reported that the effect of Fe concentrations on algal growth was interspecies dependent. In fact, algal growth of *Pseudokirchneriella subcapitata* experienced a reduction at 10 mg Fe^{2+} L^{-1} and 25 mg Fe^{3+} L^{-1}, while *Isochrysis galbana* growth was only negatively affected at concentrations above 50 mg Fe^{2+} L^{-1} and 75 Fe^{3+} L^{-1}. In any case, the absence of any toxic effect of MPs on phytoplankton community in Honda lake could be also explained by the lack of release of dissolved Fe from MPs, since its concentration in the control and treatments was below the detection limit (<10 µg L^{-1}).

The use of novel P adsorbents for restoring eutrophicated ecosystems has increased in the last years (e.g. Lürling et al., 2016) and, consequently, a growing number of studies have assessed their lethal and sublethal effects on aquatic biota (van Oosterhout and Waajen et al., 2017; Yamada-Ferraz et al., 2015; Lürling, 2013; Lürling and Tolman, 2010). At this point, it is important to consider that there exist a continuum of experimental approaches and tools, including single-species toxicity tests, natural ecosystems manipulation, experimental laboratory food chains and indoor microcosms (Caquet, 2013). As Álvarez-Manzaneda et al. (2019b) remark, in this continuum of experimental approaches, outdoor microcosms are characterized by a high complexity-ecological realism (including both direct and indirect effects) and by the possibility of replicability. However, up to date, there exist much more empirical studies focused on the responses of single phytoplankton species (Álvarez-Manzaneda et al., 2019a; Álvarez-Manzaneda and de Vicente, 2017; Wang et al., 2016; van Oosterhout and Lürling, 2013) than those focused on the response of the overall phytoplankton community (Nürnberg, 2017; Lang et al., 2016). This is especially striking considering that, obviously, potential shifts in phytoplankton community structure will influence the community structure of the higher trophic levels.

Despite the inherent limitations for comparing our results from the outdoor experiment with single-species toxicological experiments reported in the literature for the same adsorbents (MPs), we found radically different outcomes. In this sense, Álvarez-Manzaneda & de Vicente (2107) found that the algal growth of *Chlorella* sp. was strongly reduced in the presence of MPs concentrations above 0.5 g MP L^{-1} (EC_{50}: 0.15 g MP L^{-1}). These results are considerably different from ours, since no effect of MPs (when adding much higher MPs concentrations, 1.4 g MP L^{-1}) was found in any phytoplankton species, including *Chlorella* sp. One likely reason behind such great differences is that, in the *Chlorella* experiment (Álvarez-Manzaneda and de Vicente, 2017), extremely high turbidity and Tot-Fe_{dis} concentrations were observed, evidencing that MPs are much easily dissolved in BBM (algal growth medium) than in natural waters. Therefore, the inconsistency observed between our results and those found in single-species tests with *Chlorella* sp. remarks the much greater realism of outdoor microcosm experiments and the need to perform this type of experiments resembling natural ecosystems to assess the fate and effects of chemicals on different levels of organization.

Among all phytoplankton taxonomic groups, cyanobacteria constituted the dominant one along the whole experimental period. Similar results were found by Moreno-Ostos et al. (2007), who recognized the relevance of relatively high water column thermal stability in Honda lake for promoting a phytoplankton community dominated by large filamentous cyanobacteria. It is especially relevant to point out that the filamentous *S. aphanizomenoides*, which was the most abundant species in the present study, is a potentially toxic and invasive species typical of the tropical and subtropical regions, which has been recently reported in Central Europe (Zapomě;lová et al., 2009). Additionally, it is worth highlighting that, due to ecosystem and human health concerns, *R. mediterranea, Ochronomas* sp. and *Amphidinium* sp. are able to produce toxins and survive under extreme conditions of nutrient availability, solar radiation and temperature (Tanabe et al., 2011; Ismael et al., 2008; Camargo et al., 2005).

Finally, in the context of any successful restoration project for combating eutrophication, it is essential to keep in mind that, if the final goal is to limit harmful cyanobacteria blooms, a higher initial MPs dose or several MPs applications are required to achieve a lower P-DIP concentration. For stripping cyanobacteria from the water column, Jančula and Maršálek (2011) and later Lürling et al. (2016) reviewed some of the most straightforward measures, which include the classical algaecides (e.g., copper sulphate) and geo-engineering P adsorbents. In short, the use of poly-aluminum chloride (Noyma et al., 2015) as a flocculent has been demonstrated to effectively aggregate cyanobacteria and sink them out of the water column. Regarding Phoslock® addition, van Oosterhout and Lürling (2013) found, in a laboratory study, that it caused a reduction in the growth of all phytoplankton species (green alga

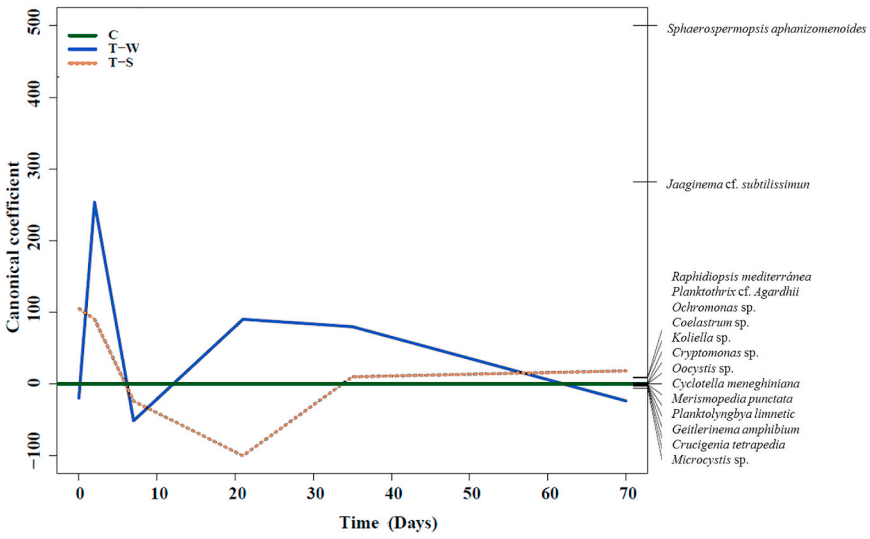

Fig. 3. Principal Response Curves (PCR) resulting from the community analysis showing the effects of MPs addition on phytoplankton biovolume from the selected species. The straight green central line represents the control treatment, the blue and brown lines represent the T-W and T-S treatments along time, respectively. The y-right axis shows the species weight, which represents the affinity of each species with the PRC response. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Scenedesmus obliquus, cyanobacteria *Microcystis aeruginosa* and *Anabaena* sp.), due to the combined effects of light limitation, flocculation with the bentonite and binding of P-DIP to Phoslock®. Despite the positive outcome of those single measures, successful restoration strategies are frequently not isolated actions (Gołdyn et al., 2014). For instance, Lürling and Faassen (2011) reported that only a combined strategy of dredging and Phoslock® addition achieved a significant decrease in both cyanobacteria biomass and microcystin concentrations. Lastly, apart from chemical methods, previous studies have also reported the convenience of changing physicochemical conditions by using bubble-plume mixing systems for mitigating water quality problems such as cyanobacteria blooms (e.g. Chen et al., 2018; Imteaz and Asaeda, 2000).

5. Conclusions

Based on the results of the present study and those obtained in previous studies focused on both assessing P adsorption capacity under different physicochemical conditions (Funes et al., 2016, 2017, 2018; de Vicente et al., 2010, 2011) and on toxicity tests on different trophic levels (Álvarez-Manzaneda et al., 2017, 2019a; 2019b; Álvarez-Manzaneda and de Vicente, 2017), we can conclude that MPs are promising P adsorbents to be used for lake restoration. More specifically, our results indicate that there is no strong evidence to infer that MPs caused an effect on the phytoplankton community, since no significant differences were found between the controls and treatments in any of the studied variables and index. However, a drawback is that cyanobacteria did not decrease despite of the notable reduction in P concentration. Thus, if the main goal of the restoration plan is to combat cyanobacteria blooms, a higher initial MPs dose or several MPs applications would be required to obtain a final P-DIP concentration low enough to limit cyanobacteria dominance.

Credit author statement

Ana del Arco: designed the methodology, carried out the sampling and the later microcosm experiment, analyzed the data, wrote the the manuscript. Inmaculada Álvarez-Manzaneda: carried out the sampling and the later microcosm experiment. Ana Funes: carried out the sampling and the later microcosm experiment. Carmen Pérez-Martínez: contibuted on phytoplankton analysis. Inmaculada de Vicente proposed the study and got the financial support, designed the methodology and she was also enrolled in the sampling, she later wrote the manuscript.

Declaration of competing interest

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

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