Biodegradable plastics can alter carbon and nitrogen cycles to a greater extent than conventional plastics in marine sediment

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HIGHLIGHTS

- Plastics promoted anaerobic sediment metabolic pathways.
- Biodegradable plastics stimulated the decomposition of buried carbon.
- Biodegradable plastics reduced the release of inorganic nitrogen.

GRAPHICAL ABSTRACT
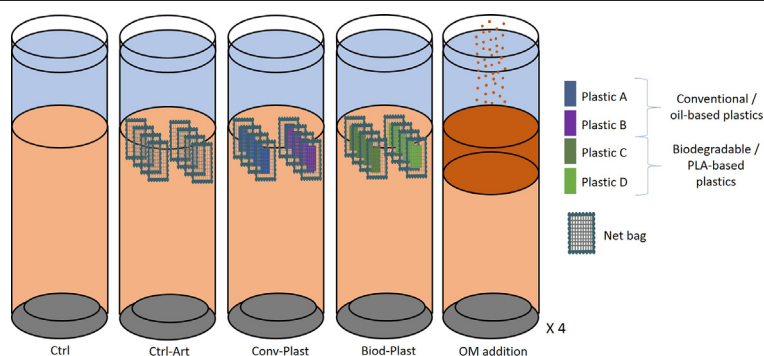
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ABSTRACT

The seabed constitutes a global sink for plastic debris, where they can remain for centuries. Biodegradable plastics offer the advantage of having less persistence in the environment than conventional ones. The seabed is responsible for key ecosystem functions related to the cycling of elements by decomposing the labile fraction of organic matter and fueling primary production, while storing the most recalcitrant part of this organic matter and limiting CO_2 emissions. Although plastics are expected to affect these processes, knowledge on this matter is scarce. In controlled microcosms, we show that biodegradable plastics can stimulate the decomposition of marine-buried carbon and reduce the release of inorganic nitrogen. We found that conventional and biodegradable plastics promoted anaerobic sediment metabolic pathways. Biodegradable plastics produced a two-fold CO_2 release to the water column, which suggests the decomposition of not only plastics, but also of buried organic carbon. The stimulation of sediment metabolism could be due to excessive carbon consumption by bacteria that derives from a rise in the carbon:nitrogen ratio. Accordingly, the NH_4^+ flux to the water column lowered. As NOx fluxes also lowered, biodegradable plastics might promote nitrification-denitrification coupling. If biodegradable plastics become a major component of marine pollution, then sediment biogeochemical cycles might be strongly influenced, which could affect the carbon sequestration of coastal ecosystems and compromise their mitigation capacity against climate change.

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1. Introduction

The exponential plastic production rate, along with poor plastic management (Geyer et al., 2017), is leading to an enormous and continuously increasing input of plastic debris in nature (Jambeck et al., 2015). Oceans are receiving large quantities of plastic debris. Despite much of

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the plastic debris that enters the ocean initially floating because its density is lower than seawater, it is colonized by fouling organisms and can become negatively buoyant and sink (Lobelle and Cunliffe, 2011). Indeed 70% of the plastic that enters oceans can end up on the seabed (Barnes et al., 2009), which would yield concentrations on the seabed of several orders of magnitude higher than in the water column (Erni-Cassola et al., 2019).

The sheer persistence of plastics in the environment, which is accentuated in deep areas like abyssal zones (Krause et al., 2020), aggravates their environmental drawbacks. On order to minimize this environmental problem, biodegradable plastics have been developed. Despite the misuse of the term biodegradability (Nazareth et al., 2019; Viera et al., 2020), biodegradable plastics can be transformed in nature into water and CO_2 or CH_4 during a period of time comparable to that of organic matter (Albertsson and Hakkarainen, 2017). Notwithstanding, their persistence in the environment can vastly vary according to the specific conditions of different environments (Beltrán-Sanahuja et al., 2020). Despite still being a small fraction of the total produced plastic, the global market for biodegradable plastics is expected to grow by more than 40% in the next 5 years (European Bioplastics, 2019).

Marine sediment receives large amounts of organic matter. In turn, sediment recycles the labile part of this organic matter by releasing the derived inorganic compounds that fuel the water column's primary production (Lavelle et al., 2005). The recalcitrant part of the organic matter that marine sediment receives is buried (Burdige, 2007). Coastal systems are among the largest carbon reservoirs on our planet. The carbon that accumulates there is termed Blue Carbon and contributes to mitigate climate change (Nellemann et al., 2009).

The capacity of plastics to act as a barrier for solutes and gases is expected to pose serious deleterious effects for the decomposition processes taking place on the seabed (Goldberg, 1994; Krause et al., 2020; Law, 2017). Additionally, inputs of biodegradable plastics to coastal sediment can have a similar effect to that of organic matter, which can boost the decomposition of carbon buried in sediment and, consequently, CO_2 release (Bianchi, 2011). Very little research has been conducted in this research field (Green et al., 2015), especially in relation to biodegradable plastics (Doering et al., 1994).

The aim of this study was to test the effects of conventional and biodegradable plastics on marine biogeochemical cycles related to carbon, oxygen, nitrogen and phosphorus. We hypothesized that both conventional and biodegradable plastics would promote sediment reduction conditions, and that the effect of biodegradable plastics would become greater due to their biodegradability. Through a microcosm experiment, the fluxes of these elements were measured according to the effects of conventional and biodegradable plastics, and were compared to organic matter pollution conditions.

2. Materials and methods

2.1. Experimental setup

Marine conditions were simulated with a microcosm experiment by using cylinders in an incubation system (Piedecausa et al., 2012), which enables environmental conditions to be controlled. Each experimental unit was a methacrylate cylinder, which was 32 cm high and had an internal diameter of 5.4 cm. Cylinders were closed at the bottom with rubber stoppers and filled with sediment up to a depth of 20 cm. In March 2018, the sediment used in cylinders was collected from the upper subtidal area in the surroundings of a beach in SE Spain in the Mediterranean Region (Santa Pola; 38°11′18.81″N 0°35′34.55″O). It was sieved through a 0.5-mm mesh to remove large particles of organic matter and macrofauna. Sediment was carbonated, which is typical of the Mediterranean, and was graded as very fine and a fine sand grain (0.063–0.25 mm) according to the Wentworth (Wentworth, 1922) classification. The rest of the cylinder was filled with seawater, which was also collected in the same area and filtered (0.5 mm). Seawater had a salinity of 37.6 PSU. Cylinders were placed inside the incubation system, which was a tank filled with the same seawater as cylinders. The tank's water level came very close to the top of the cylinder to keep the temperature of the whole water column constant, but to also prevent water mixing in it and to avoid the possible transfer of substances from plastics. Each cylinder had a cannula that was connected to air pumps for aeration. Cannulas were placed ca. 4 cm from the sediment surface, which is an optimum distance to prevent not only water stratification through the bubbling generated by the air leaving cannulas, but also sediment resuspension. Additionally, twice daily the water in each cylinder was vigorously mixed by means of a 50 mL syringe by taking special care to avoid sediment resuspension in order to minimize water stratification. The oxygen level of the water column of cylinders was maintained between 7.1 and 8.6 mg O_2 L^{-1} in order to simulate benthic normoxic conditions (>2 mg O_2 L^{-1}). The water in each cylinder was changed 3 times a week to prevent the excessive accumulation of metabolic byproducts. This water was also previously filtered and aerated. Cylinders were maintained at 16 °C by means of an aquarium chiller and a pumping system that constantly recirculated the water in the tank, and not contained in cylinders, through the chiller. This temperature resembles that of the water in proximity to the seabed in the area where sediment and water were collected, and was an approximate average of the seawater temperature close to the seabed in coastal areas, which is widely used in microcosm experiments in relation to seabed coastal biogeochemistry (Banta et al., 1999; Casado-Coy et al., 2017). Temperature was monitored twice daily with a thermometer. Sediment was left to naturally stratify for 10 days in the incubation system before the experiment began.

2.2. Plastic materials

Four commercially available film packaging plastic materials were employed as plastic is widely used for packing (Geyer et al., 2017), of which film is one the most commonly utilized plastics, and is also widespread in agriculture and for producing carrier bags (Plastic, 2018). Two of the materials were conventional petroleum-based plastics (plastics A and B). Plastic A was a composite material of several polymers that formed a multilayer polyamide, namely (PA)/polypropylene(PP)-Ethylene-Vinyl-Alcohol (EVOH)-PP. The PA side had a melting temperature (T_m) of 214 °C, an enthalpy of fusion (ΔH_m) of 8.73 J/g and a crystallization index (CI) of 3.8%. The PP side had a T_m of 185 °C, an ΔH_m of 5.5 J/g and a CI of 3.9%. Plastic B was also a multilayer material based on polyethylene terephthalate (PET)/polyethylene (PE). The PET side presented a T_m of 245 °C, an ΔH_m of 4.42 J/g and a CI of 3.3%. The PE side had a T_m of 117 °C, an ΔH_m of 64.67 J/g and a CI of 22.3%. The other two (plastics C and D) were biobased materials that contained polylactic acid (PLA), which is the largest biodegradable plastic (Tokiwa et al., 2009) produced globally (Chinthapalli et al., 2019). Plastic C was a PLA-based transparent heat-sealable compostable film. Plastic D was a bilayer of laminate paper/PLA with a T_m of 174 °C, an ΔH_m of 11.75 J/g and a CI of 12.5%. These materials were provided by plastic companies as end products ready to be used by consumers.

Each material type was cut into rectangles (3.5 per 1.5 cm), weighed and placed inside fiberglass net bags (5 per 3 cm, 1 mm mesh size; Fig. S1) to prevent loss of material. Net bags were sealed with cotton thread. Plastics that reach the ocean generally come from land (Geyer et al., 2017), and can be transported by ocean currents before reaching the seabed (Andrady, 2015). The plastics used in our experiment were placed inside the net bags and left in a 35-l bucket filled with seawater in the lab at ambient temperature for 7 days (daily variation ranged from 20 °C to 23 °C) near a window so that polymers could receive direct sunlight for approximately 2–3 h/day.

2.3. Experimental design

The experiment consisted in five treatments: a control (Ctrl); an artifact control with the net bags used to contain plastics, but without

plastics (Ctrl-Art); conventional plastics, which contained A plastics and B (Conv-Plast); biodegradable plastics, which contained plastics C and D (Biod-Plast); the treatment simulating organic matter pollution, to which organic matter was added (OM addition). All the treatments were replicated 4 times ($n = 4$, $N = 20$). Each cylinder for the Conv-Plast treatment had four plastic A pieces and four plastic B pieces, and each cylinder in the Biod-Plast treatment had four plastic C pieces and four plastic D pieces. Thus each cylinder in the Conv-Plast and Biod-Plast treatments had eight plastic pieces and each one was contained in a single net bag to be buried vertically in the first 4 cm of sediment (Fig. S2).

The plastic concentration used in this study corresponded to either 3493 particles·m^2 or 164.4 ± 1.35 and 114 ± 1.13 (mean ± standard error) g of plastic·m^2 for the conventional and biodegradable plastics, respectively. Data on small-sized macroplastic concentrations in natural environments are scarce and extremely variable. Despite this concentration being considerably high, it falls within the ranges of particles detected in coastal sediment (Erni-Cassola et al., 2019), which are expected to increase exponentially in the near future (Brandon et al., 2019), and it also falls within the same weight range as reported on the beaches of Henderson Island, one of the world's most remote and pristine islands (Lavers and Bond, 2017). Additionally, the fact that plastics were buried vertically limited the most negative expected effect of plastic on seabed biogeochemistry: lowering diffusion rates of solutes between the water column and the seabed (Goldberg, 1994). Accordingly, the effect of lowering diffusion rates of solutes was not maximized despite plastic bags sometimes totally "wrapping" the seabed surface in nature (Green et al., 2015).

To take into account any possible artifact effects of the net bags, another group of four cylinders was added, and each contained eight net bags without plastics. To know the natural levels of the response variables in this study, another group of four cylinders without net bags and without plastics was included to be used as controls. Finally, another group of four cylinders was enriched with organic matter to compare plastic pollution to organic pollution, which constitutes another source of carbon in the environment. Organic pollution was simulated by adding labile organic matter in the form of fish feed, [L-4 Alternate CMX 20 2P BB2, SKRETTING (46.5% protein, 20% fats and oils, 6.1% minerals, 2.2% fiber, 1% P, 0.9% Ca and 0.4% Na)], which was finely ground and mixed with sediment. The organic matter mixed with sediment was initially added in the first 4 cm of sediment at a final concentration of 29 g of finely ground feed per kg of sediment. These cylinders were filled weekly with 4.55 g of the same type of sediment that was initially used and with the same feed concentration on the sediment surface after extracting the water above sediment. This periodicity aimed to reach a good compromise when simulating the organic pollution conditions that occur in impacted areas, such as marine fish farms, where organic matter pulses may occur once or twice daily (Sanz-Lazaro and Sanchez-Jerez, 2020), while avoiding the experimental units being over-manipulated (Casado-Coy et al., 2017; Valdemarsen et al., 2009). Adding organic matter was done to stimulate sediment metabolism (Casado-Coy et al., 2020) and to represent a possible organic pollution level in aquaculture facilities (Sanz-Lázaro et al., 2011a).

Experiment duration was 30 days. At the end of the experiment, all the net bags were removed after extracting the water above the sediment in cylinders to limit any possible loss of plastics that could be attached to the surrounding sediment inside the net bags and to be washed by the effect of water. Then they were carefully washed in distilled water over a 0.5 mm mesh sieve to prevent any possible loss of plastics and to remove sediment. Bags were opened and plastics were left to dry at 30 °C until constant weight was reached.

2.4. Plastic material weight loss

The weight loss of each plastic material after 1 month was calculated by the weight differences recorded at the beginning and end of the experiment on a precision balance (±0.0001 g). Plastic materials were always washed twice in distilled water for 5 min and kept in a desiccator shielded from light at 30 °C until constant weight was reached. Further information on the degradability of used plastics is reported in Beltrán-Sanahuja et al. (2020).

2.5. TCO$_2$, SOU and nutrient fluxes

Total CO$_2$ (TCO$_2$), sediment oxygen uptake (SOU) and nutrient fluxes were determined by performing six incubations while the experiment was underway. Incubations were performed by sealing the upper cylinder part with a rubber stopper for specific time periods [2–3 h for organic enriched (+OM), 4–5 h for the non-organic enriched (-OM) cylinders] to prevent O$_2$ decreases below 60% in the initial concentration in the water column (Glud, 2008). First, O$_2$ samples were measured with an oximeter (Crison OXI 45 P, Hach Lange, Spain) that was placed in the core and stirred very gently to homogenize the water column and to break any possible stratification. Then water column samples were taken with a syringe for the other measurements in an attempt to minimize bubble production. Samples were taken at the beginning and end of incubations to calculate the consumption/production rates. The values of the samples taken at the beginning of incubations were always controlled to check that values fell within the range of the expected natural values to detect any possible artifact. TCO$_2$ was measured by total carbon titration with titrasol HCl (0.1 mol·L^{-1}, Applichem Panreac, Germany) within two pH ranges (Gran et al., 1950). The water concentration used to measure the nutrient (NH$_4^+$, PO$_4^{3-}$, NO$_2^-$, NO$_3^-$) was initially filtered (mixed cellulose ester syringe filter, size 0.22 μm, MS, USA), transferred to 15 mL plastic vials and frozen (−20 °C) until analyzed in an Automated Wet Chemistry Analyser - Continuous Flow Analyser (Breda, the Netherlands). Fluxes were integrated throughout the experimental time by incubations. They are reported as mmol·m^{-2}·d^{-1}.

2.6. Data analysis

The weight loss of each plastic material was assessed by a one-sample Student's *t*-test. Planned comparisons among groups of treatments were tested as part of a one-way analysis of variance (ANOVA) for the following fluxes: TCO$_2$, SOU, TCO$_2$:SOU ratio, NH$_4^+$, NO$_2^-$ and NO$_3^-$ (NOx) and PO$_4^{3-}$. The following comparisons were performed: 1) OM vs. (Ctrl, Ctrl-Art, Conv-Plast and Biod-Plast); 2) Ctrl vs. Ctrl-Art; 3) Ctrl-Art vs. Conv-Plast; 4) Ctrl-Art vs. Biod-Plast. Despite comparisons Ctrl-Art vs. Conv-Plast and Ctrl-Art vs. Biod-Plast not being orthogonal to the other comparisons, they were done because they were more relevant than the corresponding orthogonal comparisons in relation to the study objective. No adjustment was made to the significance levels because the number of comparisons was sufficiently low (Quinn and Keough, 2002).

To test if the effect of plastic materials on sediment metabolism could be explained solely by the mineralization of plastic materials, a two-tailed Student's *t*-test analysis was performed between the estimates of the carbon produced as TCO$_2$ release by the effect of both the materials and the carbon mineralized through the decomposition of materials. The effect of the plastic materials on TCO$_2$ fluxes was estimated for each cylinder by subtracting either the TCO$_2$ flux in the cylinders containing the conventional (*Conv-Plast*) or the biodegradable (*Biod-Plast*) materials from the TCO$_2$ flux in the control cylinders containing only net bags (*Ctrl-Art*). This TCO$_2$ flux was transformed into the amount of carbon produced during the experiment. In order to estimate the carbon mineralization deriving from the decomposition of materials, the total mass loss of materials during the experiment was assumed to be mineralized. The weight loss of each material was multiplied by the corresponding mass carbon ratio of the polymer that each material was made of, with 0.86, 0.63, 0.62, 0.5 and 0.44 for PP + PE, PA, PET, PLA and cellulose (paper), respectively. With the composite

materials, it was assumed that plastics A, B and D were composed mainly of PA + PP, PE + PET and PLA + cellulose, respectively, and had an equivalent per weight concentration of their forming polymers. The carbon loss of each material from a specific cylinder was summed to obtain the total carbon loss occurring during the experiment in each cylinder.

Before carrying out the statistical tests, normality and homogeneity of variance were checked by Cochran's test and p-p plots (Underwood, 1997), respectively, using the R software (v. 2.15.0) and the GAD statistical package (Sandrini-Neto and Camargo, 2015). If assumptions were not met, transformations were applied and assumptions were rechecked. By the two-tailed Student's t-test, as unequal variances were still present after transformation, the Welch test was run with function T.TEST (type 3) in Excel. The planned comparisons were calculated with Excel to ensure that the sum of squares (SS) was correctly calculated in all the comparisons. Data were reported as mean ± standard error (SE). An $\alpha = 0.05$ significance level was generally used for the statistical analyses. If the assumptions were still not met, the level of significance was lowered to $\alpha = 0.01$ (Underwood, 1997).

3. Results and discussion

In our experiment, plastic weight loss was 0.22 ± 0.09, 0.05 ± 0.05, 32.8 ± 4.58 and $8.79 \pm 0.44\%$ for plastics A, B, C and D, respectively. Only biodegradable plastics (C and D) showed significant ($p < 0.01$) weight loss. After 1 month, conventional plastics showed no sign of degradation, while biodegradable plastics remained as a single or very few pieces, that were partly transformed into a mucus-like substance. During the experiment under our organic pollution conditions, the fluxes of TCO_2, SOU, NH4+, and PO_4^{3-} were notably enhanced, while the NOx flux was markedly low. The high TCO_2 levels at the beginning of the experiment could be related to the initially large addition of OM to the first 4 cm of cylinders, which coincided with a notable release of NH_4^+ and PO_4^{3-}. The other treatments showed more homogeneous and stable trends of these fluxes during the experiment, except for the sediment with biodegradable plastics and only with net bags, with slightly higher TCO_2 and SOU levels, respectively (Fig. S3).

The time-integrated TCO_2 values in the control and control artifact cylinders, and also in the cylinders containing conventional plastics, were comparable with sediment not undergoing organic pollution. The time-integrated TCO_2 values of the cylinders containing biodegradable plastics, and of the cylinders undergoing organic pollution, fell within the range of sediment affected by organic pollution (fish farming) (Holmer et al., 2002, 2003). The time-integrated SOU values in the control and control artifact cylinders, and in the cylinders containing

conventional and biodegradable plastics, were typical of the sediment not influenced by organic pollution. The time-integrated SOU values cylinders undergoing organic pollution were similar to the sediment affected by organic pollution, such as fish farming (Holmer et al., 2002, 2003; Holmer and Kristensen, 1992) (Fig. 1).

The high TCO_2:SOU ratio in the cylinders undergoing organic pollution (4.3 ± 0.4) was comparable to the sediment affected by organic pollution (Holmer et al., 2003). Conventional plastics moderately raised the time-integrated TCO_2: SOU ratio by 1.2 units over the control artifact cylinders. This effect was much stronger for biodegradable plastics, which raised the time-integrated TCO_2: SOU ratio by 4.4 units over the control artifact cylinders and significantly differed from the control artifact cylinders ($p < 0.001$), which were higher than under the organic pollution conditions (Fig. 1). TCO_2: SOU ratios close to 1 indicate balanced biogeochemical cycling, where aerobic respiration prevails and the by-products of anaerobic pathways, such as sulfides, are re-oxidized through oxygen consumption. As the TCO2: SOU ratio increases, the sediment conditions further reduce as the prevalent metabolic pathways become anaerobic and their byproducts are not re-oxidized, but buried in sediment in the form of acid volatile sulfides that create oxygen debt (Sanz-Lázaro et al., 2011b).

These results suggest that plastics are expected to promote anaerobic metabolic pathways. This is especially notable with biodegradable plastics, which can promote anaerobic metabolic pathways to a greater extent than organic pollution despite the relatively moderate enhancement of sediment metabolism. This can be explained by the cylinders oxygen debt organic pollution not only raising the TCO_2 flux, but also the sediment oxygen uptake. With biodegradable plastics, although the TCO_2 increase was less marked than under the organic pollution conditions, the sediment oxygen uptake levels were lower than in the cylinders that contained only net bags (Fig. 1; Table S1). These results support previous research, in which a bioplastic (polyhydroxyalkanoate, PHA) promoted sulfate-reducing bacteria in marine biofilms (Pinnell and Turner, 2019).

The time-integrated SOU limitation by both plastic types was believed to be due to their barrier effect against solutions and gases (De Souza Machado et al., 2018; Goldberg, 1994; Green et al., 2015), which was likely enhanced by the type of plastics used (film) with a high surface area to volume ratio (Rillig et al., 2019). The net bags employed to contain plastics in our experiment showed the opposite effect. The cylinders with only net bags produced a non-significant 43.3% increase in the TCO_2 flux compared to the control cylinders (Fig. 1; Table S1). The mesh structure of the net bags could produce spaces in sediment similarly to sediment with coarse particles, which would increase the amount of pore water in sediment and favor its exchange with the water column rich in electron acceptors (Casado-Coy et al.,
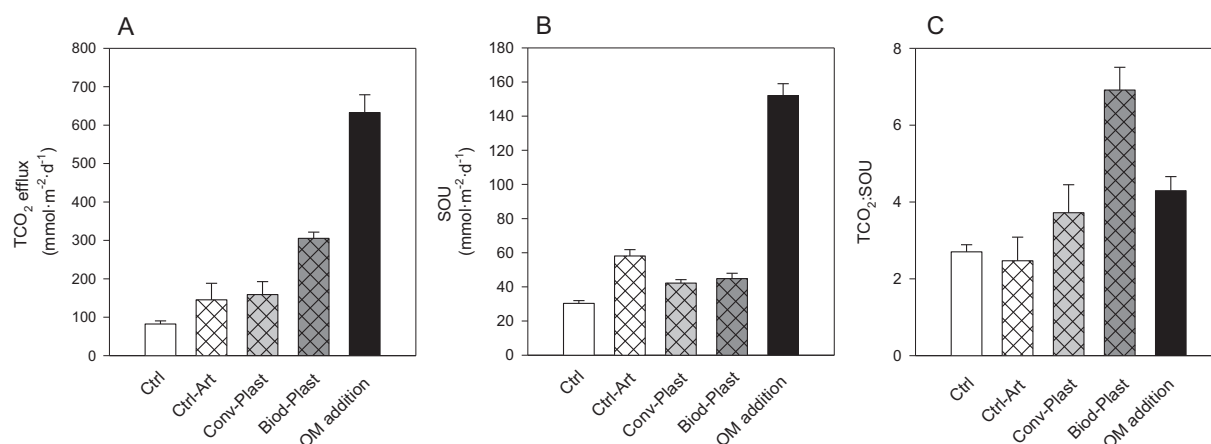

Fig. 1. Time-integrated fluxes of total CO_2 (TCO_2) (A), sediment oxygen uptake (SOU) (B), and the TCO_2 and O_2 integrated fluxes ratio (C) (mean ± SE, n = 4). Each panel has five treatments: control (Ctrl), artifact control with the net bags used to contain plastics, but without plastics (Ctrl-Art); conventional (Conv-Plast; plastics A and B); biodegradable plastics (Biod-Plast; plastics C and D); treatment simulating organic matter pollution (OM addition).

Table 1
Estimates of the carbon released as total CO_2 (TCO_2) from sediment due to the effect of plastics and carbon loss owing to their decomposition ($n = 4$, mean $\pm$ SE) per cylinder, and the potential contribution of carbon loss due to decomposition to TCO_2 release because of the effect of plastics. Values in bold indicate significant ($p < 0.05$) effects on TCO_2 release (2nd column), carbon loss due to decomposition (3rd column) and differences between the carbon released as TCO_2 due to the effect of each plastic type and the potential carbon loss owing to their decomposition (4th column).

Plastic type	Carbon (g) released as TCO_2	Carbon (g) loss from plastic degradation	Potential contribution of carbon loss from plastic degradation to carbon released as TCO_2 (%)
Conventional	0.01 ± 0.03	0.0004 ± 0.0001	3.7
Biodegradable	0.12 ± 0.01	0.02 ± 0.001	16.3

2017) which, in turn, would enhance sediment, mainly the aerobic metabolism (Martinez-Garcia et al., 2015). Our results suggest that plastic pollution can contribute to impoverish the environmental status of sediment by modifying biogeochemical conditions through oxygen depletion. Hypoxic conditions promote anaerobic metabolic pathways and the derived production of the corresponding toxic byproducts. These conditions can lead to loss of biodiversity and ecosystem functions (Sanz-Lázaro and Marín, 2011).

The TCO_2 flux in the cylinders with biodegradable plastics doubled and was significantly higher ($p = 0.004$; Table S1) than in the cylinders with only net bags (Fig. 1). This finding suggests that sediment metabolism can be greatly enhanced by biodegradable plastics. An increase in the TCO_2 flux is expected, to some extent, because biodegradable plastic is available carbon for bacteria, which is mineralized and contributes to the TCO_2 flux. The increment in carbon mineralization produced by biodegradable plastics during the experiment (0.12 ± 0.01 g carbon per cylinder) was significantly greater ($p = 0.003$) than the estimated mineralized carbon deriving from the weight loss of biodegradable plastics (0.020 ± 0.001 g carbon per cylinder). These estimates indicate that the carbon mineralization stimulation might be caused by not only the carbon mineralized from biodegradable plastics, which only constituted a minor fraction (16%) of the mineralized carbon (Table 1). Hence the carbon that was added in the form of biodegradable plastic could have stimulated the mineralization of the buried organic matter. Organic matter inputs with readily available energy can stimulate the mineralization of buried carbon (Heimann and Reichstein, 2008), especially when the available organic matter has a high carbon:nitrogen (C:N) ratio (Bianchi, 2011). Organic matter with a low nitrogen concentration lowers the carbon-use efficiency of decomposing bacteria. Accordingly, bacteria need to mineralize more carbon to assimilate the same amount of nitrogen (Manzoni et al., 2008). Plastics are a paragon example of a high C:N ratio because they generally have a very low nitrogen content compared to most organic matter types. Thus biodegradable plastics can promote the decomposition of carbon buried in sediment (Heimann and

Reichstein, 2008), which may have occurred in our experiment. A 1-fold rise in sediment metabolism above the basal level can have major consequences on the mobilization of the carbon buried in sediment.

As regards nitrogen, the time-integrated NH_4^+ and NOx fluxes reported in our cylinders fell within the range of the values observed in nature both with and without the influence of organic pollution (Holmer et al., 2002, 2003). Only the NH_4^+, values were one order of magnitude above what can be expected in cylinders undergoing organic pollution (Fig. 2). This outcome could be due to the remarkably high values obtained during incubation, especially in the first incubations that related to marked sediment metabolism, as reflected by the high TCO_2 values (Fig. S3).

The sediment with biodegradable plastics showed a significant decrease in NH_4^+ release ($p = 0.014$; Fig. 2; Table S1). Biodegradable plastics in sediment constitute a carbon input which raises the C:N ratio of sediment. As previously mentioned, the bacteria stimulated by the available carbon of bioplastics have a set nutrient ratio requirements, which may have lessened their carbon-use efficiency to exploit this allochthonous energy source (Manzoni et al., 2008) and could explain the low NH_4^+ release compared to the TCO_2 release. The low NH_4^+ fluxes could also derive from marked nitrification activity which, along with the relatively low NOx values, could explain the tight coupling of the nitrification–denitrification pathways (Middelburg et al., 1996).

In the presence of biodegradable plastics, the negative NOx fluxes (Fig. 2) indicated the sediment uptake of these nutrients, which contrasted with other treatments in which sediment released NOx. These nutrients are intermediate substrates for denitrification, whose sediment uptake is related to the occurrence of denitrification (Tenore et al., 1982). Thus the low NH_4^+ values and the NOx release to the water column could be due to the occurrence of coupled nitrification–denitrification, and also to subsequent denitrification enhancement. These results indicate that biodegradable plastics are more likely to influence nitrogen cycling than conventional ones. The observed reduction in the NH_4^+ and NOx fluxes could be owing to the promotion of denitrification, which agrees with previous research and despite them using plastics in a powdered form (Doering et al., 1994) and as microplastics (Seeley et al., 2020), and even though isotopic N tracers would be necessary to acquire conclusive results.

As regards phosphorous, the time-integrated PO_4^{3-} fluxes in not only the control and control artifact cylinders, but also in the cylinders containing conventional and biodegradable plastics, showed negative values within the -0.018 ± 0.002 to -0.028 ± 0.024 mmol$\cdot$m$^{-2}\cdot$d^{-1} range. These values were comparable to the sediment that did not undergo organic pollution (Holmer et al., 2002, 2003). In the cylinders to which organic matter was added, the time-integrated PO_4^{3-} flux was considerably higher than in the other cores (1.24 ± 0.16 mmol$\cdot$m$^{-2}\cdot$d^{-1}) and comparable to the levels found in sediment affected by fish farming (Holmer et al., 2002) (Fig. 2; Table S1). Thus conventional and biodegradable plastics are expected to more intensely affect the carbon and
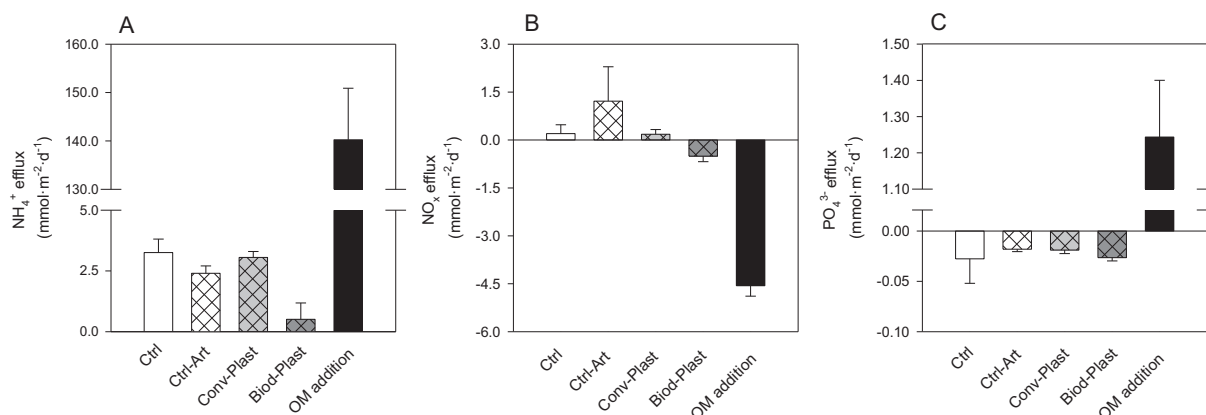

Fig. 2. Time-integrated fluxes of NH_4^+ (A), NO_2^- and NO_3^- (NOx; B) and PO_4^{3-} (C) (mean $\pm$ SE, n = 4). See the Fig. 1 caption for an explanation of treatments.

nitrogen cycle than the phosphorus cycle. Nevertheless, the PO_4^{3-} fluxes from sediment to the water column not only depend on the amount of mineralized organic matter, but also on sediment's capacity to sequester PO_4^{3-} in the upper sediment stratum by means of iron(III) hydroxides, which are present under oxic conditions (Rozan et al., 2002). As the PO_4^{3-} release to the water column can be enhanced under reduced sediment conditions (Sanz-Lázaro et al., 2015), if plastics are able to sufficiently lower oxygen concentrations, e.g. in areas with low oxygen availability, they can indirectly promote PO_4^{3-} release.

Coastal sediment plays a key role in both the cycling of elements (Lavelle et al., 2005) and climate change mitigation (McLeod et al., 2011). Currently, these ecosystems can accumulate large amounts of plastic debris, even if they are located in remote areas (Lavers and Bond, 2017). The results of this study suggest that plastics, especially biodegradable ones, can notably affect carbon and nitrogen cycles in marine sediment. The enhancement of sediment metabolism by biodegradable plastics can promote the mineralization of blue carbon sinks. The global biodegradable plastics market is relatively small compared to that of conventional plastics, as are the marine debris deriving from biodegradable plastics. Nevertheless, the exponential growth in biodegradable plastics production expected in forthcoming years (European Bioplastics, 2019) could lead to increased biodegradable plastic debris if the current mismanagement of such waste continues. If the marine biodegradable plastic debris concentration sufficiently rises in coastal sediment, it could pose a serious threat to these natural carbon reservoirs, because it would limit the mitigation capacity against climate change. Nevertheless, the potential impact of biodegradable plastics may be limited given their limited persistence in the environment compared to conventional ones. Further experiments, including fauna, different environmental setups, considering sediment type (sandy, muddy), trophic conditions (oligotrophic, eutrophic) and temperature, as well as other plastics, are needed to obtain a more complete view of the impact of plastics on marine sediment biogeochemistry.

CRediT authorship contribution statement

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.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143978.

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