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# A temporal record of microplastic pollution in Mediterranean seagrass soils☆

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ABSTRACT

Plastic pollution is emerging as a potential threat to the marine environment. In the current study, we selected seagrass meadows, known to efficiently trap organic and inorganic particles, to investigate the concentrations and dynamics of microplastics in their soil. We assessed microplastic contamination and accumulation in $^{210}$Pb dated soil cores collected in *Posidonia oceanica* meadows at three locations along the Spanish Mediterranean coast, with two sites located in the Almería region (Agua Amarga and Roquetas) and one at Cabrera Island (Santa Maria). Almería is known for its intense agricultural industry with 30 000 ha of plastic-covered greenhouses, while the Cabrera Island is situated far from urban areas. Microplastics were extracted using enzymatic digestion and density separation. The particles were characterized by visual identification and with Fourier-transformed infrared (FTIR) spectroscopy, and related to soil age-depth chronologies. Our findings showed that the microplastic contamination and accumulation was negligible until the mid-1970s, after which plastic particles increased dramatically, with the highest concentrations of microplastic particles (MPP) found in the recent (since 2012) surface soil of Agua Amarga (3819 MPP kg$^{-1}$), followed by the top-most layers of the soil of the meadows in Roquetas (2173 kg$^{-1}$) and Santa Maria (68–362 kg$^{-1}$). The highest accumulation rate was seen in the Roquetas site (8832 MPP m$^{-2}$ yr$^{-1}$). The increase in microplastics in the seagrass soil was associated to land-use change following the intensification of the agricultural industry in the area, with a clear relationship between the development of the greenhouse industry in Almería and the concentration of microplastics in the historical soil record. This study shows a direct linkage between intense anthropogenic activity, an extensive use of plastics and high plastic contamination in coastal marine ecosystems such as seagrass meadows. We highlight the need of proper waste management to protect the coastal environment from continuous pollution.



## 1. Introduction

Global plastic consumption is increasing (Neufeld et al., 2016; Wang et al., 2016) and have now become a major source of pollution in the world's oceans (Browne et al., 2011; Lebreton et al., 2012). Presently, plastics can be found in all marine habitats (Barnes et al., 2009; Thompson et al., 2004) and the concentrations are likely to increase in the coming decades (Everaert et al., 2018). The size of plastic particles in the environment is decreasing globally (Barnes et al., 2009), causing an increase in microplastic particles (< 5 mm) that could cause significant harm to the marine ecosystems. After entering the sea, large quantities of plastics remain on

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the sea surface and may be transported large distances with ocean currents (Eriksen et al., 2013; Law et al., 2014; Ryan et al., 2009) or get washed up on beaches (Browne et al., 2010; Moore et al., 2001). A large part of the debris will, however, sink to the seafloor where it can be deposited for a long time (Claessens et al., 2011; Woodall et al., 2014). The length of dispersal of plastic debris in the marine environment depends on the density, size, shape and buoyance of the particles, which might change over time due to degradation,fragmentation and biofouling (Barnes et al., 2009; Browne et al., 2010; Klein et al., 2018; Thompson et al., 2004). Microplastic particles can be both lighter and heavier than seawater depending on their density, ranging from 0.91 g $cm^{-3}$ for low-density polyethylene (LDPE) to 2.2 g $cm^{-3}$ for Polytetrafluoroethylene (PTFE) (Da Costa et al., 2018; Käppler et al., 2016). However, biofouling on plastics is known to occur rapidly once the particles enter into the marine environment (Lobelle and Cunliffe, 2011; Ye and Andrady, 1991) and this might alter the buoyancy of the plastics, causing more buoyant debris to sink to the bottom (Cózar et al., 2014).

Seagrass meadows are found on soft bottoms along the world's coastlines and are known to filter and trap organic- (Agawin and Duarte, 2002; Kennedy et al., 2010) and inorganic particles (Serrano et al., 2013, 2016) by reducing the water velocity within their canopies (Fonseca and Cahalan, 1992; Fonseca and Fisher, 1986), allowing particles to settle on the sediment or soil surface. This ecosystem function will also cause trapping and storing of plastics resulting in an accumulation of microplastics in seagrass sediment (de los Santos et al., 2021; Huang et al., 2020; Jones et al., 2020; Tahir et al., 2019). Seagrass meadows may hence potentially serve as long-term sinks for plastic particles. As benthic detritus-feeding organisms (such as crustaceans, bivalves and echinoderms) can ingest these plastic particles (Wang et al., 2019), an increased microplastic load can have negative impacts on the fauna of these ecosystems (Tahir et al., 2019), causing direct physical damage, or introducing contaminants, such as heavy metals and organic pollutants, adsorbed to the plastic particles (Gauquie et al., 2015; Hirai et al., 2011; Mato et al., 2001; Wang et al., 2017). The particles can then potentially be transferred in the food-web to higher trophic levels (Foley et al., 2018; Goss et al., 2018). Microplastic contamination has also been suggested to influence biogeochemical- and microbial processes, and alter soil properties in terrestrial soils (Rillig et al., 2019). As seagrass and terrestrial plants have similar physiology, such effects are also applicable for seagrass soils, and could significantly influence the growth and health of the seagrass plants. Microplastics have not only been found in the sediments of seagrass meadows but also attached to the seagrass leaves (Datu et al., 2019; Goss et al., 2018; Jones et al., 2020). The leaves may in turn be grazed upon by a range of herbivorous organisms (e.g. fish and crustaceans), which could be another pathway for microplastics to enter the food web (Goss et al., 2018). The seagrass *Posidonia oceanica* is endemic to the Mediterranean Sea, where it forms extensive meadows in shallow coastal areas, potentially with several meters of thick deposits underneath, known as matte (Mateo et al., 1997; Pergent et al., 2014) and with similar characteristics as terrestrial soils (Piñeiro-Juncal et al., 2020). Due to increased anthropogenic pressures in the coastal zone, including increased nutrient loads through runoff, pollution, and over-sedimentation, the species has constantly been declining over the last decades (de los Santos et al., 2019; Márba et al., 2014). The Mediterranean Sea is, as many ocean basins, highly polluted by plastic debris (Eriksen et al., 2014; Pham et al., 2014) and as much as 62 million floating macro-litter particles have been estimated for the whole basin (Suaria and Aliani, 2014) with harmful consequences for its marine ecosystems (reviewed in Deudero and Alomar, 2015). The concentrations of plastics are higher close to shore and in urban areas (Browne et al., 2011; Pedrotti et al., 2016), and as the *P. oceanica* meadows grow along the coastal shorelines they are likely to be exposed to high levels of plastic debris.

In this study, we assess the accumulation of microplastics in seagrass soils over the last century at three *P. oceanica* meadows along the Spanish coastline exposed to different levels of anthropogenic pressures: Roquetas (high disturbance) and Agua Amarga (medium disturbance), both situated close to Almería, which is known for its intensive horticultural production, and Santa Maria (National Park, protected, low disturbance) at Cabrera Island (Balearic Islands). The specific objectives are to (1) compare the concentrations, accumulation rates and types of microplastic particles and (2) quantify the contamination of microplastics in the soils since the 1930s in these three sites exposed to different levels of anthropogenic pressures, and (3) assess the relationship between greenhouse production in the Almería region and microplastic concentrations in seagrass soils since the start of the intensified horticulture production.

## 2. Methods

### 2.1. Study areas

Seagrass soil was sampled at three locations, including Roquetas (RO.S) and Agua Amarga (AG.S) in Almería, south west of Spain, and Santa Maria (SM25 and SM10) at Cabrera Island, south west of Mallorca (Fig. 1a). The seagrass meadow at Santa Maria was sampled at two different water depths (SM10: 10 m and SM25: 25 m) within a highly restricted "no-take" zone (currently closed to visitors) of the Cabrera National Park. There are no urban areas on Cabrera Island and the studied meadow is situated far from any potential sources of organic and inorganic pollution (Alomar et al., 2015). The meadow of RO.S is located at 1.5 m water depth, next to the intense agricultural industry complex of Almería, while the AG.S site (4.8 m water depth) is located approximately 62 km north east of RO.S (Fig. 1b). The Western-Almería region, which is known as the "plastic sea" due to the large area covered by greenhouses, has one of the most efficient agricultural industries in the world with about 30 000 ha of greenhouses and is a major exporter of vegetables in the European Union (Egea et al., 2018, Fig. 1b). Since the 1970s, the area has experienced a rapid transformation from primitive agriculture to an intense horticultural industry with a steady increase in vegetable production (Egea et al., 2018; Sayadi-Gmada et al., 2019). However, this industrial expansion has caused severe environmental problems, and to date, there is no adequate waste management (Sayadi-Gmada et al., 2019). The *P. oceanica* meadow of RO.S is in a degraded state, while the AG.S site is in an intermediate degree of degradation (CAGPDS, 2018; Mateo et al., 2018) and Santa Maria is considered largely undisturbed (Leiva-Dueñas et al., 2020a; Marbà et al., 2002, Table 1).

### 2.2. Field sampling and soil analysis

The seagrass soil cores were sampled by SCUBA divers in 2015 (Cabrera Islands) and 2016–2017 (Almería) using PVC-cores (7.5 cm inner diameter) with a sharpened edge that were pressed down and rotated into the soil using a hammer. Core shortening was assessed for each core by measuring the inner and outer length of the core once pressed down in the soil. The core shortening was corrected for by applying a core compaction factor, based on the field measurements, to the soil depth (as in Morton and White, 1997; Serrano et al., 2012). The soil cores were sliced at 1 cm intervals and dried at 60 °C for approximately 48 h and weighted to obtain the dry weight (DW). From the dried soil, the dry bulk


**Fig. 1.** Map showing (A) the three sampling areas and (B) satellite image over the Almería region along the coast of Spain. In Santa Maria, two sediment cores from the same seagrass meadow were sampled (SM25 and SM10). The red line (in B) is roughly indicating the area of the horticultural greenhouse industry of Almería. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**
Site locations, water depth and health status of the seagrass meadows. The sites in bold indicate which cores were continuously analyzed for microplastics at each soil layer since the 1930s.

| Site | Location | Coordinates | Water depth (m) | Soil depths analyzed (cm) | Health status |
|---|---|---|---|---|---|
| **RO. S** | Almería | 36°47′15.50″N, 2°35′20.10″W | 1.5 | 0–15 | Highly degraded |
| AG. S | Almería | 36°56′15.66″N, 1°56′0.42″W | 4.8 | 0–4, 8, 12 | Intermediate |
| **SM25** | Balearic Island | 39°9′12″N, 2°56′44″E | 25 | 0–9 | Good |
| SM10 | Balearic Island | 39°9′3″N, 2°56′55″E | 10 | 0–3 | Good |

density (DBD, g $cm^{-3}$) was derived by dividing the DW (g) with the volume ($cm^{-3}$) of the slice. To determine the mud (silt and clay particle fractions) content of the soils, a laser-diffraction particle analyzer (Mastersizer, 2000; Malvern instruments Ltd., UK) was used. Soil organic matter (OM) was determined by loss-on-ignition (LOI) at 550 °C for 4 h, and total organic carbon (TOC) was derived from the OM content using a fitted regressions for the RO.S and AG.S sites. This was calculated based on 10–12 samples from each core, which were analyzed for both OM and organic carbon content (in % DW). For the cores in SM, TOC was calculated for all soil slices. The TOC and total nitrogen (TN) in the soil was analyzed using a CN elemental analyzer (Costech analytical, US). For more details on the

soil property methods used, see Leiva-Dueñas et al. (2020b) and Mateo et al. (2018).

### 2.3. Chronological models

In order to provide a historical time frame to the plastic accumulation phenomenon and to estimate the plastic particles flux to the soil of the meadows, selected soil samples along the cores were dated combining radioisotope techniques: $^{14}C$ accelerator mass spectrometry (DirectAMS laboratory) following standard procedures (Karlen et al., 1968; Stuiver and Polach, 1977), and $^{210}Pb$ (Laboratori de Radioactivitat Ambiental, Universitat Autònoma de Barcelona) for samples older and younger than ca. 100 years, respectively. Supported $^{210}Pb$ was estimated as the average $^{210}Pb$ concentration of the deeper sediment layers analyzed wherein $^{210}Pb$ activities reached constant values. Radiometric dates of $^{210}Pb$ were calculated using the Constant Rate of Supply model as in Appleby and Oldfield (1978). Age distribution models were developed using the Bayesian approach in the Bacon software developed by Blaauw and Christeny (2011). From these models, mean soil age and soil accumulation rates (SAR) of each processed section were calculated (based on data from several years). Full details on age determination and modelling can be consulted in Belshe et al. (2019) and Leiva-Dueñas et al. (2020b).

### 2.4. Extraction of microplastics from the P. oceanica soil

The cores in RO.S and SM25 were analyzed for microplastic content in the upper 15 and 9 cm of soil, respectively. For AG.S and SM10, the topmost soil layers were analyzed as these were suspected to contain most plastic particles (Table 1). For each soil depth, one sample was analyzed and the average (±SD) dry weight of the soil samples were 21 ± 7 g. Prior to density separation, soil samples were treated with an enzyme solution to degrade organic matter, which could withhold plastic particles in the soil phase. For this, pancreatic enzyme capsules (Creon® 40 000, Abbott Laboratories GmbH, Germany, Mylan) were dissolved in a pH buffer (Trizma®, tris[hydroxymethyl]aminomethane, Sigma-Aldrich, T3038, USA) of 8, following the protocol of von Friesen et al. (2019). Ten ml of the pancreatic enzyme solution were added to the soil samples, which were placed on a shaking table (Innova 40, Incubator Shaker Series, New Brunswick Scientific) for approximately 12 h at 110–120 RPM and a temperature of 36.5 °C. This treatment, however, did not dissolve highly refractory organic material, such as seagrass roots and rhizomes, which were separated manually prior to density separation using a sieve (≥1000 μm).

After enzymatic digestion, plastic particles were retrieved from the soil samples using density separation with saturated and filtered (0.7 μm) NaI (ρ = 1.7 g/mL, GPR® Rectapur® [chemically pure] grade, VWR Chemicals). The pancreatic enzyme-treated soils were gently poured into separation funnels (1.5 L) and the glass vials were carefully rinsed (5 times) using the saturated and filtered NaI solution to make sure all of the soil was transferred to the separation funnels. An additional 1 L of the saturated NaI solution was added to the funnels and the mixture was shaken vigorously for several minutes to make sure the soil was mixed with the NaI solution. The inner surface of the funnel was rinsed with NaI solution to flush down particles that may have attached onto the sieve surface after mixing. The density of the NaI solution was measured both before and after separation to ensure that the density was satisfactory (>1.7 g/mL). The soil was allowed to settle for a minimum of 24 h, to allow complete separation of microplastics and soil/mineral particles. When the soil had settled, the uppermost layer of NaI solution containing the microplastic particles was retrieved by gently pouring the sample into a stainless-steel filter holder, in which pre-rinsed and microscopically inspected nylon filters were mounted. The samples were filtered in sequence onto nylon filters with mesh sizes of 1000, 300 and 100 μm. The minimum size limit was set to 100 μm, which defines the limit where microplastic particles are confidently identified visually and retrieved for subsequent polymeric analysis (Karlsson, 2019; Primpke et al., 2017; Setälä et al., 2019). In soils with high concentrations of microplastics (based on an initial microplastic separation and particle count-control), only a subsample of 100–300 mL of the retrieved NaI solution was filtered. To extract a subsample, the filters from the initial microplastic separation were thoroughly rinsed in separate beakers with ~70 mL of Milli-Q water and after that combined into one beaker. The beaker was filled up with Milli-Q water to a volume of 400 mL. From the beaker, a volume of 100–300 mL was collected by pipetting up and down several times (to make sure the solution was well-mixed) using a glass pipette. The retrieved subsample was poured into the stainless-steel filter holder, mounted with new (pre-rinsed and microscopically inspected) nylon filters. For each soil sample, the separation process was carried out twice and the results from the two runs were summed. In order to evaluate the extraction efficiency of the method, three control treatments (blanks) were prepared, in which five plastic particles of >300 μm in size and 20 microplastic particles with a size of 100–300 μm were added. The recovery rate after two separations was high, with 100% ± 0 (mean ± SD) of the >300 μm and 97% ± 3 of the 100–300 μm particles retrieved. All microplastic count data is presented as microplastic particles (MPP) per kg dry soil ($kg^{-1}$). For corresponding microplastic values as MPP $cm^{-3}$, see supplementary information S1. Microplastic accumulation rates (MPAR $m^{-2}$ $yr^{-1}$) were calculated as the number of particles multiplied with the corresponding SAR for each soil layer and standardized to $m^2$.

### 2.5. QA/QC

Methodological precautions were taken to minimize potential contamination of microplastics. The samples were only handled in designated laboratories and during laboratory work only 100% cotton laboratory coats were used. All samples were prepared on a laminar flow workbench (LAF) and all NaI solutions were filtered (GF/F, 0.7 μm) before adding the samples. The equipment was carefully rinsed three times in Milli-Q water before usage and covered with aluminum foil. During visual identification, washed and cleaned 50 μm nylon filters were exposed to the open air in the laboratory to detect any contamination from particles in the laboratory space. In order to assess plastic contamination from handling of the soil during field and laboratory procedures, one sample with soil older than 200 years (prior to the invention of plastic) from each core was analyzed (RO.S = 76–77 cm, AG.S = 129–130 cm, SM25 = 36–37 cm and SM10 = 27–28 cm). Core contamination was found in most soil layers, but the grey PVC particles were easily distinguishable from other particles (they had a distinct color, were not degraded or showed no indication of weathering). Using the samples from the deeper soil layers as negative controls, any particle coming from the grey PVC core used when sampling could be identified (both visually and chemically, using FTIR spectrometry) and disregarded in the other samples.

### 2.6. Microplastic analyses

The filters with the collected particles were analyzed using a stereo microscope (Leica M205C, 80-160x) connected to a camera (Leica DFC420C) and an image analyzing software (LAS, v 4.8) for measuring the length size (longest axis) of particles. Analysis was

done by a combination of visual and tactile identification (touching the particles with tweezers). Identified plastic particles were divided in morphologically different categories (shape, color, surface texture, length and tactile) according to a protocol by Karlsson et al. (2020). A representative selection of particles from each morphological category were subjected to a melting test by placing the particle on an object glass with a drop of Milli-Q water and placed over an ethanol burner. The particles were then examined under the microscope to evaluate if they had melted or curled to confirm the plastic origin (Enders et al., 2015). To determine the polymer composition of the detected microplastics a selection of particles was analyzed with Fourier-transformed infrared (FTIR) spectroscopy using a Spotlight 400 FT-IR Imaging System (PerkinElmer, USA). In total, 26 microparticles were selected, including particles representing each of the defined morphological categories and particle categories for which it was uncertain whether or not they were composed of plastic. The obtained spectra from the analyzed samples were compared to a reference polymer spectra library. Only correlative match rates with similarities over 70% were accepted (Kanhai et al., 2017; Obbard et al., 2014).

### 2.7. Statistical analysis

The statistical analyses were performed using the statistical software R (version 3.5.3) and prior to analysis the data were tested for normality and homogeneity of variance using the Shapiro-Wilk test and by visual inspection. The relationships between greenhouse surface area and production of Almería horticultural industry and microplastic particle concentrations in the soil cores were explored using linear regression models.

## 3. Results

### 3.1. Age-depth chronology and soil characteristics of the cores

While the chronological models for the RO.S, AG.S and SM10 cores were highly accurate, age uncertainty for SM25 samples was higher because the $^{210}$Pb technique was impractical. For the microplastic analysis, the upper 15 and 9 cm of the soil profile in the RO.S and SM25 were used, representing the last 94 and 89 calendar years, respectively. In SM10, the calendar age of the topmost 3 cm encompassed 2015 and 2012, and finally the top 4, 8 and 12 cm of AG.S dated from 1998 to 2012, 1976, and 1962 calendar years, respectively, were analyzed for microplastics. The SAR in RO.S varied over the timespan assessed by this study, being intermediate between 1931 and 1959 (0.34 ± 0.015 cm yr$^{-1}$), highest between 1959 and 1974 (0.43 ± 0.015 cm yr$^{-1}$), and lowest from 1974 to 2012 (0.24 ± 0.005 cm yr$^{-1}$; Fig. 2). SAR in AG.S exhibit a moderate decrease over time, being at maximum during the 1960s (0.5 ± 0.015 cm yr$^{-1}$) and decreasing towards the present (0.4 ± 0.014 cm yr$^{-1}$, from 1971 to 2012). The two cores in SM had a stable SAR throughout the time period studied (0.11 ± 0.015 and 1.23 ± 0.013 cm yr$^{-1}$ in SM25 and SM10, respectively). The soil characteristics differed among the three sites, with higher mud and TOC contents at RO.S and AG.S when compared to SM (Table 2). DBD and OM values were similar in all cores except for SM25, which showed higher DBD and lower OM compared to the other sites (Table 2).

### 3.2. Microplastic concentrations and accumulation rates in seagrass soils

The highest concentrations of microplastics were found in the surface soils (0–1 cm) of Agua Amarga (with 3819 MPP kg$^{-1}$) and in Roquetas (with 2173 MPP kg$^{-1}$) with the corresponding MPAR of 5798 and 8832 m$^{-2}$ yr$^{-1}$, respectively. In comparison, Santa Maria soils only contained 68–362 MPP kg$^{-1}$ and with an MPAR of 302–506 m$^{-2}$ yr$^{-1}$ (Fig. 3). The microplastic content generally decreased downcore, except for those from Santa Maria. According to the seagrass soil record, the microplastic concentrations and accumulation in AG.S increased rapidly and substantially in 2003; the microplastic content in AG.S became about one order of magnitude (in absolute values) higher than that for SM25 (Fig. 3A). In RO.S, there was a clear increase in microplastic concentrations

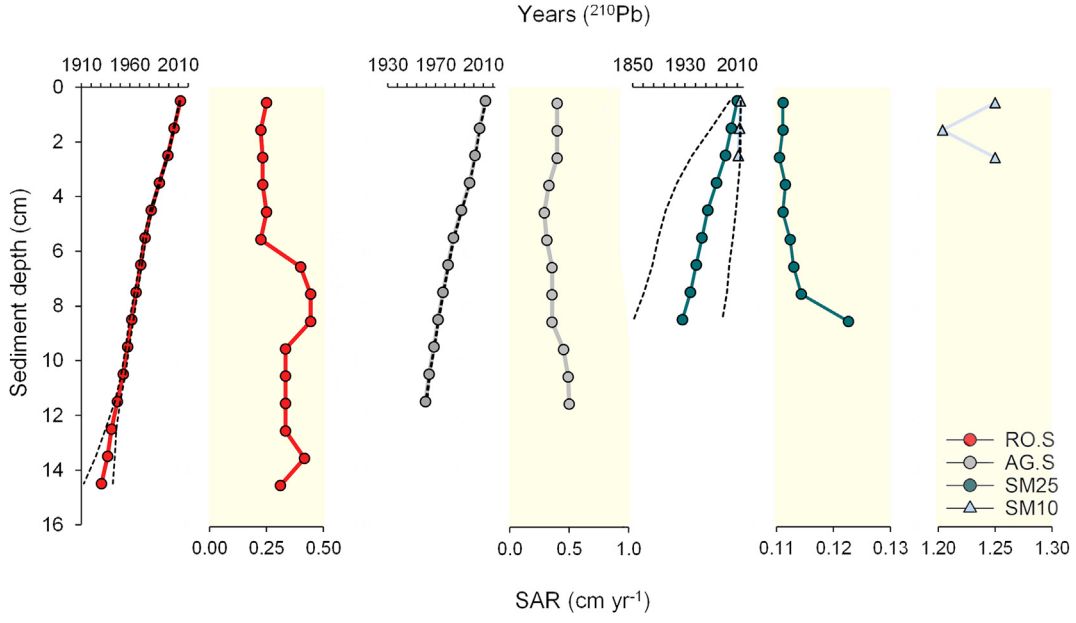


**Fig. 2.** Age-depth profiles and soil accumulation rates (SAR) (in yellow boxes) for the four cores studied. The solid lines show the mean soil age and mean SAR of each processed section and the dashed lines indicate the minimum and maximum soil age. The age of the soil is presented as a mean for the 1 cm sections; this means that for example the first slice section (0–1 cm soil depth) of each core include data from year 2008–2016 in RO.S, 2009 to 2014 in AG.S, 1996 to 2014 in SM25 and 2013 to 2015 in SM10. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**
Soil characteristics of the cores shown as mean ± SE for the soil depth profiles. Mud = % grain size particles <0.063 mm, DBD = dry bulk density, OM = organic matter, TOC = total organic carbon, TN = total nitrogen. TN content was not available for RO.S and AG.S cores.

| Site | Mud (%) | DBD (g $cm^{-3}$) | OM (%) | TOC (%) | TN (%) |
|---|---|---|---|---|---|
| RO.S | 8.2 ± 1.9 | 0.5 ± 0.04 | 8.2 ± 1.5 | 4.6 ± 0.82 | – |
| AG.S | 5.6 ± 1.7 | 0.9 ± 0.16 | 7.7 ± 0.6 | 4.8 ± 2.76 | – |
| SM25 | 0.9 ± 0.1 | 1.3 ± 0.06 | 2.5 ± 0.1 | 1.2 ± 0.06 | 0.03 ± 0.003 |
| SM10 | 1.6 ± 0.1 | 0.7 ± 0.25 | 9.2 ± 0.2 | 2.5 ± 0.03 | 0.10 ± 0.003 |

and accumulation since the mid-1970s. Before the 1970s, the microplastic contamination was very low in all of the cores and no particles were found in the majority of the soil layers, with the exception of AG.S and RO.S (from 1931 to the 1940s).

### 3.3. Microplastic properties

A comparison between RO.S and SM25 (in which the soil analyzed covered the last ~90 years) shows that there was a clear difference in the properties of the microplastics found. In RO.S, the dominant colors were green and transparent, whereas blue particles were most common in the deeper (older) soil and uniformly distributed along the core (Fig. 4A). In SM25, no dominant color was clearly distinguished (Fig. 4B). The blue colored particles were not seen in the negative control soil layer, nor was this the type of plastic used in the corers, and could therefore not derive from soil sampling and handling. The majority of the microplastic particles in RO.S was either flat, irregular or of fibrous shapes, while in SM25 fewer irregular particles were found in comparison to those of flat shape and fibers. Considering all four cores, the most common particle colors were transparent (30%), white (18%) and green (15%) and the dominant shapes were irregular (58%) and flat (30%) (Fig. 4C and D). Most particles were in the size range (based on maximum length) of 500–1000 μm (29%), followed by 300–500 μm (23%) and 150–300 μm (22%) when assessed for all sites. In RO.S and SM25, the largest size fraction (2000–5000 μm) was only found in the recently deposited soil layer (at surface), while the smaller length sizes (100–500 μm) dominated in older soil layers (Fig. 5).

### 3.4. Microplastic polymeric composition

FTIR analysis was used to identify the polymeric composition of a selection of particles representative of different microparticles with distinct morphological characteristics. Fifteen of the 26 selected particles were identified as 5 specific plastic polymers, i.e. polyurethane (PU), polystyrene (PS), vinyl chloride/acetate copolymer (VC), polyethylene (PE), and polytetrafluoroethylene (PTFE) (see supplementary information S2 for the FTIR spectra for the identified polymers). Five particles were best matches to the additives benzyl butyl phthalate (BBP), a plasticizer used almost exclusively in polyvinyl chloride (PVC) products, and Crelan®, a cycloaliphatic polyisocyanate used as a hardener for polyurethane (PU) powder coatings. These particles were likely PVC and PU since BBP and Crelan® are identifiable with FTIR and often constitute a substantial portion of the plastic polymer they are mixed with. Therefore, these particles were classified as the polymers PVC and PU, respectively (Fig. 6). Four of the particles that were difficult to determine with stereomicroscopy and melting tests were identified as other materials than plastic polymers (i.e. cellulose and carbonate). These particle categories were discarded from the results. One major group of microplastic particles found in the soils of RO.S were green with either an irregular or flat shape, and were identified as PVC (Figs. 4 and 6). All PU particles in RO.S were irregular shaped, the majority being yellow, and only a few transparent. In turn, in AG.S the PU particles were white and also irregular shaped.

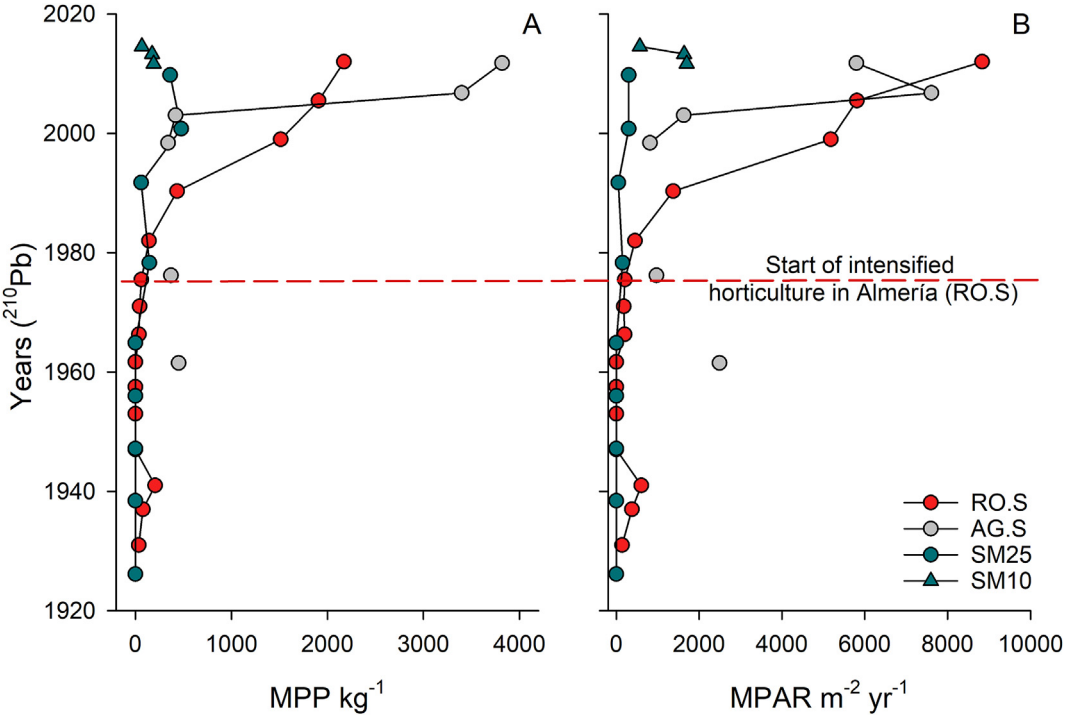


**Fig. 3.** Seagrass soil record of (A) microplastic particles (MPP $kg^{-1}$) and (B) microplastic accumulation rates (MPAR $m^{-2}$ $yr^{-1}$) for the different cores. The dashed red line indicates the start of the intensified agricultural development in the Almería region in the mid-1970s. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

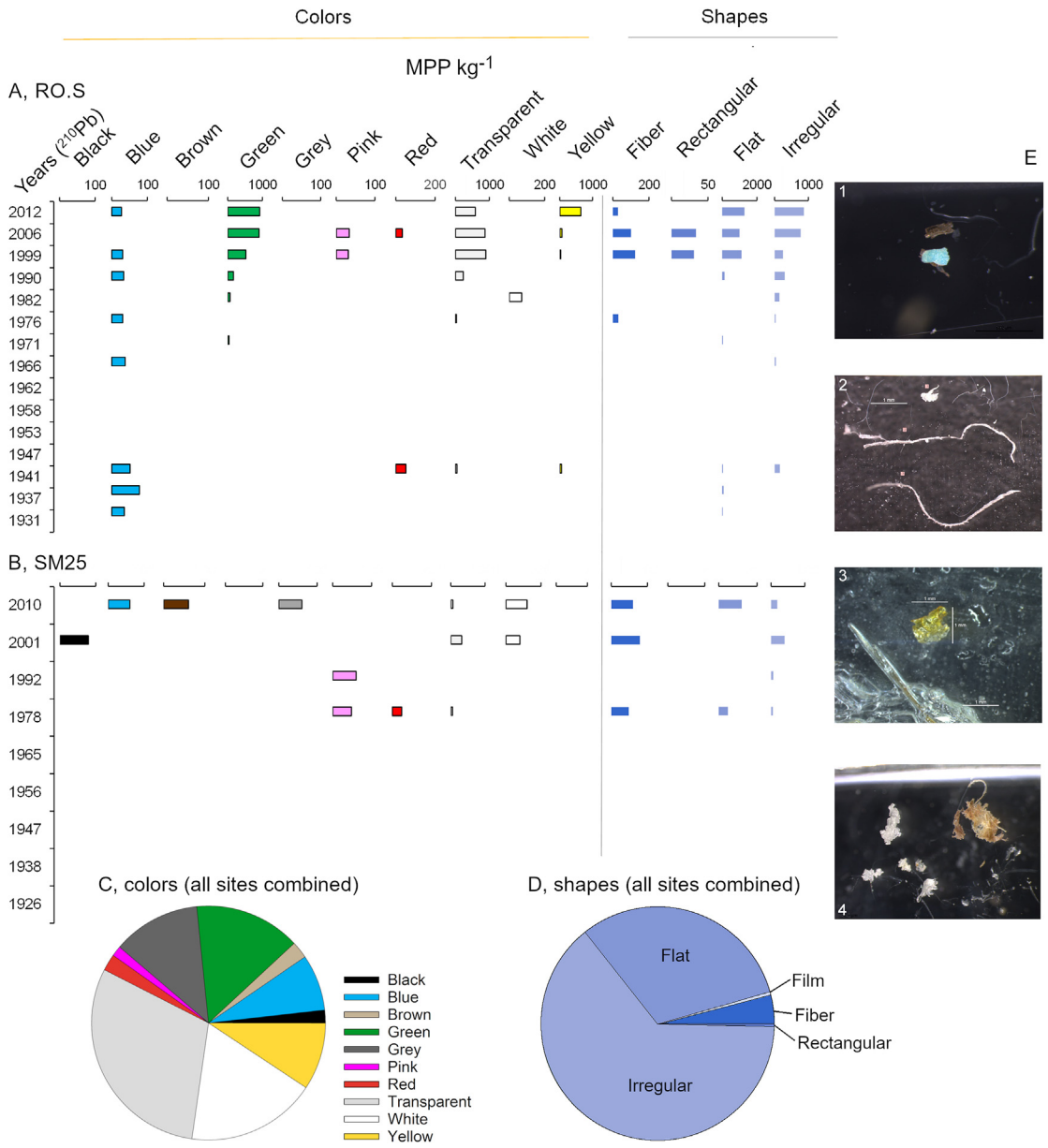


**Fig. 4.** A, B: The color and shape of microplastic particles (MPP $kg^{-1}$) in relation to soil age for the cores from Roquetas (RO.S) and Santa Maria (SM25); C, D: summary of the color and shape properties of MPP for all cores combined (n = 4); E: examples of particles found at the different sites; 1: blue and rectangular, 2: transparent and fiber, 3: yellow and irregular, and 4: transparent and brown, and irregular particles. Note that the shape class "film" was only located in Agua Amarga (AG.S). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Blue microplastic particles were also common and were observed in both RO.S and AG.S. These particles were usually in a brittle condition, either flat or irregular shaped, and identified as PS polymers (Fig. 6). PS was the most widely distributed microplastic polymer in the soils and was present in all cores except SM10.

### 3.5. Agricultural development vs soil microplastic content

The microplastic contamination in the soil of RO.S was strongly correlated to the area covered with greenhouses ($F = 47$, $df. = 4$, $p < 0.01$, $R^2 = 0.90$) and greenhouse production ($F = 340$, $df. = 4$, $p < 0.001$, $R^2 = 0.99$) since the beginning of the intensified agricultural industry in Almería region in 1975 (Fig. 7).

## 4. Discussion

The *Posidonia oceanica* soils assessed in this study showed increasing concentrations and accumulation of microplastics since the mid-1970s. Converting the microplastic particles found in the seagrass soils to mass (based on data from Collignon et al., 2012; Fries et al., 2013; Martin et al., 2020) implies that the MPAR in recent soil layer in RO.S would generate a microplastic uptake ranging from 1 to 160 kg $ha^{-2}$ $yr^{-1}$, which indicates the microplastic sink potential of seagrass meadows. There was a clear correlation between the development and production of the greenhouse agricultural industry in the Almería region and the microplastic accumulation in the nearby seagrass soils of RO.S, with

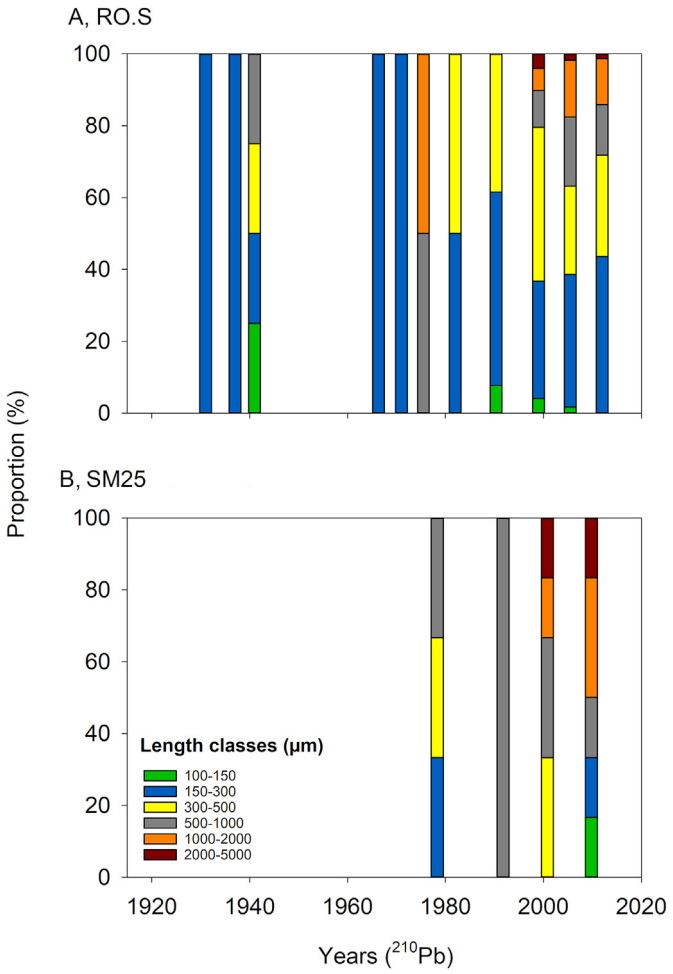


**Fig. 5.** Length classes of microplastic particles (MPP) in (A) RO.S and (B) SM25 cores, for which the soil was analyzed since the 1930s. In SM25, no microplastic particles were found before 1976.

an average (±SD) MPAR ($m^{-2}$ $yr^{-1}$) of 3642 ± 3497 from 1976 until 2012 in relation to an average accumulation of 167 ± 209 prior to the expansion of the agriculture in the area (1931–1971). This demonstrates the impact of land-use change and increased anthropogenic pressure, and highlights the urgent need to advocate proper waste management to protect the coastal environment from continuous microplastic pollution from land.

### 4.1. Concentrations of microplastic particles in seagrass soils

The most common source of plastic debris in Spain is terrestrial discharge, corresponding to about 89% of particles found in the marine environment (Liubartseva et al., 2018), with equivalent values found globally (Andrady, 2011). In the Mediterranean surface waters, the plastic concentrations are particularly high close to the shore and in areas adjacent to urban development (Pedrotti et al., 2016). The highest microplastic concentrations found in the present study were also found at sites along the Spanish mainland coast (RO.S and AG.S) as compared to Santa Maria (SM10 and SM25), situated in Cabrera Island. The comparatively low plastic content of the soils of Santa Maria is in agreement to what has previously been found in the area (244–897 particles $kg^{-1}$ DW; Alomar et al., 2016). In contrast, in the mainland sites, the microplastic concentrations were substantially higher than what has previously been found in other seagrass meadows (Huang et al., 2020) and in most coastal sediments and beaches (0–660 MPP $kg^{-1}$; Clunies-Ross et al., 2016; Horton et al., 2017; Naji et al., 2017; Martin et al., 2020). However, even higher values (more than 18 000 MPP $kg^{-1}$) have been reported in other coastal environments situated next to major cities or in areas with low hydrodynamic activity (Karlsson, 2019; Manalu et al., 2017). As the RO.S and AG.S sites contained high concentrations of MPP, the accumulation rates were also proportionally higher compared to Santa Maria as well as to other benthic habitats (Turner et al., 2019). AG.S displayed a drastic increase in microplastic contamination in the upper (most recent) two cm of soil (2007–2012). This pattern was not observed in RO.S nor in SM25, which both showed a gradual accumulation of particles over the last 45 years. This massive increase of particles in AG.S might be related to flooding events occurring in the region when large amounts of debris have been washed off from land (Agustín Barrajón, pers. com.). Flooding events can substantially increase the amount of microplastic particles to the coastal environment, with as much as 3–14 times higher levels being reported (Gündoğdu et al., 2018; Veerasingam et al., 2016). The risk of these flash floods is also expected to increase in the future due to climate change combined with land-use change and urban development in the region (Jodar-Abellan et al., 2019), thus resulting in further plastic pollution of coastal areas. It has been suggested that in high amounts, microplastic particles could reduce the density and increase the porosity of the soil (as in terrestrial soils; Rillig et al., 2019), resulting in higher turnover by the microbial community on the organic matter, which in turn could negatively affect the carbon sink function of seagrass meadows. On the other hand, microplastic particles could act as a capping of the soil, disrupting diffusive gas transports over the soil surface, possibly resulting in reduced oxygenation and thus increased levels of sulfides (Borum et al., 2006) and greenhouse gases (George et al., 2020). However, no clear patterns of changes in soil DBD and microplastic content could be seen and the variation in soil properties for the cores is likely due to the difference in SAR between the sites.

### 4.2. Microplastic characteristics and composition

Size and color of plastic particles will greatly influence the bioavailability and probability of ingestion of microplastics by macrofauna (Wright et al., 2013), and light colors, such as white, beige and yellow particles are likely to be more favored by many marine organisms (of lower trophic level) since they have a closer resemblance to natural food sources (Shaw and Day, 1994). Almost 50% of the microplastic particles identified in this study were indeed transparent and white, and these light colors were found in all cores. There was a distinction of polymer types, shapes and colors between sites, which strengthens the interpretation that the sources of microplastics to the soils differ due to distinguished anthropogenic pressure among the different sites. For instance, both soil records at Santa Maria (SM25 and SM10) contained VC, which was not found in the two sites of Almería, in which PVC, PU and PS were instead detected in high amounts. In RO.S, the blue colored PS plastics were present throughout the soil record, indicating that the usage of this plastic has been constant since the 1930s, and since they appear in the soil record before the 1970s, they could not be related to the development of the intensified greenhouse production in the area. PS was discovered already in the 1800s, being manufactured in large quantities for decades (Geyer et al., 2017), and found in sediment records dating back to the 1950s (Turner et al., 2019). Flat or irregularly shaped green and transparent particles were the most common plastic particles in the recent soil layers of RO.S, increasing steadily since the 1980s and 1990s, respectively. The green colored particles were identified as

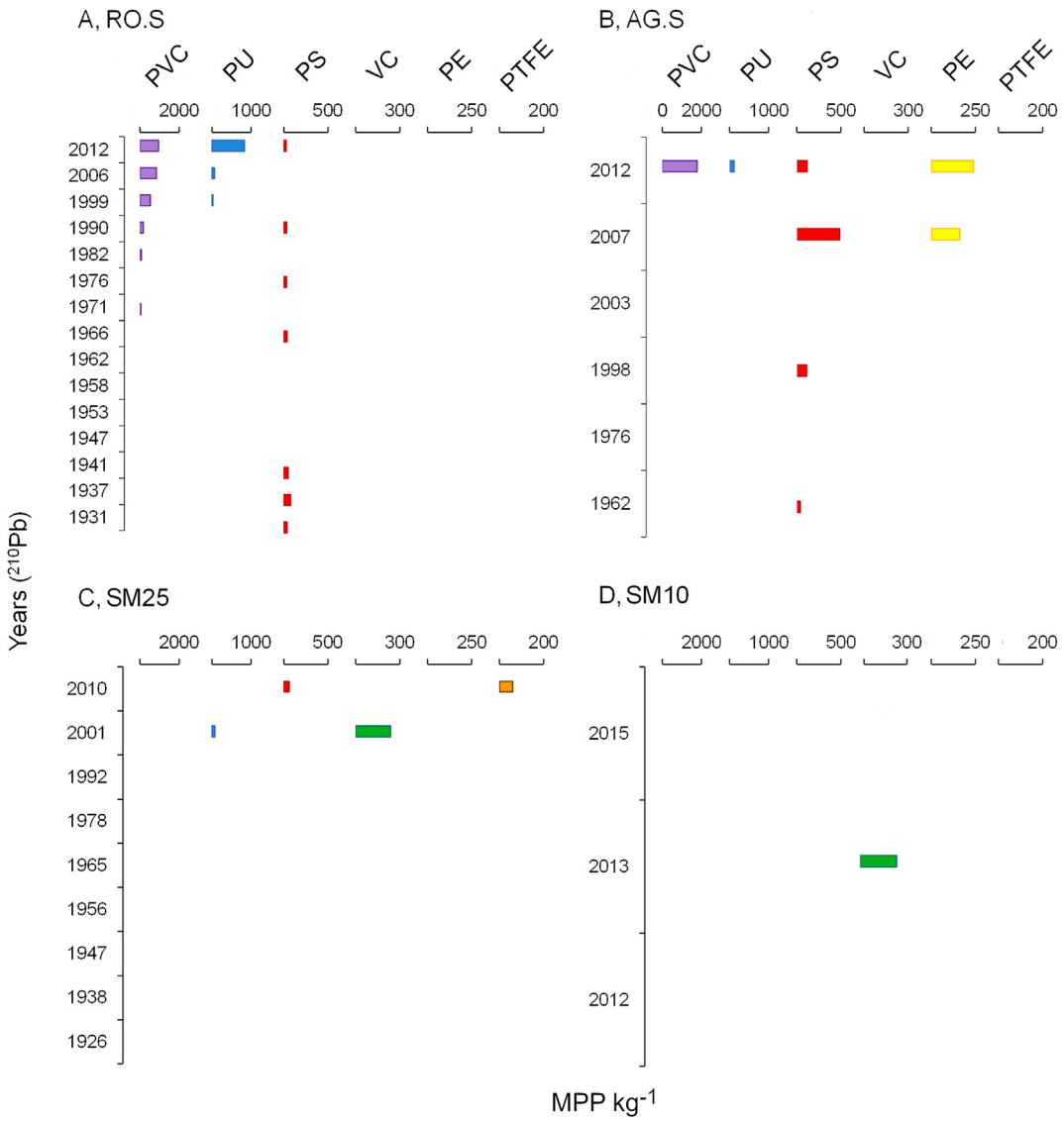


**Fig. 6.** Plastic polymers (MPP kg$^{-1}$) found along the cores of the four different sites. PVC = polyvinyl chloride. PU = polyurethane, PS = polystyrene, VC = vinyl chloride/acetate copolymer, PE = polyethylene, PTFE = polytetrafluoroethylene.

PVC, which is commonly used in irrigation systems (Rodríguez-Seijo and Pereira, 2019) and in mulch films in the agricultural industry (Mormile et al., 2017). Once entering the soil environment, PVC is rather stable with a low degradation (i.e. weight loss) compared to other polymers (Kumar et al., 2020). The transparent particles could originate from the films used in present agricultural production as cover of the greenhouse structures (Espí et al., 2006). However, this could not be confirmed as the plastic composition of the majority of the transparent microplastics could not be determined (although some were classified as PU). As not all particles were analyzed for polymer composition, and the FTIR analysis could only identify part of the microplastic particles to specific polymer types, the origin of all particles identified as plastics using the melting test could not be confirmed. This limitation in polymer classification is common in marine and lake sediments (Cluniess-Ross et al., 2016; Martin et al., 2017; Turner et al., 2019). The difficulty in identifying polymer types in soil and sediment records using FTIR might be related to hydrolysis and/or oxidative weathering that microplastics may go through over time (Galloway et al., 2017; Karlsson et al., 2018), which might change the particles' spectra (Käppler et al., 2016). Also, some microplastics found, mainly in RO.S, were identified as the additive BBP and the coating Crelan® and not to specific polymer types, and although this confirms the plastic origin of the particles, it also adds some uncertainty to the polymer type identification. Additives and coatings are commonly used in agricultural plastic products to increase the durability against weathering or to change the surface properties, e.g. to improve the UV resistance of the plastic (Mormile et al., 2017). In SM25, there were no clear patterns in specific colors, shapes or polymers of microplastics, although the number of plastic particles has increased exponentially since 1976. This indicates that there is no point source of microplastic pollution and likely reflects the continuous contamination of microplastic seen in the world's ocean, and fits well with our understanding of the ubiquitous nature of plastic pollution and the large dispersal range of microplastic debris in the marine environment, as highlighted in many studies (e.g. Eriksen et al., 2014; Ryan et al., 2009). In both RO.S and SM25, size classes were evenly distributed, with the exception of the largest (2000–5000 μm) and smallest sizes (100–150 μm), that were less abundant. In the present study, the largest size range was

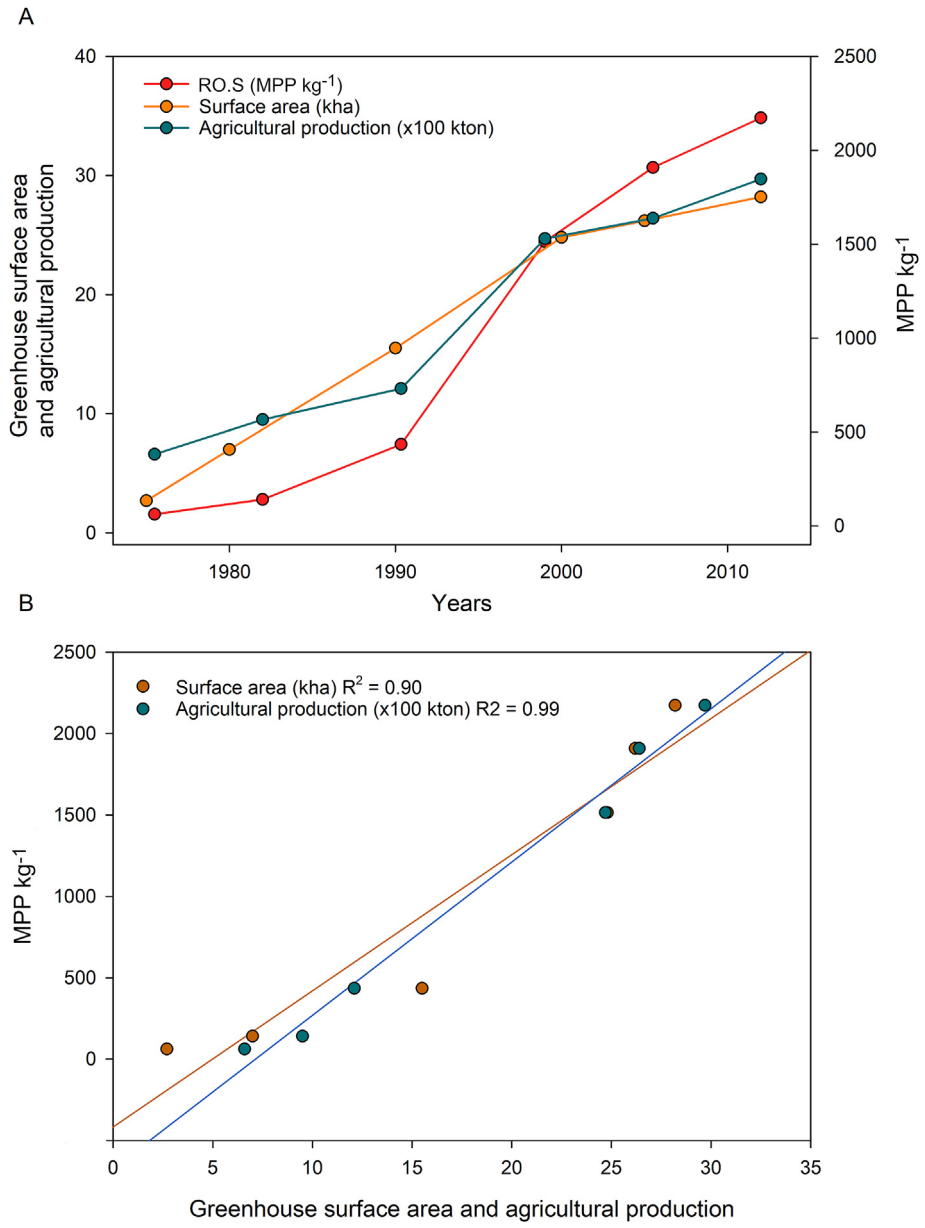


**Fig. 7.** The development of the agricultural industry in Almería since the mid-1970s seen as (A) amount of greenhouse area, greenhouse agricultural production, and microplastic particles (MPP) in the seagrass soil at RO.S and (B) the relationship between greenhouse surface area (kha) and greenhouse agricultural production (kton) with microplastic concentrations at RO.S (MPP $kg^{-1}$). Data of the greenhouse surface area and production is taken from Egea et al. (2018).

only found in the recent surface layers, while smaller particles (ranging from 100 to 500 μm) were most commonly found in deeper soil layers. The generally low presence of larger plastic particles has also been noted in lake and mangrove sediment records (Martin et al., 2020; Turner et al., 2019), which has been related to water depth and hydrodynamic processes (Vaughan et al., 2017). The water depth is known to influence the accumulation of organic matter in seagrass meadows (Dahl et al., 2020; Lavery et al., 2013) and thus might have an influence on the microplastic accumulation. The seagrass meadows in this study were situated at different water depths but as the shallowest site (i.e. RO.S) did not have the highest microplastic particle concentrations, nor did the shallower SM10 in comparison to SM25, although located at the same site. Therefore, depth seems not to be the main explanation to the differences in microplastic accumulation between the sites.

### *4.3. Temporal trends of microplastic contamination in relation to agricultural development in Almería*

We found higher concentrations and accumulation of microplastic particles in RO.S and SM25 since the mid-1970s compared to older soil deposits (1920s–1960s). The exponential increase of microplastics in RO.S correlated well with the development of the agricultural industry in the Almería region, which since the 1970s has been transformed from a low-productive agricultural area into an intensive horticultural complex with about 30 000 ha occupied by greenhouses at present (Egea et al., 2018). Most of the plastic used in the greenhouse horticulture is high- and low density polyethylene (HDPE and LDPE) and to a lesser extent ethylene vinyl acetate (EVA), polypropylene (PP), PS and PVC (Sayadi-Gmada et al., 2019). LDPE is commonly used as cover for the greenhouses, but has to be replaced continuously due to weathering and therefore

generates large amounts of plastic waste (Rodríguez-Seijo and Pereira, 2019). The greenhouses of Almería produce about 1390 kg $ha^{-1}$ plastic waste each year, whereof LDPE stands for about 70% of this plastic residual (Sayadi-Gmada et al., 2019). Even though plastic films are being recycled, the concentrations of microplastic in greenhouse soils can be considerably increased by weathering and fragmentation of plastics, wastewater irrigation and the use of sewage sludge contaminated with microplastics (Kong et al., 2012; Nizzetto et al., 2016; Zhang and Liu, 2018). Most of this plastic debris aggregates to soil particles, but some remain mobile (Zhang and Liu, 2018) and can potentially be transferred through the soil to nearby rivers, streams or directly to the ocean. The plastics used in the Almería greenhouses are low-density polymers (<1 g $cm^{-3}$), except PVC and PS, which were both present in the soil of the RO.S site. The particle density is important for the microplastic distribution in the marine environment (Erni-Cassola et al., 2019; Kowalski et al., 2016) and dense PVC and PS have the potential to sink to the bottom without alteration of the buoyancy of the particle (for example through biofouling; Artham et al., 2009), which might increase the accumulation in soils adjacent to areas of high plastic pollution. The high microplastic pollution seen in this study can lead to plastic ingestion in suspension-feeding and grazing macrofauna, and increase the exposure of toxic heavy metals (Brennecke et al., 2016) and persistent organic contaminants (Koelmans et al., 2014) to marine organisms. Seagrass meadows are already under immense threats from human activities (Orth et al., 2006; Waycott et al., 2009), and plastic pollution can additionally impact the habitat (Rillig et al., 2019; Tahir et al., 2019) and reduce the ecosystem services that they provide.

## 5. Conclusions

The *P. oceanica* soil paleo-archive revealed a clear accumulation of microplastics since the mid-1970s, with the most recently deposited layers showing the highest microplastic contamination, demonstrating a direct relationship with the increasing anthropogenic pressure on the coastal environment over the last four to five decades. Although the seagrass soil at the insular bay of Santa Maria had a drastically lower particle load, there was also an increase of microplastic contamination over time, showing that plastic pollution is spreading far from its sources, even into remote protected areas like the national park of Cabrera. The seagrass soils along the Spanish mainland coast had the highest microplastic concentrations evidencing that land-based sources are the main vectors in Spain for plastic debris to the ocean (Liubartseva et al., 2018). This finding was further strengthened by a highly significant correlation between the sediment microplastic concentration (in the RO.S site) and greenhouse agricultural development and production in the Almería region, since the start of the intensified agricultural industry of the area in the mid-1970s. In the light of this finding, we conclude that the transformation of the Almería region into a highly productive agricultural complex over the last 45 years has come to a high environmental cost in terms of microplastic contamination.

## Main findings

We found a dramatic increase in microplastic pollution in seagrass soils since the mid-1970s and a clear linkage between intense agricultural production and high plastic contamination in the adjacent seagrass soils.

## Author contribution

MD, MB, EDA, CLD, CMM, MGr, MGu, NPJ, MAM: Conceptualization; MB, MGu, MAM: Funding acquisition; MD, SB, EDA, CLD, CMM, MGr, KM, NPJ, MAM: Investigation; SB, EDA, CLD, CMM, MGr, KM, NPJ, MAM: Methodology; MD: Visualization; MD: Writing - original draft; SB, MB, EDA, MGr, MGu, CLD, KM, CMM, NPJ, MAM: Writing - review & editing

## 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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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.116451.

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