Science of the Total Environment 573 (2016) 532–540

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Using a polymer probe characterized by MALDI-TOF/MS to assess river ecosystem functioning: From polymer selection to field tests

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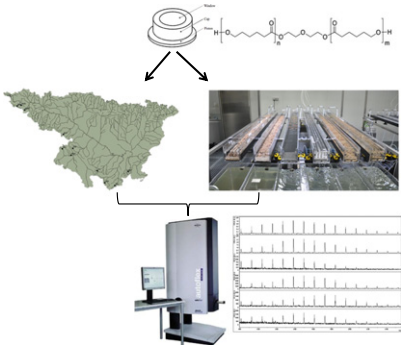
HIGHLIGHTS

- Four polymers were characterized by MALDI-TOF/MS.
- Polycaprolactonediol 1250 polymer was used as probe to assess ecosystem functioning.
- Polycaprolactone diol 1250 probes were tested in mesocosoms and in river.
- MALDI-TOF/MS spectra reflect changes undergone by probes after exposure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form 12 August 2016
Accepted 19 August 2016
Available online 27 August 2016

Keywords:
Ecosystem functioning
Organic matter decomposition
MALDI-TOF/MS
Polymer degradation

ABSTRACT

Characterization of river ecosystems must take into consideration both structural and functional aspects. For the latter, a convenient and simple approach for routine monitoring is based on the decomposition of organic matter measured in terms of breakdown of natural organic substrates like leaf litter, wood sticks. Here we extended the method to a synthetic organic material using polymer probes characterized by MALDI-TOF/MS. We first characterized several commercial available polymers, and finally selected polycaprolactonediol 1250 (PCP 1250), a polyester oligomer, as the most convenient for further studies. PCP 1250 was first tested at mesocosms scale under conditions simulating those of the river, with and without nutrient addition for up to 4 weeks. Differences to the starting material measured in terms of changes in the relative ion peak intensities were clearly observed. Ions exhibited a different pattern evolution along time depending on their mass. Greatest changes were observed at longest exposure time and in the nutrient addition treatment. At shorter times, the effect of nutrients (addition or not) was indistinguishable. Finally, we performed an experiment in 11 tributaries of the Ebro River during 97 days of exposure. Principal Component Analysis confirmed the different behavior of ions, which were clustered according to their mass. Exposed samples were clearly different to the standard starting material, but could not be well distinguished among each other. Polymer mass loss rates, as well as some environmental variables such as conductivity, temperature and flow were correlated with some peak intensities. Overall, the interpretation of field results in terms of environmental conditions remains elusive, due to the influence of multiple concurrent factors. Nevertheless, breakdown of synthetic polymers opens an interesting field of research, which can complement more traditional breakdown studies to assess river ecosystem functioning.

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http://dx.doi.org/10.1016/j.scitotenv.2016.08.135

1. Introduction

It is widely recognized that an adequate characterization of river ecosystems must take into consideration both structural and functional aspects (Gessner and Chauvet, 2002) (Palmer and Febria, 2012). However, classical health assessment of freshwater ecosystems is mainly based on physicochemical variables (dissolved oxygen, nutrients, acidity, conductivity, pollutants), river hydromorphological characteristics, and other structural properties such as the composition of biological communities (Bunn et al., 1999), as it is reflected in the current legislation (Water Framework Directive, Directive 2000/60/EC). Despite its relevance as driver of ecosystem services (Costanza et al., 1997) (Sweeney et al., 2004), much less attention has been devoted to ecosystem functioning (Feld et al., 2011) (Friberg et al., 2011). Stream ecosystem functioning encompasses a variety of processes such as production, retention and decomposition of organic matter, or retention and recycling of nutrients (Elosegi et al., 2010). The use of leaf-litter decomposition as a functional tool is greatly spread because of its sensitivity to a great variety of stressors such as nutrient addition (Ferreira et al., 2015), land-use changes (Martínez et al., 2013) or water regulation (Mendoza–Lera et al., 2012), and its experimental simplicity (Bärlocher, 2005). Thus, decomposition of organic matter measured in terms of breakdown of leaf litter has been often preferred to other indicators for routine monitoring (Feio et al., 2010); (Gessner and Chauvet, 2002). Alternatively, leaves have been replaced by other more standardized materials such as cotton strips (Imberger et al., 2010); (Tiegs et al., 2007) or wooden sticks (McTammany et al., 2008); (Tank and Winterbourn, 1996);(Young et al., 2008);(Arroita et al., 2012). While all these substrata reflect the decomposition of natural organic matter, they yield little information about the degradation of synthetic organic matter. Since many freshwater ecosystems are nowadays heavily exposed to anthropogenic pollution (Vörösmarty et al., 2010) it seems worth developing probes to assess how stream ecosystems respond to such perturbation and to what extent they are able to degrade synthetic organic matter. To this end, polymers may constitute suitable candidates to be used as substrates.

Polymer degradation is a complex process encompassing both abiotic (mechanical, thermal, chemical, photolysis) and biotic (biodeterioration, biofragmentation, assimilation) phenomena that often are concurrent (Göpferich, 1996) (Molero et al., 2008). Typically after an initial abiotic step, biotic degradation proceeds through the action of enzymes excreted by the microorganisms (bacteria, fungi etc.). In contrast to abiotic processes, biotic degradation usually starts at the polymer's surface where an initial depolymerisation step takes place by means of extracellular enzymes. When broken polymer chains are small enough, they may be uptaken by the microorganisms and metabolized until complete mineralization (Eubeler et al., 2010). Polymer biodegradation has been the object of several reviews (Swift, 1997; Shah et al., 2008; Lucas et al., 2008; Eubeler et al., 2010). These reviews usually focus on two main aspects, namely, the biodegradation of the different polymer groups and standardized test methods and procedures employed to characterize polymer biodegradation. Studied groups include, among others, polyolefins, polyethylene glycols, polyurethanes, polyamides, polyiimides, polyisoprene, acrylic polymers, polyvinyl alcohol, polyvinyl pyrrolidone and polyesters, being the latter by far the most studied (Tokiwa and Calabia, 2007) (Eubeler et al., 2010) as they are the main constituents of biodegradable polymeric materials ("bioplastics"). Remarkably, most of the research on the biodegradation of polyesters has been focused on soil and compost, while water environments (seawater, freshwater and wastewater) have been much less investigated (Eubeler et al., 2010).

Polymer degradation studies include monitoring of changes occurred in physico-chemical and rheological characteristics of the material (intrinsic viscosity, average molecular weight, (Pitt and Zhong-wei, 1987)), as well as the application of analytical chemical methods such as infrared spectroscopy, nuclear magnetic resonance, gas chromatography coupled to mass spectrometry, liquid chromatography mass spectrometry and Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Eubeler et al., 2009). These analytical techniques provide information about the molecular structure of polymers, and maybe useful for monitoring changes over time. MALDI-TOF/MS, originally developed to study peptides and proteins, has also been successfully applied to synthetic polymers (Favier et al., 2004). This technique enables analysing polymers in a broad range of molecular weight. However, its resolution and mass accuracy are best for relatively low-mass polymers (Nielen, 1999) (Schriemer and Li, 1996). MALDI-TOF/MS is particularly suitable for polymer analysis because of simple acquisition of the mass spectra which show mainly single-charged quasi-molecular ions with hardly any fragmentation (Rizzarelli and Carroccio, 2014). Moreover, simple sample preparation, short analysis times, the variety of available matrices and low sample consumption are further advantages of this technique (Montaudo et al., 2006). Nevertheless, MALDI-TOF/MS has been seldom used to study polymer degradation in water. Some examples of MALDI-TOF/MS to study polymer degradation include the hydrolytic degradation of poly(ethylene terephthalate) in water (Weidner et al., 1997), the aerobic biodegradation of poly(vinylpyrrolidone) on a laboratory-scale fixed-bed bioreactor run with river water (Trimpin et al., 2001) and the aerobic biodegradation of polyethylene glycols in seawater and wastewater (Bernhard et al., 2008).

The general aim of the present article was to use a polymer substrate as a probe for characterizing river ecosystem functioning. To the best of our knowledge, this approach had not been yet tested for that purpose. Specific objectives sought in the present study were: (i) to select commercially available synthetic polymers to be used as probes in degradation studies in freshwater; (ii) to compare the starting polymers with potentially modified structures following its exposure to aquatic environments using MALDI-TOF/MS at lab (mesocosm) scale; (iii) to use the selected polymer probe in real field experiments. To this end, an exposure experiment was carried out in the Ebro river basin in parallel to another study using leaf litter (Monroy et al., 2016).

2. Methods

2.1. Materials

Selected polymers: ε-Polylysine (POL) 4000 Da (physical aspect: powder), was obtained from Zhengzhou Bainafo Bioengineering CO LTD (Zhengzhou city, Henan Province, P.R. China). Polycaprolactonediol (PCP) 1250 Da (physical aspect: waxy), and PCP 2000 Da (physical aspect: waxy), were obtained from Polysciences USA (Warrington, PA18976) and poly(ethyl vinyl ether) (PEVE) 3800 Da (physical aspect: viscous oil), from Aldrich (Steinheim, Germany). Matrices: all-trans retinoic acid (RA), trans-3-indoleacrylic acid (IAA), 2-(4-hydroxyphenylazo)benzoic (HABA), dithranol (DIT) and trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) were obtained from Aldrich (Steinheim, Germany). 2,5-dihydroxy benzoic acid (DHB) was obtained from Bruker (Bruker Daltonik GmbH, Bremen, Germany). Metal Salts: silver trifluoroacetate (Ag-TFA), sodium trifluoroacetate (Na-TFA) and potassium trifluoroacetate (K-TFA) were purchased from Aldrich (Steinheim, Germany). Solvents: the employed solvent Tetrahydrofuran (p.a.) (THF) and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and water (p.a.) were obtained from J. T. Baker (Teugseweg 20-Deventer, Netherlands). The cap units used in the degradation exposure experiments were purchased from Exposmeter AB (Tavelsjo, Sweden). Each capsule unit consists of a plastic piston (3.9 cm external diameter) a plastic cap (2.5 cm diameter) with a window (2 cm diameter) (Fig. S1, Supplementary data).

The Four commercially available polymers selected (POL, PEVE, PCP 1250 and PCP 2000) were characterized using MALDI-TOF/MS as described in Section 2.4.

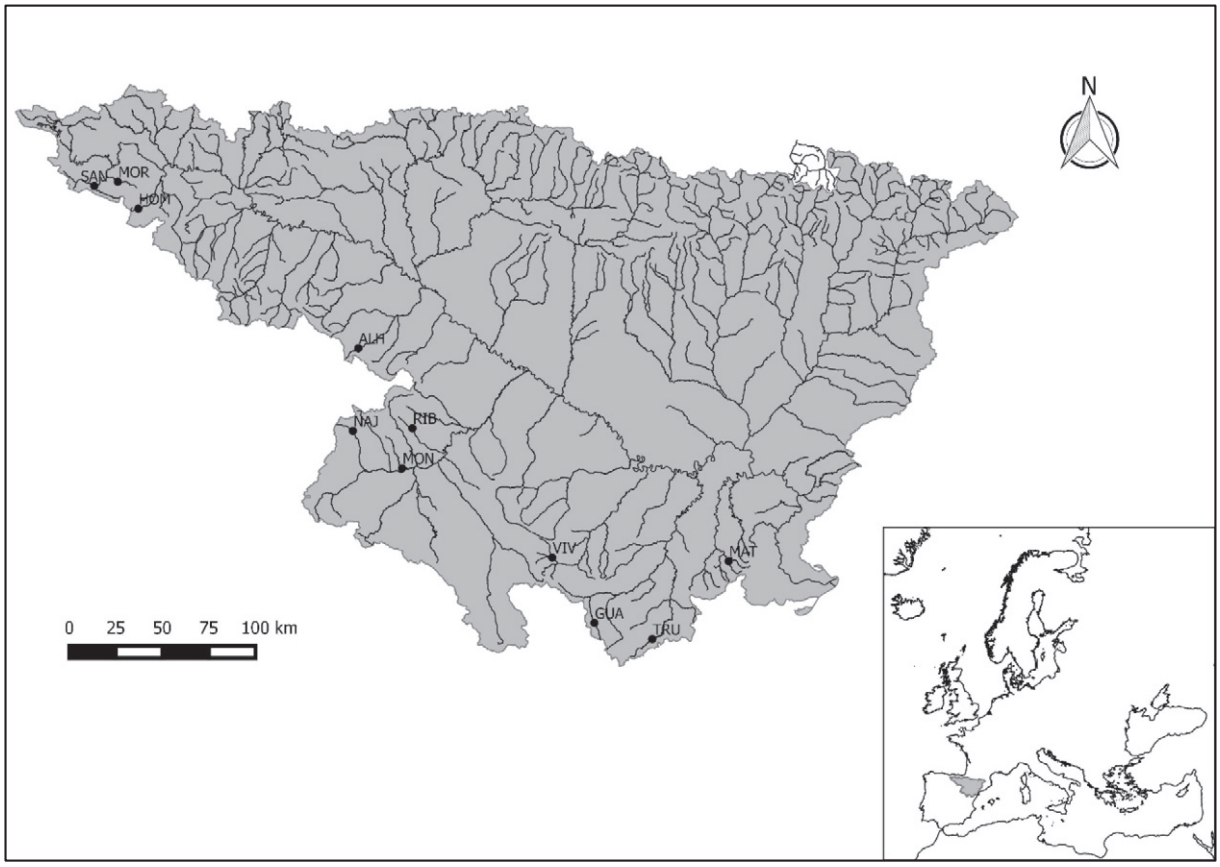

Fig. 1. Ebro River basin map showing the location of the study sites.

2.2. Mesocosm degradation experiments

Mesocosm degradation experiments were carried out by placing capsules filled with PCP 1250 (20–80 mg) in two artificial streams (mesocosm) located at the indoor Experimental Streams Facility of the Catalan Institute for Water Research (Girona, Spain), with two treatments differing in their nutrient concentration. The artificial streams were 2 m long, had a rectangular cross-section of 50 cm^2 and were set up as an open system with constant slope and steady, uniform flow. They operated with water at a constant flow of 60 mL s^{-1}, resulting in mean water velocity of 0.9 ± 0.3 cm s^{-1}, and a water depth over the plane bed between 2.2 and 2.5 cm. The artificial streams were filled with approximately 100 L of sand (mean diameter = 0.37 mm). Sand was sterilized in a Presoclave-II 30 L autoclave (120 °C for 20 min) (JP Selecta S.A., Barcelona, Spain) and evenly distributed in the artificial stream to create a plane bed that facilitated the growth of biofilm. Biofilm inocula were obtained from an unpolluted segment of Segre River (Puigcerdà, Girona, Spain), after scraping 10–12 cobbles. New inocula were provided twice a week to each channel during the first three weeks of the colonization period.

The artificial streams run with rainwater collected in a tank and filtered through activated carbon filters. One of the streams had no nutrients added, whereas in the other the concentrations of phosphate and ammonium were raised (0.038 mg L^{-1} P-PO_4^{3-} and 0.035 mg L^{-1} N-NH_4^+; Table S1, Supplementary data), by means of an injection of concentrated solutions (KH_2PO_4 and NH_4Cl, respectively) via a peristaltic pump (IPC Ismatec, Glattbrugg, Switzerland). Daily cycles of photosynthetic active radiation (PAR) were defined as 9-h daylight + 15-h darkness. PAR was held constant at 150 μE m^{-2} s^{-1} during the daytime. Water temperature was held constant at 20 °C by means of a cryo-compact circulator (Julabo CF-31, Seelbach, Germany). Total exposure time was 28 days. Samples (three capsules) were collected every 7 days. The following physico-chemical and biological parameters were monitored: Temperature, pH, conductivity, dissolved oxygen, nitrates, nitrites, chlorophyll, fluorescence, effective quantum yield (Yeff) and total organic mass (AFDM). (See Figs. S2–S5 and Table S1, Supplementary data).

Table 1
Different parameters to optimize for MALDI-TOF/MS characterization of the target polymers.

Parameters							
Matrix	Name	RA[1]	IAA[2]	HABA[3]	DIT[4]	DHB[5]	DCTB[6]
	Solvent	THF		30%ACN/0.1%TFA (TA30)			
	Concentration	10 mg/mL		20 mg/mL		50 mg/mL	
Analytes	Name	PCP[7]	PCP[7]	PEVE[8]		POL[9]	
	Mw	1250	2000	3800		4000	
	Concentration	1 mg/mL		5 mg/mL			
Cationization agent	Metal	K		Na		Ag	
	Concentration	2 mg/mL					
Matrix : analyte : Cat. agent		10:10:01	10:25:01	10:50:01		10:100:01	

RA[1]: All-trans retinoic acid, IAA[2]: trans-3-indoleacrylic acid, HABA[3]: 2-(4-hydroxyphenylazo) benzoic, DIT[4]: dithranol, DHB[5]: 2,5-dihydroxybenzoic acid, DCTB[6]: t-2-(3-(4-t-Butyl-phenyl)-2-methyl-2-propenylidene)malononitrile, PCP[7]: polycaprolactonediol, PEVE[8]: poly (ethyl vinyl ether), POL[9]: ε-polylysine.

Table 2
Optimized conditions for MALDI-TOF/MS characterization of the target polymers.

Sample	Proportion	Matrix	Cationizing agent	Solvent
POL[1]	10:10:01	DHB[4]	AgTFA	TA 30
1 mg/mL		10 mg/mL	2 mg/mL	
PEVE[2]	10:10:01	All trans retinoic acid	KTFA	TA 30
1 mg/mL		10 mg/mL	2 mg/mL	
PCP[3] 1250 MW	10:10:01	DCTB[5]	NaTFA	THF
1 mg/mL		10 mg/mL	2 mg/mL	
PCP[3] 2000 MW	3:20:01	DCTB[5]	AgTFA	THF
1 mg/mL		20 mg/mL	2 mg/mL	

POL[1]: ε-polylysine. PEVE[2]: poly (ethyl vinyl ether). PCP[3]: polycaprolactonediol. DHB[4]: 2,5-dihydroxybenzoic acid, DCTB[5]: t-2-(3-(4-t-Butyl-phenyl)-2-methyl-2-propenylidene)malononitrile.

2.3. River exposure experiments

Once the degradation device was evaluated in the mesocosm study, a decomposition experiment was performed at 11 sites across the Ebro River basin, which had relatively good water quality (Ccanccapa et al., 2016; López-Serna et al., 2012; Roig et al., 2015), well preserved channel and riparian zones, but which were spatially distributed along a variable precipitation gradient, ranging from 380 up to 621 mm/y (Fig. 1). We deployed three caps filled with PCP 1250 (20–80 mg/caps) at each site, and recovered them 97 days later. After collection, samples were stored at low temperature (4 °C), air dried at room temperature and weighted prior to MALDI-TOF/MS measurements (described in Section 2.4). Degradation rate constants K_d (d^{-1}) were calculated from weight loss assuming first-order kinetics at time = 97 days.

Water temperature was recorded every hour during the experiment with data loggers (Onset Optic StowAway or ACR SmartButton). Electrical conductivity, dissolved oxygen and pH were measured (WTW Multi 350i) on three occasions at each site. On the same occasions, water was collected and transported chilled to the laboratory. Unfiltered water was used to determine ammonium concentration and alkalinity within 48 h of collection, while filtered (Millipore 0.45 μm) water was frozen for later analyses of nitrate, nitrite and soluble reactive phosphorus (SRP) concentrations. Nitrate was determined by capillary ion electrophoresis (Agilent CE); ammonium and nitrite concentrations were determined with automatic analyzers (Dionex DX-120 and Traacs 800). SRP concentration was determined by the ascorbic acid method (APHA, 1998). Alkalinity was estimated by titration with H_2SO_4 to an end point pH of 4.5 (APHA, 1998). Daily mean flow values for all studied sites were obtained from nearby gauging stations for the period 2005–2015 (data from Ebro Hydrographic Confederation; http: \\www.chebro.es). Results of physico-chemical and environmental variables are given in Table S2 (Supplementary data).

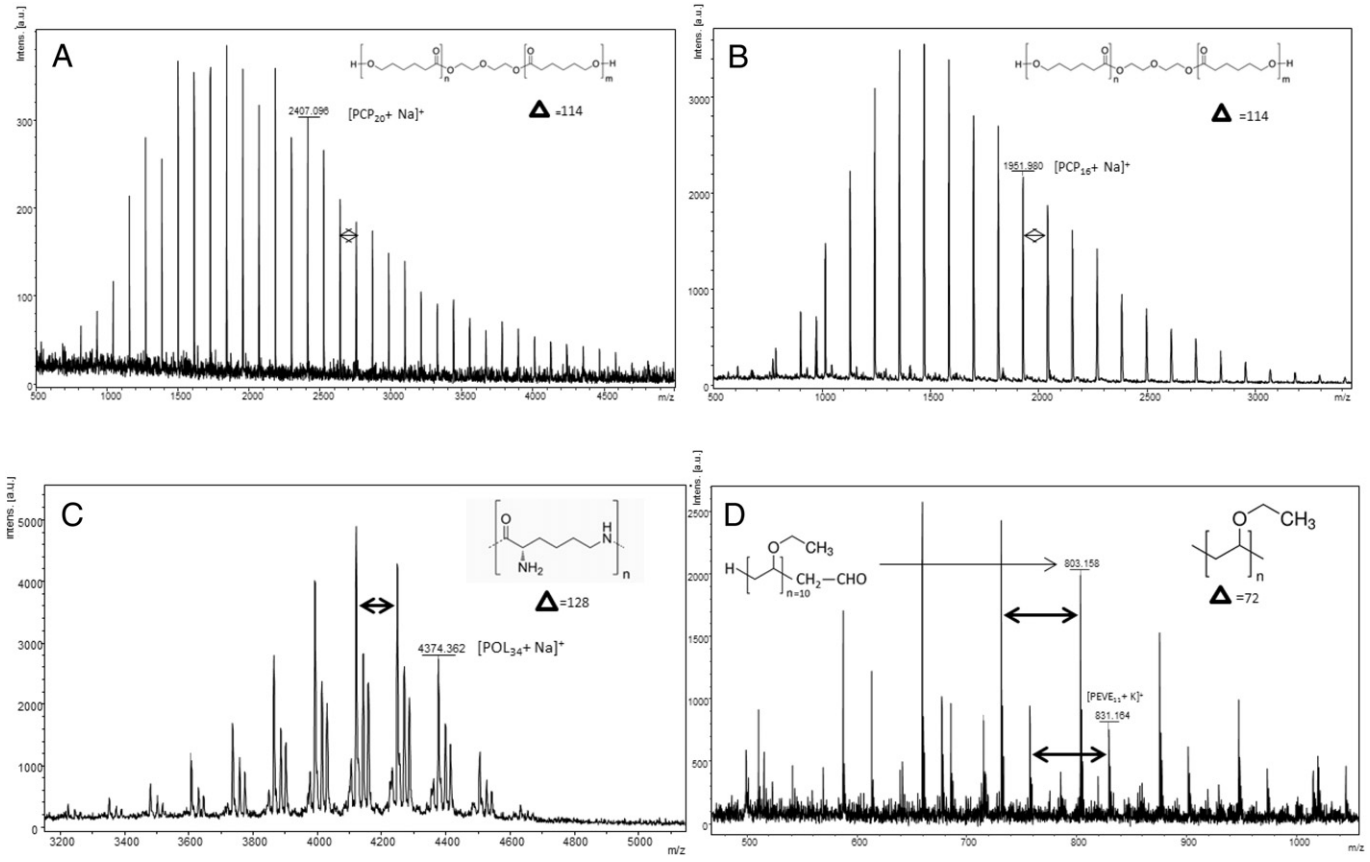

Fig. 2. MALDI-TOF/MS spectra of the studied polymers. (A) polycaprolactonediol 1250 (PCP 1250); (B) polycaprolactonediol 2000 (PCP 2000); (C) polylysine (POL); (D) polyethylvinyl ether (PEVE). M + Na and M + K adducts are observed for PCP 1250, PCP 2000 and POL. Main species observed for PEVE are tentatively identified as K adducts of structure shown in the insert (Katayama et al., 2001; Kumagai et al., 2008).

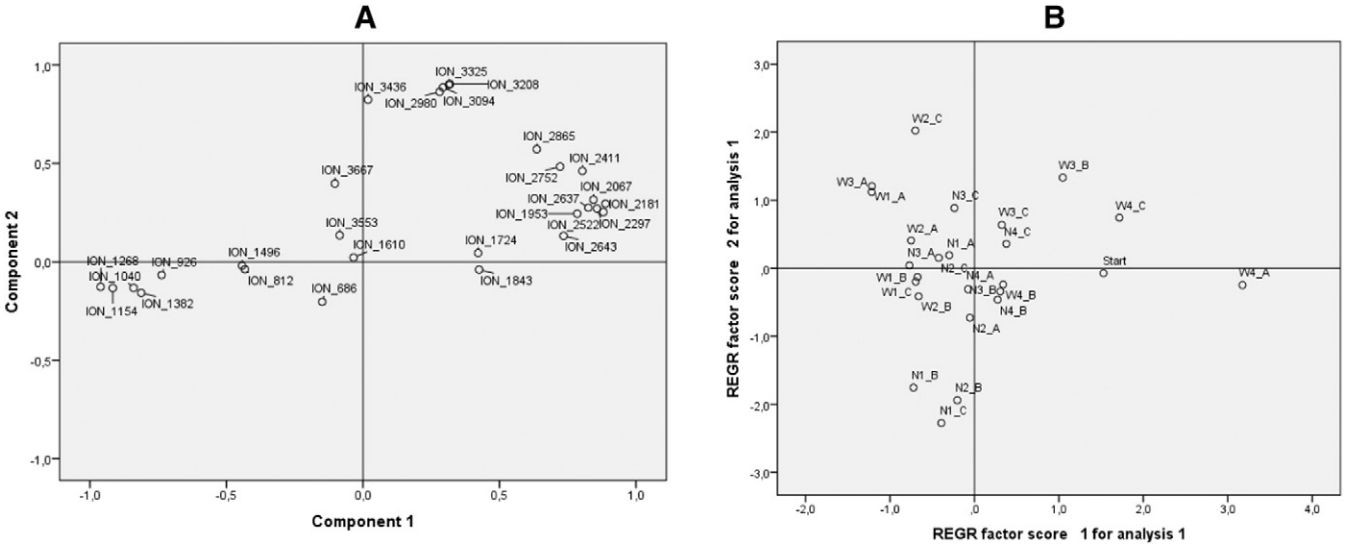

Fig. 3. Principal Component Analysis plots of mesocosms experiments. Samples corresponding to the treatment with nutrient addition are labeled 'N', those of the treatment without nutrient addition are labeled 'W'. Ions are indicated by their m/z. (A) Loadings plot; (B) Scores plot.

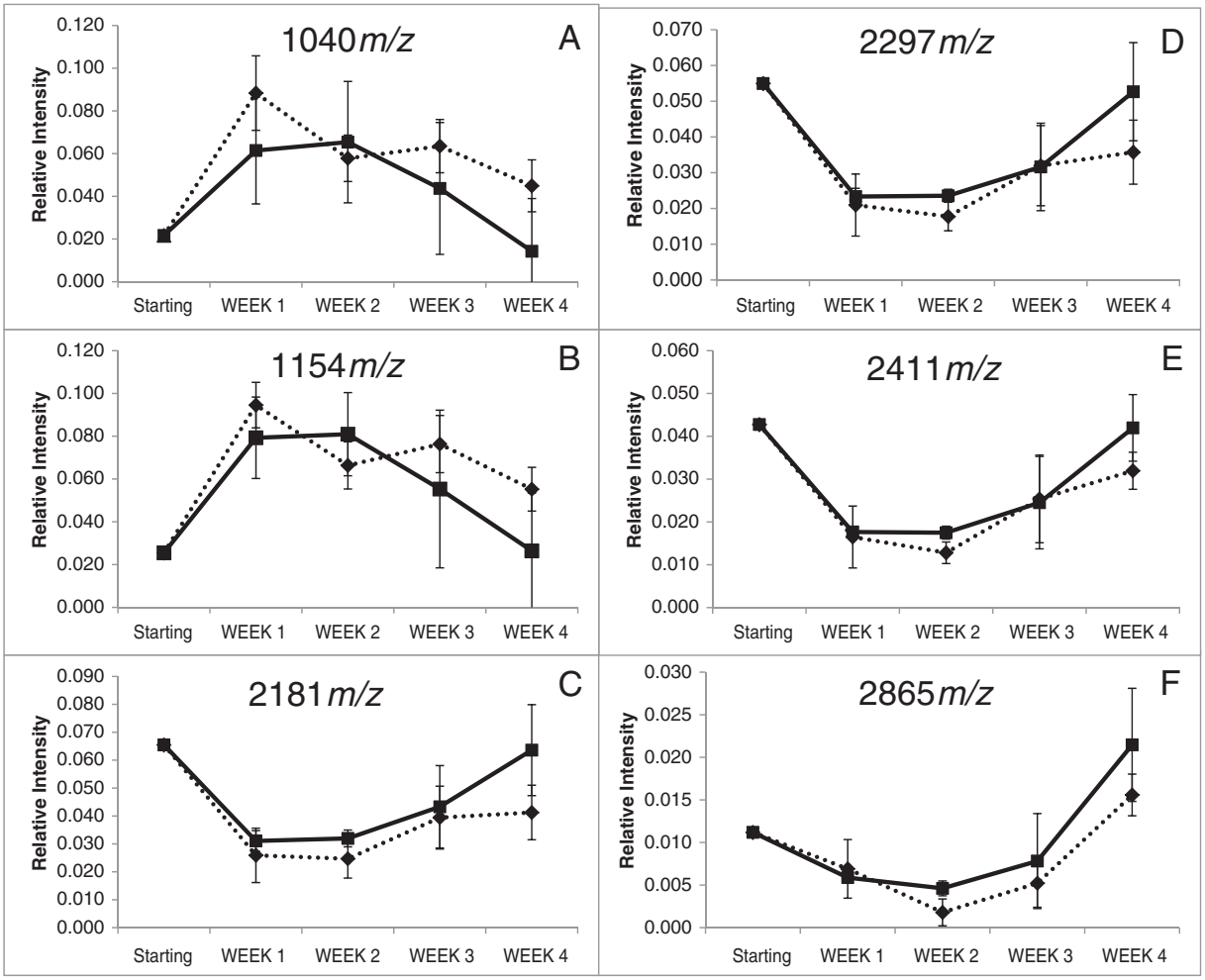

Fig. 4. Time course of relative peak intensities of some representative ions in mesocosm experiments with nutrients (full line) and without nutrient addition (dotted line). Error bars correspond to mean ± standard deviation ($n = 3$).

Table 3
Summary of ANOVA results comparing experiments with and without nutrient addition against standard material. Relative peak intensities of each ion at a given time (week 1 to 4) are compared to those of the standard material.

Week	Number of statistical significant peaks[a]	
	Nutrient	Without nutrients
1	7	11
2	4	6
3	1	1
4	11	3

[a] Levene test of homogenity of variance.

2.4. MALDI-TOF/MS

MALDI-TOF mass spectra were recorded using an Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 200 Hz smartbeam laser (337 nm). Different parameters were tested in order to find the optimal conditions for further analysis (Table 1). Six different matrices (RA, IAA, HABA, DIT, DHB and DCTB) were prepared in THF or in 30%ACN/0.1%TFA at different concentrations in order to analyze four polymers with different average molecular weight that oscillated between 1200 and 4000 Da. Three metal salts (Ag-TFA, K-TFA and Na-TFA) were tried as cationization agents. Matrix, polymer and cationization agent were mixed at three different ratios (10:10:1,10:25:1, 10:50:1 and 10:100:01), then 1 μL of the mixture was deposited onto the stainless steel target plate, which was then dried in air before insertion into the ion source chamber. The analytical conditions for the MALDI-TOF/MS analysis were set as follows: positive reflector ion mode; ion source 1 voltage, 19.2 kV; ion source 2 voltage, 16.2 kV; lens voltage, 8.5 kV; and mass range, 600–4000 m/z. The mass spectra were acquired by averaging 1000 laser shots. Laser irradiance was maintained slightly above threshold.

2.5. Statistical analysis

Data analysis was carried out on both mesocosm and river experiments using as variables the normalized ion intensities z_i defined as $z_i = x_i / \sum x_i$, where x_i designates the intensity of ion i in a given sample. Statistical treatment was performed with IBM SPSS Statistics Package 21.0. For mesocosm exposure experiments *Levene test* was first applied to evaluate the homogeneity of variances of the results. Significant differences were determined by one-way ANOVA (with treatment as a factor) at a significance level lower than 0.05 ($p < 0.05$). When significant differences were found for treatment factor, the *Tukey and Games - Howell* test (significance, $p < 0.05$) was used as post hoc comparison between controls and treatment. Principal Component Analysis was applied to both mesocosms and river exposure experiments.

3. Results and discussion

3.1. Polymer selection and characterization

In the present study, four polymers were chosen for further MALDI-TOF/MS characterization, namely, POL, PEVE, PCP 1250 and PCP 2000. Selection was done on the basis of two criteria: (a) molecular weight range (1000–10,000 Da), as it is the most suitable for MALDI-TOF/MS analysis and (b) their commercial availability and economic affordability. It is worth noting that their MALDI-TOF/MS characterization was not previously reported.

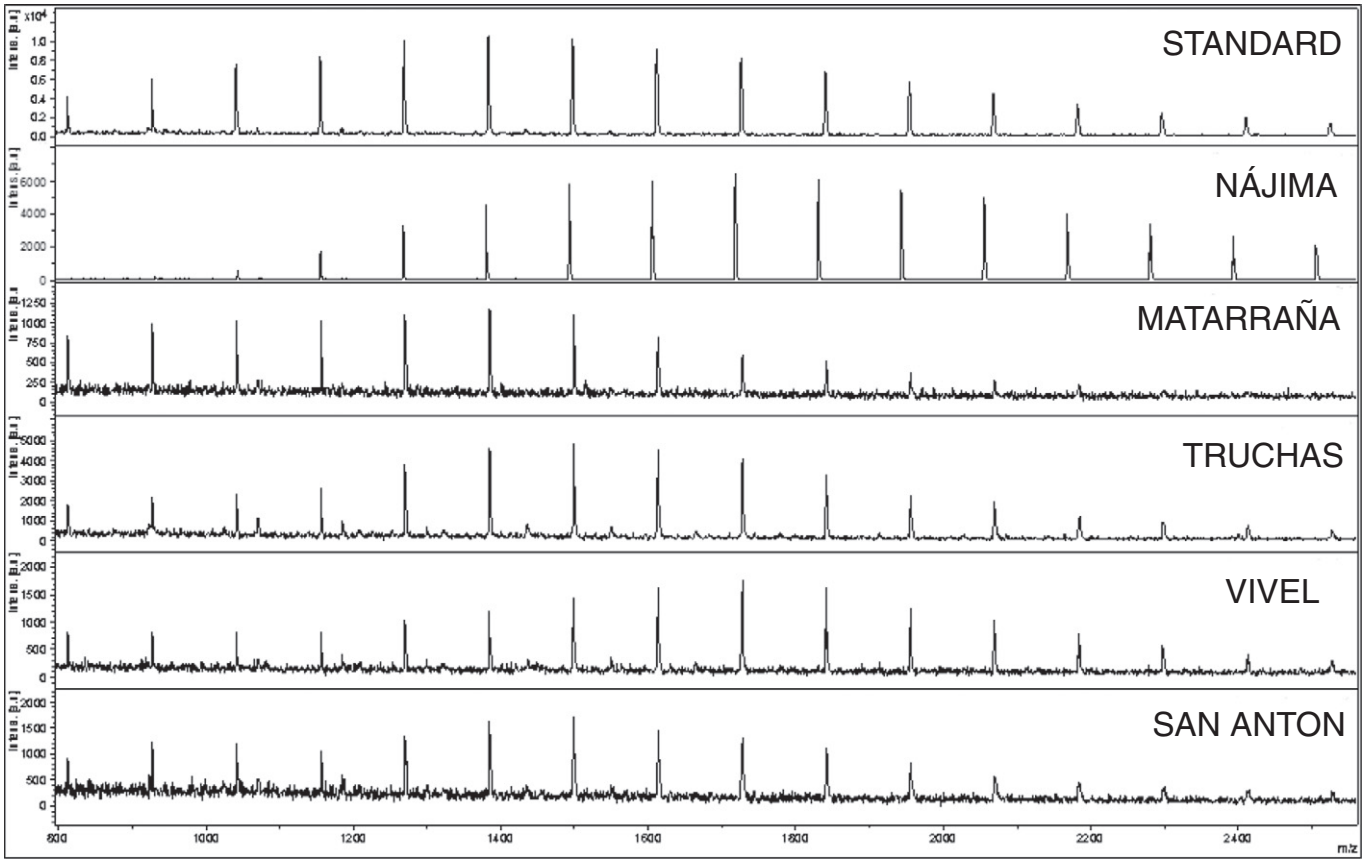

Fig. 5. Representative MALDI-TOF/MS spectra of polymer probes of different river exposed samples. Spectrum of standard material is included for comparison purposes. Note that intensity scales correspond to different attenuation factors. Differences among spectra of are perceptible both in absolute intensities of peaks and in their respective distribution (shifting of maxima).

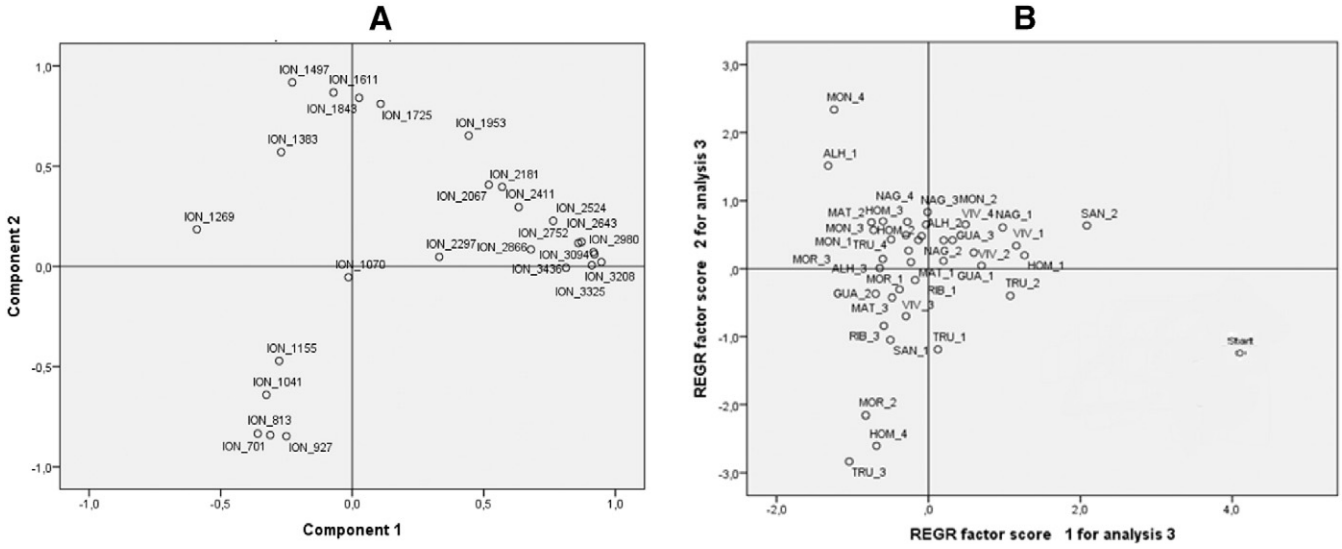

Fig. 6. Principal Component Analysis plots of experiments in the Ebro river basin. Ions are indicated by their m/z. (A) Loadings plot; (B) Scores plot.

Sample preparation is crucial in MALDI-TOF/MS and greatly influences the quality of the spectra. In a typical preparation approach, appropriate amounts of polymer, matrix and cationizing agent dissolved in compatible (preferably identical) solvent are mixed to yield a matrix: analyte molar ratio in a specific range for each polymer.

In MALDI-TOF/MS analysis, a dilute solution of the analyte polymer is mixed with a more concentrated matrix solution. The choice of a matrix tailored for a particular kind of polymer sample is crucial for its successful characterization. Optimal matrix selection is usually found by trial and error since the exact role of the matrix is still not fully understood. General requirements for an ideal matrix performance are the following: high electronic absorption at the employed laser wavelength, good vacuum stability, low vapor pressure, good solubility in solvents that also dissolve the analyte, and good miscibility with the analyte in the solid state. In contrast to biopolymers, the ionization of syntethic polymers usually occurs by cationization rather than protonation. Different cations (such as lithium, sodium, potassium and cesium) and transition metal salts (such as $Ag^{+}X^{-}$, $Cu^{2+}X^{2-}$) that efficiently wrap around the polymer can be used and are added in the form of cationizing agents. Finally, after the selection of the MALDI matrix, cationizing agent and solvent, several options are available for transferring the mixture onto the MALDI target plate (Aparna et al., 2015). In the present study the dried droplet method was selected. In this method the three solutions are mixed, and 1 μL of the mixture is applied to the target plate and air-dried at room temperature. Under these conditions crystallization is relatively slow, thereby increasing the risk of segregation among sample, matrix and cationizing agent (Montaudo et al., 2006). Matrix, cationizing agent and solvent as well as optimal experimental conditions are summarized in Table 2. Detailed MALDI-TOF/MS spectra of the four polymers studied are given in Figs. 2A to 2D. It is

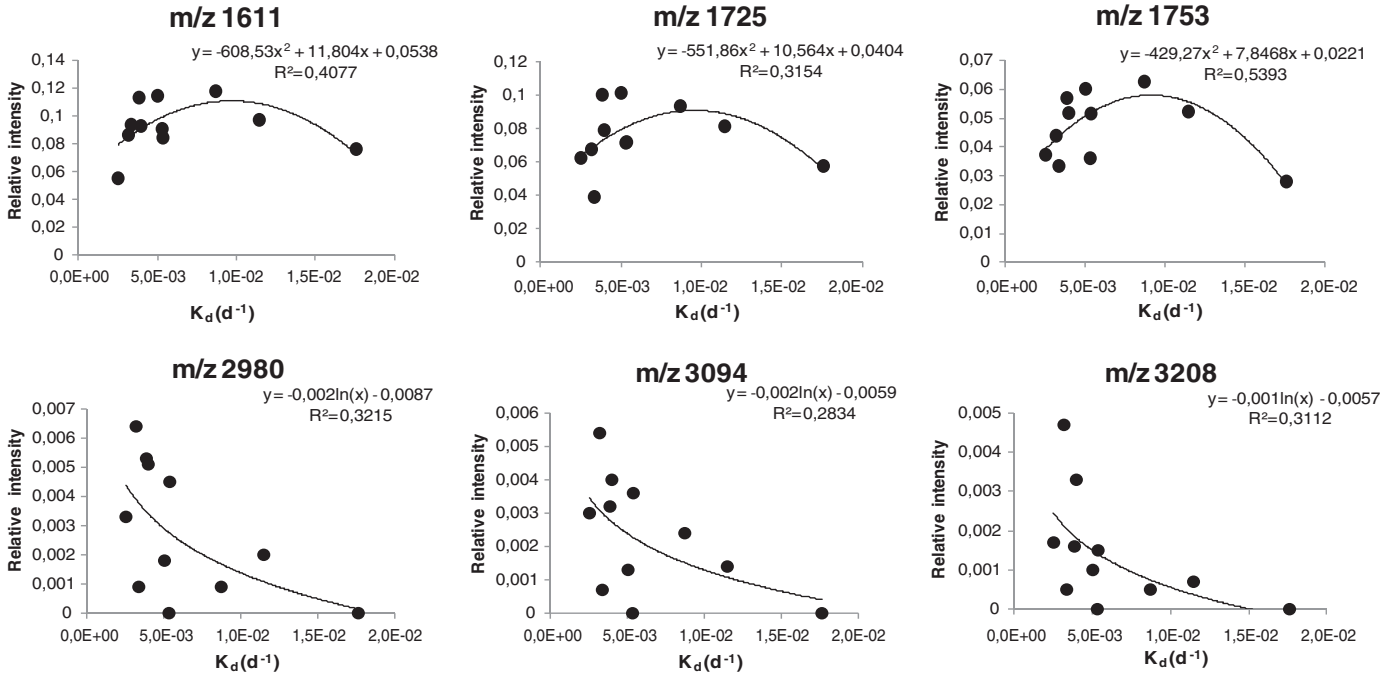

Fig. 7. Correlation between of polymer weight loss (expressed as K_d) and intensities of some representative peaks.

worth noting that even though Ag was added as cationizing agent, sodium and potassium adducts ($M_n + 23$ and $M_n + 39$ respectively) are the predominant species appearing in the spectra in the case of PCP 1250, PCP 2000 and POL (Figs. 2A–C) (Liu et al., 1999). Main peaks observed for PEVE were tentatively identified as K adducts of HM_{n-1}–CH_2–CHO (see Fig. 2D and the insert therein) (Katayama et al., 2001; Kumagai et al., 2008).

High matrix effects were observed for PEVE when the polymer:matrix:cationizing agent ratios used were of 10:50:01 and 10:100:01. This means that if high laser energies are used only very weak spectra intensities are obtained (Rizzarelli and Carroccio, 2014). The best conditions for this polymer were finally set at a ratio 10:10:1 (Table 2). These data suggest that the polymer might have undergone degradation by some cause (i.e. photo-degradation) (Göpferich, 1996; Rizzarelli and Carroccio, 2014). As seen in Table 1, even though the only apparent difference between PCP 1250 and PCP 2000 is molecular weight, the optimal conditions for MALDI-TOF/MS characterization of PCP 2000 were remarkably different, thus indicating that the polymer molecular weight may influence the crystallization process (Bahr et al., 1992).

Owing to its physico-chemical properties, commercial affordability, degradability, manipulation advantages and easy MALDI-TOF/MS characterization, PCP 1250, a polyester oligomer, was finally selected as testing material to be further used in mesocosms and field experiments.

3.2. Mesocosms experiments

In the artificial streams experiment we focused on the possible role of nutrients, since the development of river biological communities is strongly dependent on nutrient availability (Ponsatí et al., 2016; Sabater et al., 2016). Physico-chemical and biological variables measured are reported in Table S1 and Figs. S2–S5 respectively (Supplementary data). The three first components of a PCA performed using the normalized intensities of the 25 most abundant ions in the range m/z 700–3436 present in the MALDI_TOF/MS spectra captured 79.7% of the total variance (51.9%, 16.3% and 11.5% respectively) (Fig. 3). In the loading plot ions appeared well separated (Fig. 3A) in three groups corresponding to low (m/z 700–1700), medium (m/z 1700–2800) and high masses (m/z > 2800). The scores plot (Fig. 3B) did not differentiate samples neither in terms of exposure time nor treatment (nutrient concentration). However, the starting material seemed different from the rest, indicating that exposed samples had undergone some structural changes.

The evolution of the relative intensities of different ions along time depended on the ion mass (Fig. 4). Whereas small ions apparently increased their relative intensity during the first two weeks and decayed afterwards, the opposite behavior was observed for larger ones. Note that relative intensities only allow a comparison of the mass distributions (in the studied range) of the remaining polymer, but do not inform about other possible deeper absolute changes undergone by the polymer during the water exposition. The differences in relative intensities between a given ion of an exposed sample with respect to the standard material at different exposure times were checked for both treatments (ANOVA, Levene test $p < 0.05$) (Table 3). In the first three weeks, the number of ions showing statistically significant differences with respect to the standard was similar in both treatments and decreased over time. However, in the fourth week the trend was reversed and the number of statistically different ion intensities with respect to the standard material increased, but in the presence of nutrients the change was remarkably more pronounced (3 different peak intensities in the treatment without nutrients; 11 different peak intensities with nutrients). Furthermore, a direct comparison of peak intensities for both experiments at a given time was performed (ANOVA, Levene test $p < 0.05$) only showing statistically significant differences for peaks m/z 2411 and 2865 at week 4. Overall, under the conditions examined, differences in the degradation of polycaprolactonediol attributable to the presence of nutrients were seemingly statistically relevant only at longest exposure times. This result is consistent with the differences observed on biofilm development between both treatments (see Supplementary data, Figs. S2–S5, parameters Chlorophyll-a, organic biomass, photosynthetic activity and fluorescence) that were likewise more perceptible at longer exposure times (weeks 3 and 4).

3.3. River experiments

After 97 days of exposure, the composition of the polymer probes differed from the starting material, but the extent of changes was highly variable among samples. In general, changes were evident in terms of weight loss Table S3 (Supplementary data) as well as in their MALDI-TOF/MS spectra (see some representative examples in Fig. 5). The spectral changes detected were of two types: (i) different distribution of the polymer chain lengths, the most transformed samples showing a shift of the maximum towards longer chains and (ii) a decrease of the overall peak intensity (in the examples shown in Fig. 5, this fact is manifested in the different attenuation factors needed to acquire the spectra). A preliminary interpretation of the results was obtained by PCA using the normalized intensities of the main 25 ions in the m/z range 700–3436 present in the MALDI-TOF/MS spectra of the samples. The first three axes explained 78.5% of the total variance (51.2%, 21.4% and 5.9%, respectively) (Fig. 6). Remarkably, the loadings plot (Fig. 6A) followed a similar pattern to that obtained in the mesocosm PCA (Fig. 3A), i.e. ions clustered in three groups corresponding to low (m/z 700–1300), medium (m/z 1300–1900) and high masses (m/z > 2000), with ranges slightly shifted towards low masses. As observed in the mesocosm experiments, the PCA scores plot (Fig. 6B) allowed differentiating samples among sites. Starting material, characterized by positive PC1 and negative PC2 scores, appeared clearly separated from the river-exposed samples. Polymer weight loss (quantified as K_d) was comprised between $17.6 \cdot 10^{-2}$ and $2.52 \cdot 10^{-3}$ $days^{-1}$ corresponding the lowest value to stream TRU, one of the most severely affected (together with VIV) by flow reduction during the experiment (VIV K_d was $3.97 \cdot 10^{-3}$ $days^{-1}$). This behavior was also observed in litter degradation measurements done in parallel (Monroy et al., 2016), thus pointing to the relevance of local-scale hydrological conditions as proposed by these authors. The relative intensities of some peaks were correlated to K_d (Fig. 7); thus, whereas larger m/z peaks were negatively correlated, medium ones showed a less pronounced trend. Regarding the influence of environmental conditions, conductivity, mean temperature, river flow and nutrients (N-NH_4^+, DIN and SRP) showed a correlation with the intensities (Table S4, Supplementary data). Again, as previously mentioned, low and high mass ions exhibited in some cases opposite trend correlations.

4. Conclusions

The study of degradation of synthetic organic matter by freshwater ecosystems can be approached by means of polymer probes, used in a similar way as natural organic materials (leaf litter, wood sticks, etc.), yielding thus a complementary information on river metabolism. PCP 1250 can be effectively used as a probe and its transformation conveniently monitored by MALDI-TOF/MS. The interpretation of field results in terms of environmental conditions remains elusive, probably reflecting the influence of multiple concurrent factors (Sabater et al., 2016). In that respect, the use of MALDI-TOF/MS IMAGING techniques might open new possibilities (Rivas et al., 2016). In any case, the use of probes based on polymer degradation characterized by MALDI-TOF/MS seems a novel and promising tool whose potential uses are not restricted to river ecosystems, but can be applied as well to other biological systems such as wastewater treatment plants.

Acknowledgements

This study has been financially supported by the EU through the FP7 project GLOBAQUA (Grant agreement No 603629), and by the Generalitat de Catalunya (Consolidated Research Groups "2014 SGR 418—Water and Soil Quality Unit" and 2014 SGR 291—ICRA). It reflects only the author's views. The Community is not liable for any use that may be made of the information contained therein. The authors are thankful to Maria Casellas (Experimental Streams Facility, Catalan Institute for Water Research, Girona, Spain) for her technical help in the operation of the artificial streams.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.08.135.

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