Assessing the role of DOC released by macrophytes, algae and sediments on pesticide adsorption.

Alba Arenas Sánchez[1], Paul van den Brink[1], John Deneer[2], Bert Jan Groenenberg[3] and Gertie Arts[2]

(1) Department of Aquatic Ecology and Water Quality. Wageningen University. Wageningen. The Netherlands.

(2) Environmental Risk Assessment group (Alterra). Wageningen University and Research Centre. Wageningen. The Netherlands

(3) Department of Soil Quality and Environmental Chemistry (Alterra). Wageningen University and Research Centre. Wageningen. The Netherlands.

Abstract

Pesticides are considered as hazardous compounds for the aquatic environment. Several studies have proved that Dissolved Organic Carbon (DOC) can bind to different organic contaminants. Most likely, this binding is related with the hydrophobic acids present in the DOC mixture, as well as the hydrophobicity of the compound. Thus, this topic can be considered highly important as DOC can act as a vehicle for the movement of pesticide residues in surface water. DOC released by different compartments present in an aquatic ecosystem will be analyzed, together with the analysis of the affinity between DOC and the pesticide λ-cyhalothrin. First, four experiments were carried out to measure DOC release from sediments, submerged plants (*Myriophyllum spicatum*), floating plants (*Lemna minnuscula* and *Lemna gibba*), emergent plants (*Glyceria maxima*) and algae (*Scenedesmus obliquus)*. Collected water samples were used to determine the fraction of hydrophobic materials in DOC, by measuring UV absorption at 260 nm and by chemical fractionation. Finally, the most representative samples from the different plant treatments and algae treatment were collected and used to analyze the interaction with the pesticide by mean of the Equilibrium Dialysis Method. Results showed that sediments were likely the most important contributor to the DOC pool, but also plants were contributing to the DOC concentration. Algae showed the lowest DOC concentration values. Among plant treatments, *Myriophyllum* treatment had the highest DOC concentrations, however in *Lemna* treatment we measured the highest total amounts of DOC expressed in milligram dissolved C per gram of biomass. The influence of *Glyceria* on DOC concentrations was negligible in this experiment. Sediments in the *Glyceria* experiment were reaching similar DOC values than other treatments with plants. The *Glyceria* protocol required higher light intensity and temperature. Therefore, it may be that not only biotic factors, but also abiotic factors as temperature and light could influence DOC release and concentrations. Differences in hydrophobic fractions were small between treatments and also the affinity of the pesticide λ-cyhalothrin for DOC was similar for all treatments. Furthermore, we observed that sediments could contribute importantly to the DOC hydrophobic fraction. However, *Myriophyllum* and *Lemna* also do. Therefore, they could contribute to the pesticide mobilization in surface waters. Nevertheless, we suggest that sediments play the most important role in the regulation of DOC in surface waters and more attention should be paid to the role played by the sediments in this process.

Introduction

Pesticides are widely used as a method for the control and prevention of pests and diseases (Thomas & Hand, 2011; Crum *et al*., 1999). These hazardous chemicals can enter into adjacent aquatic ecosystems by means of processes such as spray drift, runoff, and drainage (Thomas & Hand, 2011) and, consequently affect biotic communities of such aquatic systems (Brock *et al*., 1992). The techniques normally used for remediation of polluted water are based on physical and electrochemical treatments (Jia *et al*., 2006), but these methods are expensive and not always effective (Business Publishers Inc. 2004, cited in Olette *et al*. 2008). De Carvalho *et al*. (2007) stated that concentrations of pesticides can be attenuated by sorption on sediment and aquatic plants for the more lipophilic compounds (log Kow >3) and ultimately degraded in these compartments. Additionally, the algal community has been considered metabolically active and competent in the degradation of pesticides and other organic xenobiotics several times (Caceres *et al*., 2008; Barton *et al*., 2004). Besides, there are many studies on organic contaminants binding to Dissolved Organic Matter (DOM) (Ogner & Schnitzer, 1970; Keskitalo & Eloranta, 1999). Previous studies stated that this interaction can be described as similar to a partition-like process (Chiou *et al*., 1986), employing an organic carbon partition coefficient, Koc (Merkelbach *et al*., 1993). Other studies related organic compounds binding to DOC with hydrophobic acids and hydrophobic neutral fractions (Akkanen *et al*., 2004; Kukkonen *et al*., 1990). Hydrophobic acids are usually identified as humic and fulvic acids (Akkanen *et al*., 2004; Chiou *et al*., 1986). DOM is often quantified by its carbon content (ca. 67 %) and referred to as Dissolved Organic Carbon (DOC) (Bolan *et al*., 2011). Moreover, recent experiments discovered that pesticide concentrations in surface water were higher than expected when plants and sediment were present. A possible explanation was the binding of hydrophobic contaminants to DOC.

Sources of DOC can be allochthonous or autochthonous. Allochthonous sources are of major importance in open systems like catchment areas (run-off) or downstreams (Allan, 1995; Robertson *et al*., 1999). Autochthonous DOC can be significant depending on the characteristics of the system; in our case we have considered a closed system without any external input. These sources can be aquatic plants, algae and sediments (among others), by means of processes as exudation, excretion, decomposition or diffusion (Baker & Farr, 1987; Thomas, 1997). Algae have a short generation time, small size (high potential to grow) and high surface area/volume ratio (Wang *et al*., 1997). Also, they have a high Net Primary Productivity of 3 gC/m2*day (Roberson *et al*., 1999) and exudate a significant portion of this photosynthetically fixed carbon (Kritzberg *et al*., 2004). Thus, they could be potentially important sources of DOC compared with aquatic macrophytes. However, it has been found that macrophytes can be as productive as algae in a macrophyte dominated clear water phase (Allende *et al*., 2009). On the other hand, despite DOC released by phytoplankton could reach a range between 10-50 % of the net primary production; much of this may be sequestered or consumed by bacterio-plankton as well as by heterotrophic algae (Sondergard & Jensen 1986, cited in Thomas 1997). Flynn *et al*. (2008) stated that it is inevitable to include the role of bacteria when modeling phytoplanktonic DOC at the ecosystem level. Moreover, also DOC coming from macrophytes may be lower than expected when associated with epiphytic bacteria and heterotrophic algae (Thomas, 1997). Therefore, it seems more accurate to speak about net DOC release than about gross values when analyzing DOC concentrations with the presence of uncontrolled variables. Among plants, data show a higher Net Primary Production by emergent plants (41.6 g DW/m2*day) (Westlake, 1975) when compared with submerged and floating plants (1.9 and 1.4 g DW/m2*day respectively) (Robertson *et al*., 1999). Considering that NPP and DOC release are related (Godmaire & Nalewaijko, 1989), we could expect higher DOC concentrations coming from emergent plants than from floating and submerged plants. On the other hand, floating plants are smaller in size and have faster growth rate than emergent and submerged plants (Hillman, 1961). Therefore, as they are more similar to algae than the rest, they may be the plant type leading to the highest DOC concentrations. With respect to sediments, soil type is important. More clay is related with more DOC sorption to sediment (Wilson *et al*., 1991). On the other hand, detritus accumulated in deposing sediments has been proved to have an important contribution to the DOC pool (Thomas, 1997). Therefore, it could be stated: the more organic matter (o.m.) content, the more DOC release. Additionally, it has been found that aerobic conditions promoted by emergent plants (oxygen translocation from surface) increase DOC concentration in pore water (Mann & Wetzel, 2000). Moreover, diffusion of this DOC into the water phase (Liss 1975, cited in Thomas & Eaton 1996a) could lead to an increase of the effects of emergent plants on DOC concentration in the water phase. In relation to these variables affecting DOC release from sediments, only the increase of DOC in sediment pore water was analyzed in our research. Variables as organic matter or clay content were not analyzed due to lack of time and the evidence that results will fit with existing data.

Diverse information exists on the release of DOC by aquatic macrophytes, algae and sediments. However, it is not clear which of them could be releasing more DOC than the others, as well as what could be inducing differences among and within these compartments. This study will be focus on getting a deeper knowledge on DOC release by the different compartments in the aquatic environment, as well as the variables which determine final DOC concentration. We consider this topic highly important as pesticides and other organic compounds can be partitioned onto DOC (depending on their hydrophobic fraction), and this can acts as a vehicle for the movement of pesticide residues in surface water (Barriuso *et al*., 1992). Hence, affinity of a selected pesticide (λ-cyhalothrin) will be also analyzed in this study.

Based on previous information, we hypothesise that sediments could have the largest contribution to the DOC pool. Additionally, emergent plants could increase DOC concentration in sediment pore water. Algae may be the most productive alive organism. Among plant species, floating plants could be the most productive ones if DOC release is more related to the growth strategy than to the NPP. If NPP is more related to DOC than the growth strategy, emergent plants could release more DOC than floating and submerged plants. With respect to pesticide interaction with DOC, it is expected that the more hydrophobic DOC, the higher Koc value of the pesticide will be observed.

In order to test these hypotheses, five experiments were carried out. The first three were focused on macrophyte DOC release (macrophyte species were *Myriophyllum spicatum* (*Myriophyllum*), *Glyceria maxima* (*Glyceria*) and a mix of *Lemna minuscula* and *Lemna gibba* (*Lemna*)). A fourth experiment was conducted to study DOC release by algae under algae dominating conditions. All experiments were carried out under environmental conditions (light, temperature, pH, nutrients) that allowed the optimal growth of the different species. Finally the interaction of DOC with the pesticide λ-cyhalothrin was assessed by means of the application of the Equilibrium Dialysis Method, as used by Merkelbach *et al*. (1993) and Akkanen *et al*. (2005). This method enables the study of the interaction of hydrophobic organic chemicals with DOC and their partitioning. With this method, the affinity of the hydrophobic compound with DOC can be estimated calculating its organic carbon sorption coefficient (Koc). Koc will change depending on the hydrophobic fraction present in the DOC. These data allow us to analyse whether the hydrophobic DOC fraction coming from specific compartments differs from others and evaluate if this could lead to variations in the persistence and transport of the pesticide in surface water.

Materials and Methods

Experimental set up

<u>Macrophyte DOC release</u>

Each experiment consists of two treatments; one including sediments and plants (plant treatment) and one including only sediments (control). The growth of algae in these treatments could not be avoided. Thus, we had algae concentrated to a certain extent in all vessels, except in the *Lemna* control (covered with dark plastic to simulate a layer of *Lemna* plants). Algae concentration was never as high as in the algae experiment, where it was dominating.

Plant pots were filled with 500 mL of standard sediment type (5% peat -dry weight according to 2±0.5% O.C-, 20% clay, 75% sand) (Maltby *et al.*, 2010). Each plant pot was placed inside a 1.8 L test vessels (based on an updated version of the test method proposed for *Myriophyllum*, in Maltby et al. (2010)). In order to maintain a pH range of 7±0.5, $CaCO_3$ was added to the sediment (7 g/kg dry weight sediment) (Maltby *et al*., 2010). An aqueous nutrient medium was added to the sediment in order to achieve concentrations of 200 mg/kg sediment (dry weight) of both NH_4Cl and Na_3PO_4 and a 30%-50% moisture level (Maltby *et al*., 2010). Smart & Barko medium (Smart & Barko, 1985), with an initial pH of 7.5-8 (updated version of the ring-test protocol from Maltby *et al*. (2010)) was used as a water growth medium. The volume of growth medium was 1.8 L for *Myriophyllum* and *Lemna* and 1.1 L for

Glyceria, taking into account that the latter species is an emergent plant species (helophyte) and should not be totally submerged (Davies, 2001). Considering that 500 mL of this volume was occupied by the plant pot with sediments, real volumes for plants were 1.3 L and 0.6 L respectively.

To ensure that all test vessels received the same light intensity, the test vessels were rotated on a weekly basis.

Accounting for variability within treatments, extra vessels were used for plant treatments and for controls in each experiment. The initial number of vessels was 11 for *Myriophyllum* and *Lemna* plant treatments and 12 for *Glyceria* (more room space and high quality shoots available). 8 vessels were initially used as control treatment (less variation expected) per experiment. After a pre-treatment period of 11 days, 5 vessels with plants and 5 control vessels were selected and used to continue with the definitive experiment (criteria explained in following sections).

Myriophyllum spicatum (Myriophyllum)

Myriophyllum spicatum was collected from one of the ditches at the Sinderhoeve experimental station (Renkum) and placed in a bucket with Smart & Barko (S&B) medium and a defined experimental light intensity of 120±30 µE m^{-2} s =7400-10360 lux in PAR, for one day (adaptation phase).

The vessels were placed under a light intensity of 120±20 µE m^{-2} s =7400-10360 lux in PAR, 16:8h light dark ratio and a temperature of 20.5±0.5°C. 5 shoot apices (10 cm long each) were planted in every plant pot with two nodes inside sediments. Before planting them, fresh weight of the five shoots selected per vessel was estimated by weighing on a precision balance (5 decimals) (not >30% variation from the mean). After 11 days, the vessels that showed optimum plant growth and had lower Chl-a levels were selected to continue with the experiment.

Lemna minnuscula and *Lemna gibba* (*Lemna*)

Lemna minnuscula and *Lemna gibba* were sampled from an urban pond in Wageningen (Emmapark, Wageningen, The Netherlands). The adaptation stage was carried out in the same way as for *Myriophyllum*, but test conditions differed in light intensity (110±20 µE m^{-2} s = 6660-9620 lux in PAR). Additionally, nutrients were added to the water medium. This phase took 4 days, since in between a trial was performed in order to determine which were the best conditions for growing *Lemna* (based on a treatment with sediments + nutrients in water, a treatment with nutrients in sediments and water and third one without sediments, all of them including three replicates). Finally, the best growing option was to add nutrients in sediments and water.

Sediment composition and nutrient concentration in the soil solution (same moisture level) were similar to those for *Myriophyllum* and *Glyceria*. Since *Lemna* requires additional N and P, a single dose of N and P was applied: 400 mg NH_4NO_3 and 30 mg K_2HPO_4 L^{-1}, which equals 140 mg N and 6.83 mg P L^{-1}. 10 mL of this nutrient solution were weekly added to the water medium (in plant treatment and control).

The control vessels were completely covered with aluminium foil with small holes on top facilitating air exchange, to simulate the covered surface by *Lemna* and to avoid algae growth due to extra nutrient concentration. The vessels were placed in the same climate room as *Myriophyllum*, at a light intensity of 110±20 µE m^{-2} s = 6660-9620 lux in PAR, a 16:8h light dark ratio (OECD, 2002b) and a temperature of 18.4±0.5°C. A number of leaves from *Lemna minuscula and Lemna gibba* (mixed) enough to cover the test unit surface were defined as initial biomass. Fresh weight of the biomass in the first vessel was determined with a precision balance and approximately the same weight was applied on the rest of the vessels with *Lemna*. 11 days later, the vessels with *Lemna* that were healthier and had lower Chl-a level were selected to proceed with the experiment. Control vessels did not differ substantially, therefore they were chosen at random.

Glyceria maxima (Glyceria)

Glyceria maxima plants were obtained as seedlings of about 7 cm (raised emersed) from an external source. To adapt them to the laboratory conditions, all seedlings were placed in a bucket with water covering the substrate surface and left growing for 20 days, with a light intensity of 420±20 µE m^{-2} s = 29600-32560 lux in PAR. This experiment was carried in a separate climate room in which light and temperature were different.

The vessels were placed under a light intensity of 420±20 µE m^{-2} s = 29600-32560 lux. in PAR, a 16:8h light dark ratio and a temperature mimicking the seasonal conditions (Davies, 2001), in this case 23±1°C was the temperature according to our conditions. 12 medium size and good quality shoots were selected, flushed (taking out original substrate) and planted individually in each 12 pots corresponding to the plant treatments. Initial fresh weight and length was determined for each shoot with a standard balance (1 decimal) and a ruler respectively. On day 11, the vessels where plants showed high growth and less yellow colouring were selected to continue with the experiment. Also, the Chl-a level was taken into account (control vessels were selected based on their Chl-a apparent similarity with the plant treatment vessels, always looking for the lower Chl-a levels).

Algae DOC release

Two treatments were also set up for this experiment (5 replicates per treatment). However, in this case no sediments were included since the main objective was to determine the algae DOC release under optimal conditions of light and nutrients. Hence, it consisted of one treatment with algae (algae treatment) and one treatment with only water medium (control). Dark conditions were achieved in the control by wrapping with aluminium foil.

The selected species was the green alga *Scenedesmus obliquus*, since it is a fast growing species, and it is well known they develop under light conditions very well. In order to adapt the test alga to the test conditions, an inoculum culture was prepared in the test medium 7 days before start of the test. The algal biomass was adjusted in order to allow exponential growth to prevail in the inoculum culture until test start (2-5x10^3 cells mL^{-1}). The inoculum culture was incubated under the same conditions as the test cultures in a glass flasks of sufficient volume to obtain a surface volume ratio of at least 0.15 cm^2 m^{-1} (OECD, 2002a). For the inoculum culture a 300 mL flask was filled with a volume of 200 mL. The light intensity was in the range of 82±2 µE m^{-2} s = 5920-6216 lux in PAR. A 16:8h light dark ratio (Maltby *et al*., 2010) and a temperature within the range of 23.5±0.9°C were applied. The initial pH of the water medium was 7.5-8 (OECD, 2002a). The growth medium was WC medium, derived from the COMBO medium described in Kilham *et al*., (1998), not using the animate component and adding buffer TES, trace element solution (ATE) and vitamin stock solution (after autoclaving 20 minutes at 121°C and cooling down below 60°C, then 0.5 mL VIM, vitamin solution, was added). Sterilisation of the medium was performed to avoid the presence of any disturbing organism at the beginning, despite later on the flasks were open as in previous experiments.

Finally, 10 conical flasks (500 mL flasks filled up to 300 mL with WC medium) were placed in a climate room with controlled environmental conditions. In 5 of them an inoculum of *Scenedesmus obliquus* was applied, with a final concentration of 2-5x10^3 cells mL^{-1} at t = 0. No inoculum was included in the controls, and to avoid algae growth, the 5 flasks in the control treatment were fully covered with perforated aluminium foil. The algae were left to grow for 3 days before measurements started. To ensure that all systems received the same light intensity, the test vessels were rotated every 3 days.

Pesticide interaction with DOC

The method was applied once all DOC samples from previous experiments were analyzed, selecting water from the three plant treatments, as well as from the algae treatment. Differences in concentration of hydrophobic DOC among species were considered as important information to understand pesticide behavior when interacting with DOC. The pesticide selected was λ-cyhalothrin, as it is a well known substance for analysis and it could add new information to previous studies developed within the Environmental Risk Assessment group at Alterra (linked group in the project).

Before that, another experiment took place to determine equilibration time of λ-cyhalothrin. Four dark flasks were filled with 200 mL of Mili-Q water, and a dialysis membrane (Spectra/Por 6, MWCO 1000 Da) placed inside and also filled with Mili-Q water. The flasks were closed to avoid volatilization. 2 mL of λ-cyhalothrin from the stock solution (500 µg/L), previously diluted in acetone and water, was applied with a syringe outside the membrane, diluting solution 100 times to get a final concentration of 5 µg/L in the systems. The flasks were used to estimate how far the equilibrium was after 72h. Final values were corrected for an equilibration rate equal to 1. Water in each of the flasks was sampled at separate but sequential dates.

Once the equilibration time was estimated, 11 new flasks were prepared in total. Three replicates for *Myriophyllum, Glyceria* and *Lemna,* and two for *Scenedesmus obliquus*. The samples with the highest DOC concentration were selected. Exceptionally, due to delay on DOC analysis in the case of algae, samples with a representative Chl-a concentration were selected. Each water sample was used to fill in one flask with 150 mL including the DOC released during the treatments. To have enough replicate data, three dialysis membranes were placed inside each flask. For pesticide application, the final concentration (5 µg/L) was reached from the same stock solution concentration (500 µg/L) and equal to the equilibration experiment. However, in this case, 1,5 mL was added to the outside water solution.

After 72h samples were taken from solutions inside and outside the dialysis tubes. Concentrations measured inside and outside were corrected for the fact that the system was presumably no yet at equilibrium. From the determination of equilibration time it was concluded that the concentration inside should be multiplied by 1.4 (or 1/0.7). The concentration outside was corrected for the mass transfer implied in the previous correction. The following formula (Eq. 1) was applied to determine the corresponding Koc of our pesticide with different DOC natures (the higher Koc, the more affinity, the more persistence).

$$Koc = \frac{Ca - Cb}{Cb * DOC} \qquad (1)$$

(Ca= concentration outside the membrane; Cb: concentration inside the membrane. The difference is equal to what is fixed to DOC).

The empty flasks, filled membranes and outside water, were weighted to have an accurate value of recovery after extractions at the end.

Also a testing protocol to determine the efficiency of the extraction method was developed (recovery experiment described in the Analytical method section).

Sampling and measurements

pH, temperature (T) and dissolved oxygen (DO) were measured as control parameters in macrophyte experiments, at days 1, 3, 11, 18, 24 and 29 for macrophytes, and at days 0, 3, 7 and 10 for algae.

Analytical measurements for macrophyte experiments started at day 11, without transplanting of plants to prevent losing DOC released during the pre-treatment period. After 11 days, plant biomass was estimated from five of the removed extra vessels. The vessels where plants were growing better were selected to do this measurement. In the case of *Lemna*, due to bad quality of plants in extra vessels, only initial and final biomass was measured. Additionally, for all experiments, three extra biomass samples were prepared at day 0 to determine initial dry weight. DOC and Chl-a concentration, as well as UV-absorption at 260 nm were measured in water samples at days 11, 18, 24 and 29. UV-absorption at 260 nm was measured to determine the concentration of hydrophobic DOC. The hydrophobic DOC was identified with the sum of fulvic acids, humic acids and neutral hydrophobic fractions. Additionally, per vessel 150-200 mL water samples were prepared in duplicate at last sampling date. One of the samples was preserved with NaN_3 1M for future DOC fractionation analysis. In this analysis, fulvic and humic acids, as well as neutral fractions were measured as the hydrophobic fraction of DOC. Also, hydrophilic fractions were measured in this fractionation analysis. The final NaN_3 concentration in water was 0.001M. The other water sample was frozen for future Equilibrium Dialysis Method (EDM) application. Later on, the samples with the highest DOC concentration were selected to apply the analyses. Pore water extraction from *Myriophyllum* and *Glyceria* sediments was performed at last sampling date. In this phase, the DOC concentration was also analysed.

In the Algae experiment, at sampling dates days 3, 7 and 10, DOC and Chl-a concentration in water was measured. At these sampling dates, also the number of particles per mL and UV-absorption at 260 nm were measured. At the last sampling date 150 mL water samples were prepared and preserved with NaN_3 1M for future DOC fractionation analysis. The final NaN_3 concentration in water was 0.001M. The selected sample contained an intermediate but representative concentration of Chl-a. Two other samples with the higher Chl-a concentration were stored at 4°C for EDM. They were not frozen as the analysis took place 3 days later.

In the pre-experiment to determine equilibration time, pesticide samples were extracted with n-hexane after 5h (flask 1), 24h (flask 2), 48h (flask 3) and 72h (flask 4). At each sampling date two extractions were performed, one from the inside Mili-Q water and a second one from the outside Mili-Q water.

In the final sorption experiment, three samples were taken per flask to extract the pesticide from the water outside the membrane (water + DOC) and one sample was taken from water inside each membrane. In total we had three membranes per flask, adding up to three replicates for Ca/Cb per flask.

Analytical method

To measure pH we used a pH-meter (WTW pH23, model Sentix-81). An OxiGuard sensor was used to control T and DO. The sensors were submerged to a medium depth where conditions were considered to be homogeneous.

For Chl-a measurements in plant experiments, two methods were used. Until day 24, a fluorimeter (AquaFluor™) was used to avoid disturbance of the systems. At day 29, Chl-a concentration was measured with a spectrophotometer. For this last method, a known volume of homogeneous sample was filtered through a GF/C filter, subsequently stored in the dark and frozen until extraction. Next, the filters were rolled, placed inside a glass tube filled with 10 mL of 80% ethanol and shaken on a vortex for 5 seconds at 80 rpm. Hereafter the samples were placed in a stove at 75°C for 5 min, cooled on ice to decrease temperate to 20°C, shaken and centrifuged for 5 minutes at 3000 rpm at 5 degrees. Afterwards, a small amount of the sample was pipetted into a quartz cuvette and absorption at 750 and 665 nm was measured. Subsequently, one drop of 0.4 N HCl was added and absorption at the same wavelengths as before was measured. This step was used as a correction factor for disturbing particles. The final step for this analysis was to calculate Chl-a concentration in µg/L following Eq. 2

$$chl - a\ (\mu g/L) = 29.6 * [(E665 - E750) - (E665, HCl - E750, HCl)] * {}^{v}/_{V * l} \quad (2)$$

(v = volume extract in mL (= 10 mL), V = volume sample in litter, L = air path in cuvette in cm)

Macrophyte biomass increase was determined by estimating the initial dry weight of total planted biomass per pot (average from dry weight of three extra replicates) and weighing fresh and dry weight after 11 days and at the end of the experiment at day 29. Finally, mean dry weight over time was plotted for *Myriophyllum*, *Glyceria* and *Lemna* (Graphs available on excel file `sedweights and biomass´). A linear growth was defined for *Myriophyllum* and *Glyceria* with Eq.3 and Eq.4 respectively. For *Lemna*, only two biomass values were available, what makes difficult to determine the population growth of this specie. However, we decided to estimate this growth with an exponential function (Eq.5), based on the assumption that *Lemna* species normally show an initial exponential growth under optimal conditions (Blundon, 1994; Clark, 1924). Growth rates allowed us to estimate plant biomass at days 18 and 24. In the case of *Lemna*, the biomass at day 11 had to be estimated as well. Dry weight was measured by placing the fresh biomass samples into the oven at 105°C and weighing them (4 decimals precision, 1 decimal for *Glyceria*) every 24 hours until the weight was stabilised.

$$y = 0.0146x + 0.2852 \quad (3)$$

$$y = 0.0155x + 0.4074 \quad (4)$$

$$B = 0.139 * e^{0.028t} \quad (5)$$

Increase in algae biomass was calculated in a similar way (Figure 1), however measurements were based on Chl-a concentration (µg/L) and number of particles per mL. Chl-a analysis was performed using a PHYTO-PAM analyser (Heinz Walz GmbH, Effeltrich, Germany) at days 0, 3, 7 and 10. 2 mL of a 10 times diluted sample was analysed with the PHYTO-PAM. Chl-a concentration in algae culture was diluted 10 fold to avoid saturation of the apparatus. The number of particles per mL was determined by using a CASY cell counter, Model TT (CASY-Technology, Germany) based on the principle that intact cells can be generally considered isolators and increase the level of resistance when a low voltage field with 1MHz is applied. In this case, a 201 dilution factor was applied (50 µL sample + 10000 µL CASYton solution). The apparatus gave us the results based on this 201 dilution factor. Nevertheless, these values had to be corrected for the previous 10 fold dilution factor.

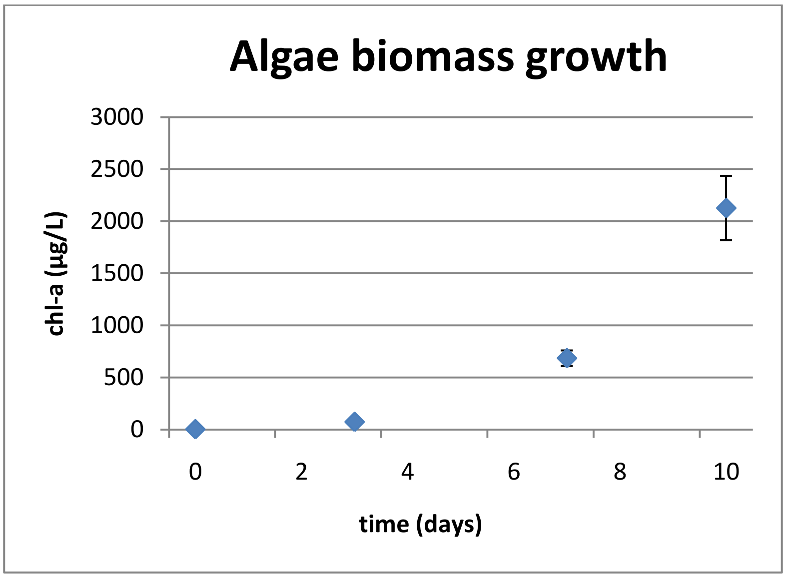

Figure 1. Graph showing the exponential growth of *Scenedesmus obliquus.*

For DOC analysis approximately 10 mL of pore water was extracted per sample. Sediment was collected, placed in tubes and weighed (130-150 g of wet sediment), to ensure an equilibrated weight in the centrifuge. Samples were centrifuged for 15 minutes at 2200 rpm and supernatant was sampled with a glass Pasteur pipette.

Prior to the analysis of DOC the water was filtered over a Watman cellulose filter, with a pore size of 0.45µm. Two tubes of approximately 10 mL per sample, plus two blanks were prepared and placed in the DOC/DIC analyser (Total Organic Carbon analyser, O.I.C International BV, Model 700), with a LOD (limit of detection) of 0.1 mg C/L. The last samples (algae treatment), were analysed for DOC in the biological and chemical laboratory of the Soil Science Centre using the same technique but with a different apparatus, with 1.8 and 2.2 as LOD values for IC and TOC. The apparatus at the Soil group laboratory calculated DOC concentration (mg C/L) as TOC (Total Organic Carbon)-IC (Inorganic Carbon). Both apparatus measure TIC and TOC. A blank prepared with filtered nano-pure water gave a value of 1.4±1 mg C/L in the first apparatus at the AEW laboratory, while in the other at the Soil department laboratory, with a higher detection limit, values for these blanks were 0. Moreover, blanks with only WC medium (filtrated) were measured with both apparatus. The first cited apparatus gave a value of 30.12 mg C/L, while the second showed a value of 29 mg C/L. This implies that these apparatus are working in the same way. Thus, they could give comparable results. Furthermore, as an important remark we should explain that the high DOC concentration in the WC medium was assumed to come from the disturbance caused by the EDTA present in the medium. This compound has high carbon content in its structure ($C_{10}H_{16}N_2O_8$) and that could explain that it is measured as DOC. Hence, DOC concentrations in the algae experiment were corrected by subtracting the mean value of these blanks.

Additionally, total DOC mass in water was calculated multiplying DOC concentration values by the water volumes used per experiment.

UV-absorption at 260 nm is linearly related with the concentration of hydrophobic organic matter present in water samples. Only hydrophobic particles are going to absorb at this wavelength (Dilling & Kaiser, 2002). Therefore, UV spectroscopy at 260 nm was applied to the filtered samples (before DOC analysis). In order to transform the obtained signal into a concentration of hydrophobic DOC, a calibration experiment was performed. In this experiment, we measured the absorbance as a function of the concentration of hydrophobic material, for which we used purified humic acid. Three dilution series were made out of an initial concentration of humic acid (HA) of 10 g/L (5 g C/L). Each series contained 5 solutions and one blank containing the solvent. In series 1, solvent was S&B to make it as similar as possible with the measured absorption values from the experimental systems; series 2 followed the same criteria, but adding same nutrient concentration per diluted sample as the *Lemna* experiment had, since these nutrients could interfere on the signal; finally, series 3 was made with the WC medium used for algae, following the same criteria as previously. The solutions were 100 mg C/L (10 mL HA 10 g/L in 0.5 L), 50 mg C/L, 20 mg C/L, 10 mg C/L and 5 mg C/L (last 4 dilutions were made out from 100 mg C/L). Nutrient addition in the second series was calculated based on the final volume, the final concentration (400 mg NH_4NO_3 and 30 mg K_2HPO_4 L^{-1}) that every diluted sample should have, and the dilution factor that should be applied to the HA concentration. The final volumes were 500 mL in the solution with 100 mg C/L and 100 mL in the rest of the solutions. To finalise this experiment, absorbance at 260 nm was measured with UV spectrophotometry, and together with the measurement of the concentration of DOC in the diluted samples using the Total carbon analyser, a calibration graph was made (absorbance vs. hydrophobic DOC). Consequently, extrapolation of the absorption values measured in previous experiments allowed the determination of the related mg C/L, and therefore, the expression of the hydrophobic DOC as a fraction (%) from total DOC. In parallel, also the NO_3^- concentration in one water sample from each macrophyte species in the experiment and in WC medium was analysed, as this ions can interact with the signal when concentration is above 25 mg/L. With this we were able to justify the use of three calibration series when nitrate values are over the limit in 2 of the 3 growth medium used (not in S&B).

Fractionation analysis was developed at the Energy research Centre of the Netherlands (ECN). Speciation of DOC was determined by measurement of the humic acids, fulvic acids concentration, as well as the concentration of neutral and hydrophilic compounds. These measurements were developed according to the rapid batch technique of van Zomeren & Comans (2007). This technique is based on currently recommended guidelines for organic matter characterization from the International Humic Substances Society (Swift, 1996).

Extraction of pesticide concentrations inside and outside the membranes was made by liquid-liquid extraction. Adding 2-3 mL n-hexane to 7-10 mL of water sample (both of them applied with Pasteur pipettes and weighted) and shaking vigorously for 30 seconds. Also, glass tubes used for these extractions were weighted empty. Differences in weights were used to have accurate values of our volumes and the final concentrations extracted. Extracts were analysed using gas chromatography with electron capture detection (GC-ECD). An Agilent 6890B GC equipped with an electron capture detector, an autosampler and a Agilent DB-5MS 30 m length, 0.53 mm I.D., 1.50 µm film thickness column was used. The injector (splitless injection with of 5 µL sample) and detector were operated at 250 and 325°C, respectively. The GC oven condition had a 275°C isotherm. The carrier gas was helium and the make-up gas was nitrogen. Run time was 20 min and retention time of lambda-cyhalothrin was about 7 minutes. External standard calibration was used to quantify lambda-cyhalothrin. The stock solution of 500 mg/L was made in acetone from 98 % Dr. Ehrenstorfer reference material. Working standards were made by dilution of the stock solution, with hexane, with a concentration ranging between 0.5 and 10 ppb. Method detection limit (LOD) has been established for extract in hexane 0.08 ng/mL and limit of quantification (LOQ) for about 0.20 ng/mL. In this case (with the extraction volumes indicated before) the detection limit (LOD) for water was about 0.026 ng/L and limit of quantification (LOQ) about 0.066 ng/mL.

To conclude with the pesticide extraction and analysis procedure, a recovery experiment was performed at the end. 10 glass tubes were weighted empty, adding hexane to 5 of them and water with DOC (this does not have to be the same but similar to the water we used before) from *Lemna* treatment in the other 5 tubes. All of them were weighted again, and then 50µL of λ-cyhalothrin from a stock solution of 200 µg/L was added to 4 tubes with hexane and 4 tubes with water with DOC. Finally, about 3 mL n-hexane was added to the five tubes with water and they were weighted again. All tubes were shaken (except for the one with only hexane) and analysed later on with GC. Recoveries of fortified blanks ranged between 95 to 111 percent with an average of 106.2%. The spike solution used during the recovery experiment was made in acetone by diluting the stock solution 500 mg/l subsequently (25 times and afterwards 100 times) to a 200 µl/l lambda-cyhalothrin solution in acetone. The dosage solution was made by diluting the same stock solution, 1000 times with Milli-Q water.

Data analysis

DOC released by the different compartments was analyzed as a `net release´, considering the different uncontrolled variables that could affect this process. This analysis was done in terms of DOC concentration (mg C/L), DOC total mass (mg C) and mg C/g plant or µg Chl-a).

The statistical method used to analyse the data was an Univariate analysis (One-Way ANOVA) applied to two treatments with different variables or end points. The following variables were tested: Chl-a, hydrophobicity, DOC concentration and DOC total mass. Before this analysis, normality and homogeneity of variances were tested for the different variables. Normally, we used logarithmic transformed data to reduce variations within the original data. However, when the variance of the non-transformed data was clearly more homogeneous, the statistical analysis was applied to the original data. In general, our data followed a normal distribution, but more variation was found in the homogeneity of variances. When this homogeneity of variances was not proved, a non parametric test was applied. Within the ANOVA analysis, comparison between 3 plant experiments was applied, as well as between these plant experiments and algae experiments. A post-hoc analysis was applied, using a Bonferroni test. Additionally, linear correlations among Chl-a and DOC, as well as Chl-a hydrophobicity and DOC-hydrophobicity were analysed. The objective of this last analysis was to approach a nicer view of direct, indirect or no correlation among variables. This analysis was applied for all sampling points until day 24 when correlation with Chl-a was analyzed in plant experiments. We did not apply this correlation until day 29 since the Chl-a measurement method changed at last sampling date. For the algae experiment, all sampling points at all sampling dates could be considered as we had a common measurement method (PHYTO-PAM analyser). The correlation analysis between hydrophobic fraction and DOC concentration were applied for all sampling points and dates together. Also, these analyses were done for all experiments and variables per sampling date. Additionally, linear correlation between DOC concentration and plant biomass was applied. The same was done for algae biomass. The coefficients of determination were considered as indicators of the association of our data with a linear distribution.

Results

Net DOC release (DOC concentration, DOC total mass and mg DOC/g of plant or µg Chl-a)

DOC concentration increased over time in *Myriophyllum* plant treatment and control until day 24 (Figure 2 a), when equilibrium seems to be reached. DOC concentration in the *Lemna* plant treatment increased until day 24 as well. However, the *Lemna* control did not show an increase in concentration during the experimental period (totally dark treatments) (Figure 2 c). DOC concentration in *Glyceria* plant treatment and control increased over time (Figure 2 b). In this experiment, no equilibrium was observed at day 24, despite variation in DOC concentration increased at day 29. With respect to the algae treatment, increase in DOC concentration over time was noticeable on day 10 (last sampling date). In this experiment the control treatment did not show a significant increase in DOC concentration (Figure 2 d).

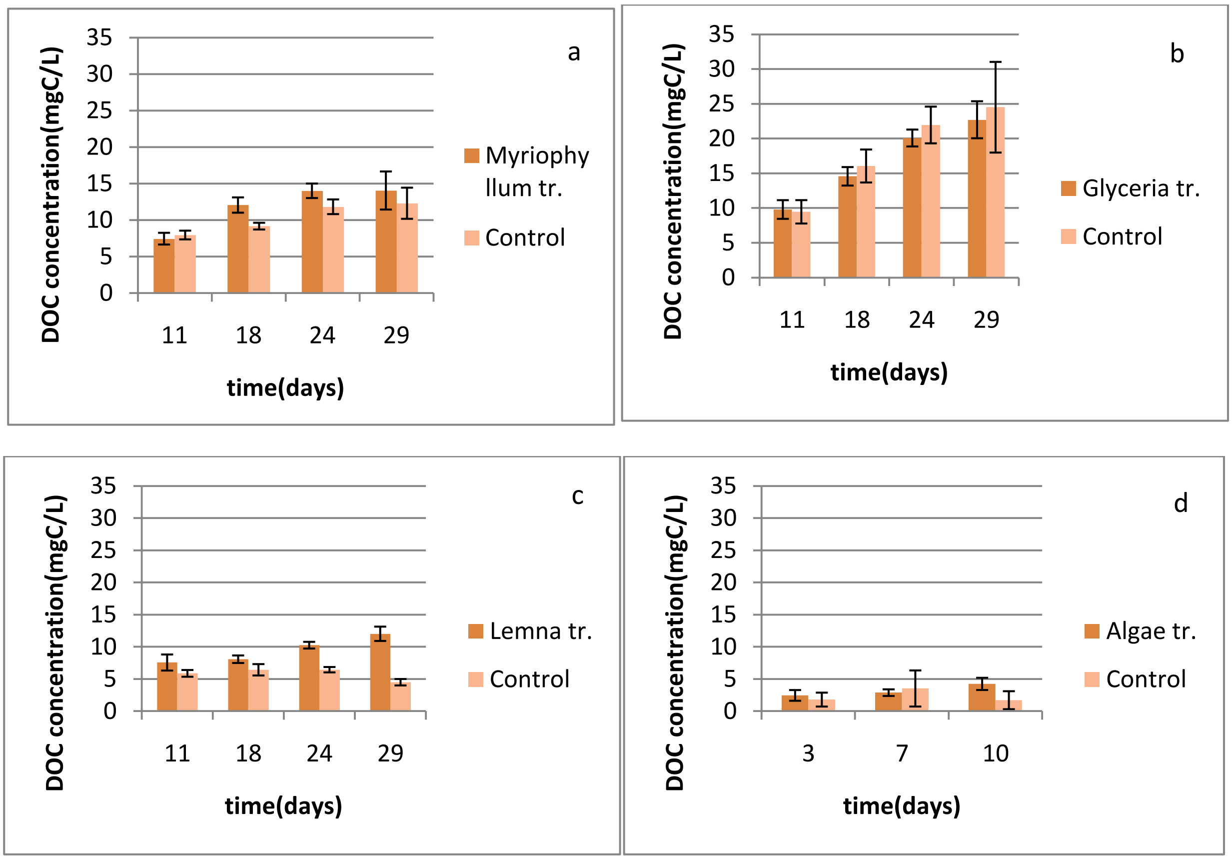

Figure 2. DOC concentration (mg C/L) over time for *Myriophyllum (a), Glyceria (b), Lemna (c) and algae (d)* experiments (plant treatment vs. control). Plant treatments are mixed systems with algae + sediments (substrate). Controls contain only sediments, also mixed with algae. *a* and b are rooted plants, *c is a floating specie. a and c* have the same water volume. Sediments are the same in *a, b, and c*: but in c, the control was covered with dark plastic*.* *d* has no sediments. The light intensity is higher in *b.*

When DOC concentration in plant treatments were compared with their respective controls, we found significant differences in the *Lemna* experiment at all sampling dates ($F = 7.027$, $p < 0.05$; $F = 10.019$, $p < 0.05$; $F = 124.939$, $p < 0.05$ and $F = 197{,}859$, $p < 0.05$). *Myriophyllum* plant and control treatments were significantly different on days 18 and 24 ($F = 29.165$, $p < 0.05$ and

F = 9.675 p <0.05) but no differences were found on days 11 and 29. All significant differences were associated with a positive relation, that is, plant treatments had larger DOC concentrations or net DOC release than controls. (See ANOVA table 1 and 3 in ANNEX 1). At day 24 the *Myriophyllum* plant treatment showed a concentration of 14±1 mg C/L, while DOC concentration in the control was 12±1 mg C/L. In the case of *Lemna*, at day 24 DOC concentrations of 10.3±0.5 mg C/L and 6.5±0.4 mg C/L were found in the plant treatment and the control respectively. *Glyceria* plant and control treatments did not show differences in DOC concentration at any sampling date (See ANOVA table 2 in ANNEX 1). At day 24, DOC in the *Glyceria* plant treatment had a concentration of 20±1 mg C/L, while the control showed a close value of 22±3 mg C/L (See excel file `parameters+results´).

The algae treatment had significantly higher DOC concentration than its control on day 10 (F = 8.988, p <0.05). These values ranged from 2.5±0.8 mg C/L to 4.2±0.9 mg C/L. In the algae treatment the Chl-a level was always significantly different when compared with the control. (See ANOVA table 4 in ANNEX 1).

With respect to the algae contribution to DOC concentration in our mixed systems, we analyzed the linear relation between Chl-a and DOC in *Myriophyllum, Glyceria, Lemna* and algae experiments. The coefficients of determination or R^2 were below 0.5 when all sampling points until day 24 were considered, with the exception of the *Lemna* experiment. When DOC was plotted against Chl-a per experiment and sampling date, the highest values were reached in the *Lemna* experiment (around 0.8). Also, an exceptional value was observed in the *Myriophyllum* experiment at day 24. With respect to the algae experiment, all R^2 values were below 0.5 (See ANNEX 2). Additionally, at the last sampling date, Chl-a concentration in different plant experiments (in plant treatments and controls) was analyzed (Figure 3). No significant difference in Chl-a was detected between *Myriophyllum* plant treatment and control (See ANOVA table 1 in ANNEX 1). However, this should be coupled with a low $R^2 = 0.03$ (See ANNEX 2) on this sampling date for future conclusions. *Glyceria* showed a significant difference in Chl-a concentration between treatments (F = 9.662, p <0.05. See ANOVA table 2 in ANNEX 1), being higher in the plant treatment. Nevertheless, this should be compared with the absence of significant differences in DOC concentration between treatments at this sampling date and a low correlation between Chl-a and DOC ($R^2 = 0.05$. See ANNEX 2). On the other hand, the *Lemna* experiment seemed to have a stronger correlation between Chl-a and DOC in this last sampling date. In this case, significantly higher Chl-a concentration in the plant treatment was found (p <0.05 -non parametric-), as it occurred for the DOC concentration at this last sampling date.

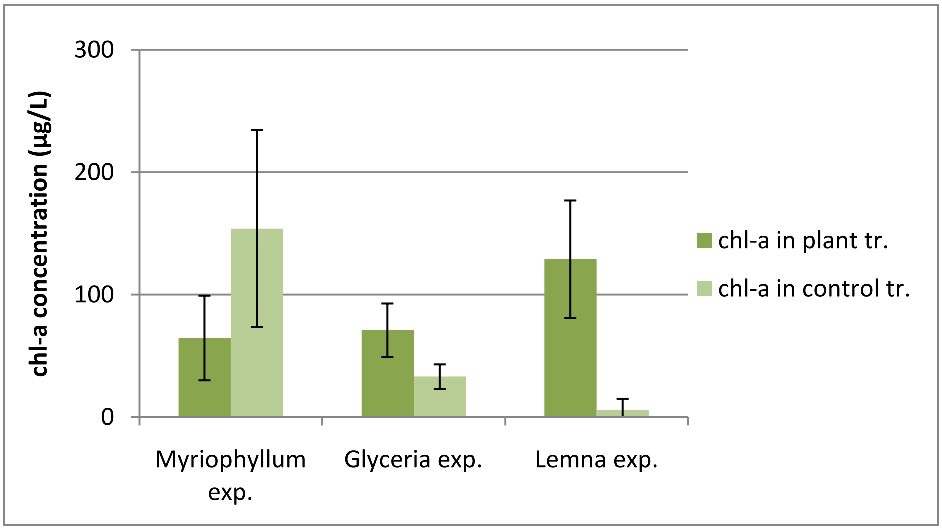

Figure 3. Chl-a concentration (µg/L) in plant and control treatments on day 29, in *Myriophyllum, Lemna* and *Glyceria* experiments.

Considering plant and algae biomass and their correlation with DOC, we found that *Lemna* and algae had the strongest correlation. Coefficients of determination (R^2) were 0.99 and 0.87 for algae respect to Chl-a concentration and number of cells/mL respectively. A R^2 value of 0.96 was found for *Lemna,* 0.78 for *Myriophyllum* and 0.98 for *Glyceria* . This last value is meaningless as the plant is not fully submerged and there was no evidence of plant contribution from previous data. Based on this, mg C/g plant or µg Chl-a (better association than number of cells) were compared between species. However, it has to be considered that *Myriophyllum* had a low correlation value. The value mg C/g of plant in the *Glyceria* experiment was neglected. Results showed that *Lemna* had the largest net DOC release per gram (Figure 4). Algae values were negligible and significantly different when we compared them with the rest of the species (F = 3,045.687 and p <0.0001 in both comparisons. See ANOVA table 5 in ANNEX 1). *Myriophyllum* and *Lemna* net DOC release per gram of plant differed significantly at all sampling dates (See ANOVA table 7 in ANNEX 1).

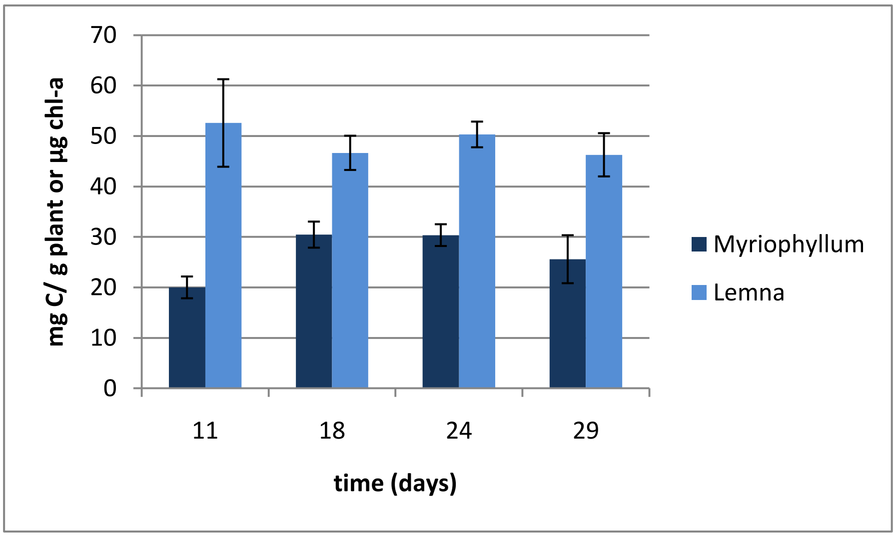

Figure 4. mg C/g of plant for *Myriophyllum* and *Lemna* plant treatments at different sampling dates.

Differences in DOC concentrations among experiments were highly significant when we compared plant treatments and algae treatment (Figure 5). The algae treatment had the lowest net DOC release in terms of concentration. This was also observed in the control treatment (Figure 6; p-values from Bonferroni test <0.0001. See ANOVA table 5 in ANNEX 1). Among plant experiments (Figure 5), DOC concentration in the *Myriophyllum* plant treatment was significantly lower than in the *Glyceria* plant treatment at all sampling dates (p-values from Bonferroni test <0.05. See ANOVA table 6 in ANNEX 1). DOC concentrations at day 24 (maximum values) were 20±1 mg C/L, 14±1 mg C/L and 10.3±0.5 mg C/L in *Glyceria*, *Myriophyllum* and *Lemna* plant treatments respectively. The algae treatment showed a final concentration at day 10 equal to 4.2±0.9 mg C/L (See excel file `parameters+results´). *Myriophyllum* and *Lemna* treatments, as well as their respective controls, showed a significant difference in concentration in most of the sampling dates (p-values from Bonferroni test <0.05). However, on the last sampling date, differences between these plant treatments were not significant (See ANOVA table 7 in ANNEX 1). *Myriophyllum* plant treatment and control had a larger net DOC release than the respective *Lemna* treatments when these differences were given. In general, differences among controls (Figure 6) in plant experiments were significant, with larger contribution of *Glyceria* controls to the DOC pool in terms of concentration (p-values from Bonferroni test <0.05. See ANOVA table 6 and 8 in ANNEX 1).

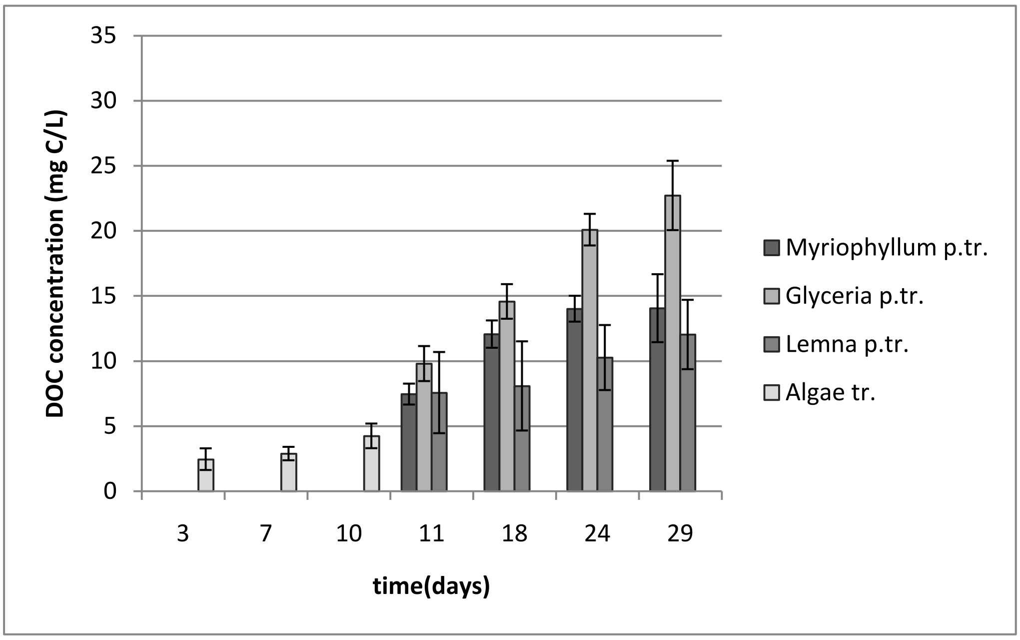

Figure 5. DOC concentration (mg C/L) in plant treatments at different sampling dates, per

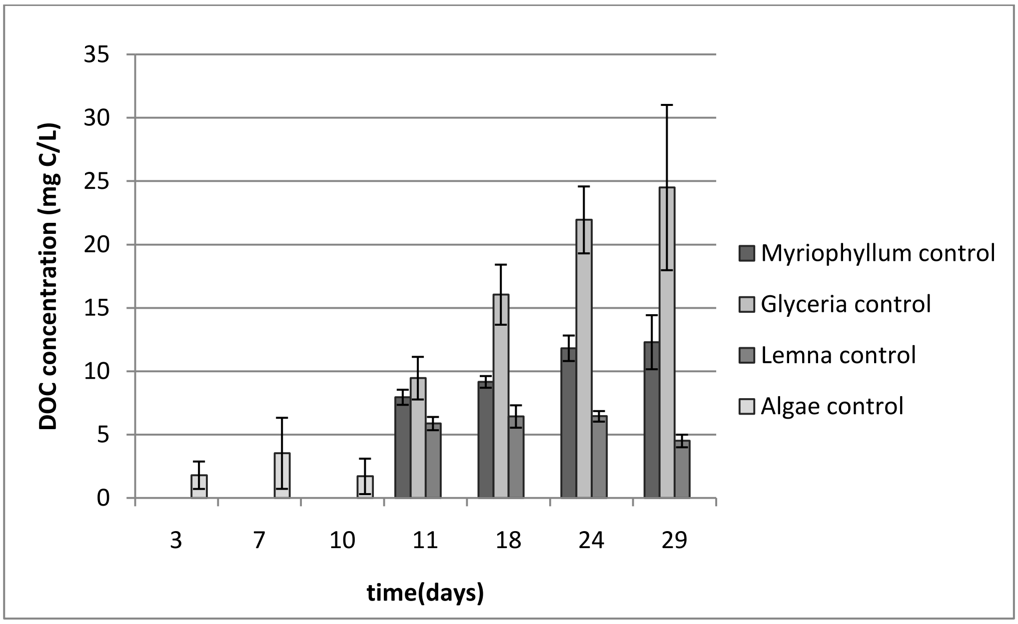

Figure 6. DOC concentration (mg C/L) in control treatments at different sampling, per species.

DOC total mass did not follow the same distribution as the concentration. Different water volumes were used for *Glyceria* and algae experiments. Based on that, from day 11 to day 24 the largest net DOC release is not coming from the *Glyceria* plant treatment, but from *Myriophyllum* and *Lemna* treatments (Figure 7). Mostly significant differences were found between the three treatments at these sampling dates (p-values from Bonferroni test <0.05. See ANOVA table 6, 7 and 8 in ANNEX 1). We should take into account that in the *Glyceria* plant treatment DOC is coming mainly from sediments, as we did not find differences between this plant treatment and its control with only sediments. The DOC total mass at day 24 reached values equal to 18.2±1.3 mg, 13.3±0.7 mg, 12.1±0.7 mg respectively coming from *Myriophyllum, Lemna* and *Glyceria* plant treatments (See excel file `parameters+results´). At day 29 these differences have disappeared and the net DOC release in terms of mass is not significantly different among treatments any more (Figure 7; ANOVA tables 6, 7 and 8 in ANNEX 1). We observed clear significant differences between the algae treatment and plant treatments, with 1.3±0.3 mg coming from the algae treatment. Also, significant differences were found between algae control and plant controls at all sampling dates. Algae treatment and control were less productive treatments (Figures 7 and 8; p-value from Bonferroni test <0.0001. See ANOVA table 5 in ANNEX 1). Analyzing the net DOC release in controls in terms of DOC total mass, *Glyceria* and *Myriophyllum* controls were always releasing significantly more than the *Lemna* control (Figure 8; p-value from Bonferroni test <0.05. See ANOVA table 7 and 8 in ANNEX 1). As an exception, the *Glyceria* control was not significantly different from the *Lemna* control at day 18. Total DOC mass was significantly higher in the *Myriophyllum* control than in the *Glyceria* control until day 24, with the exception of a p-value = 0.055 at day 18. This difference was reduced over time and at day 29 no significant differences were found between *Myriophyllum* and *Glyceria* controls.

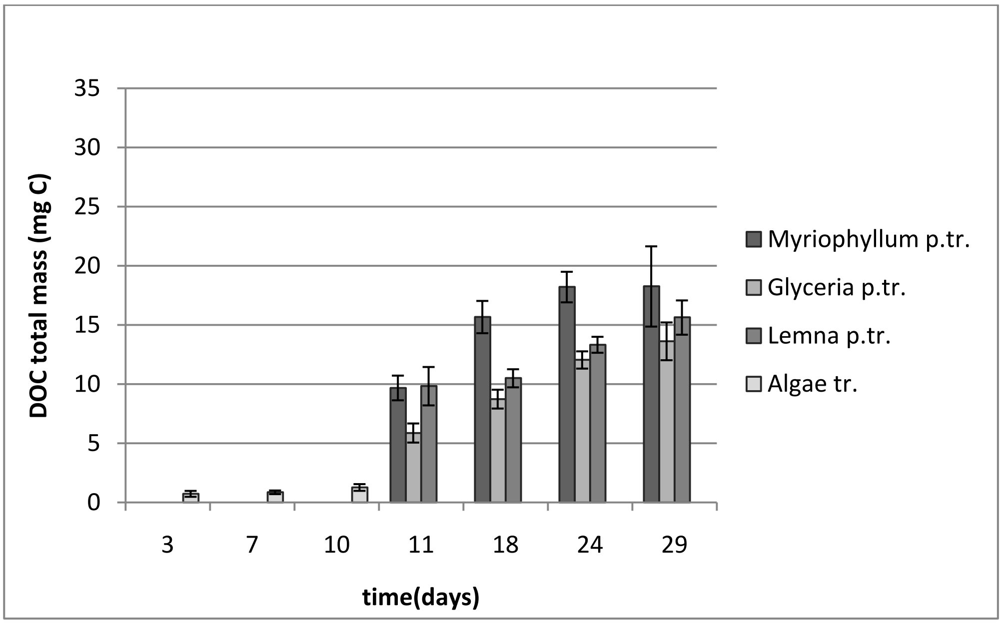

Figure 7. DOC total mass (mg C) in plant treatments and algae treatment at different sampling dates.

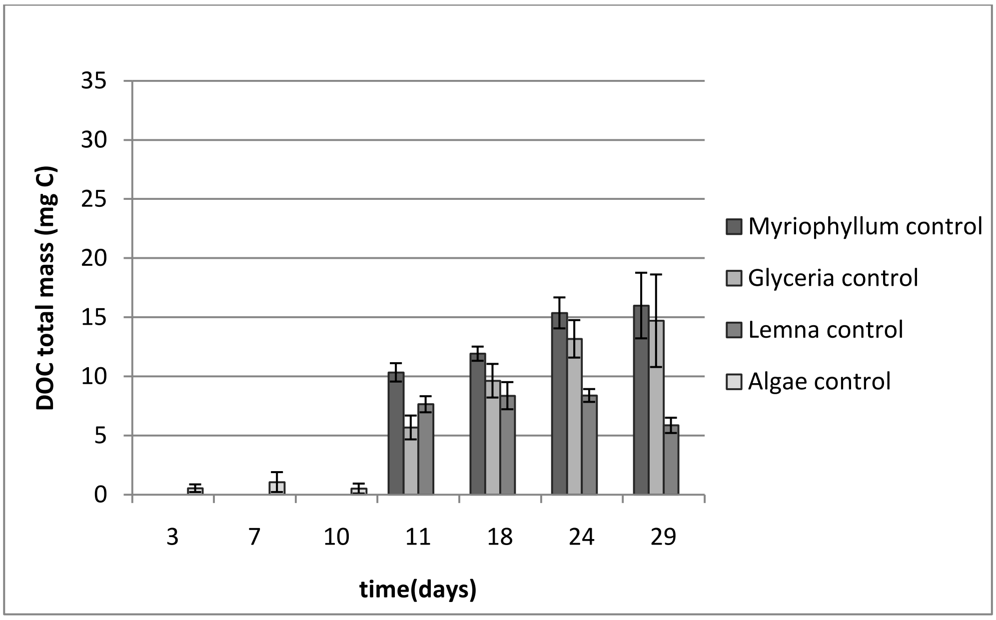

Figure 8. DOC total mass (mg C) in control treatments at different sampling dates, per species.

DOC in pore water

In *Myriophyllum* and *Glyceria* experiments, pore water DOC concentration (Figure 9) did not show significant differences among plant treatments and controls. However, when comparing plant treatments and controls separately, significant differences were observed (among plant treatments: $F = 65.862$, $p < 0.05$; among controls: $F = 20.068$, $p < 0.05$. See ANOVA table 9 in ANNEX 1).

Final DOC concentration in pore water in the controls of *Myriophyllum* and *Glyceria* experiments showed values of 49.2±7.7 mg C/L and 117.5±32.5 mg C/L. The concentrations of DOC in pore water from plant treatments were 41.5±4.7 mg C/L and 85.1±11.2 mg C/L respectively (See excel file `parameters+results´).

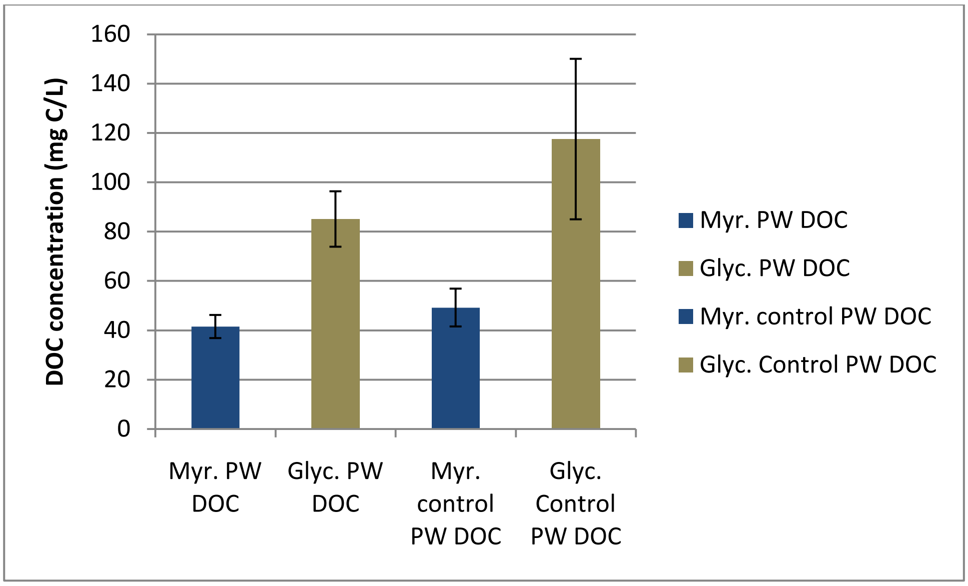

Figure 9. DOC concentration (mg C/L) in sediment pore water, in *Myriophyllum* and *Glyceria* experiments (plant and control treatments)

Hydrophobic fractions

Calculated hydrophobic fractions from UV spectroscopy at 260 nm showed that the concentration of hydrophobic DOC of the different samples was stable from the beginning (day 11). No significant increase in hydrophobic fractions over time was observed (Table 1). Comparing plant treatments with controls for the different species experiments, significant differences were found at all sampling dates in *Myriophyllum* ($F = 67.755$, $p <0.05$; $F = 27.596$, $p <0.05$; $F = 22.097$, $p <0.05$ and $F = 9.560$, $P <0.05$) and until day 24 in *Lemna* experiment ($p <0.05$ -non parametric test-; $F = 38.610$, $p <0.05$ and $F = 51.183$, $p <0.05$). At day 29, a value of $p = 0.05$ ($F = 5.330$) was obtained in the *Lemna* experiment when homogeneity of variances was assumed in this case at $p =0.048$. However, applying the strict statistic criteria, a non-parametric test gave a p-value =0.151. All significant values indicated a larger hydrophobic fraction in plant treatments than in controls (See ANOVA table 1 and 3 in ANNEX 1). The *Glyceria* experiment did not show significant differences between plant and control treatment at any sampling date (See ANOVA table 2 in ANNEX 1). Results from the algae experiment are below the detection limit; thus, a statististical analysis was not performed. Comparing different plant species treatments at day 29 *Myriophyllum* and *Lemna* treatments had similar hydrophobic DOC concentration. Nevertheless, they both had significantly larger hydrophobic fraction than the *Glyceria* plant treatment. All significances were based on a p-value <0.05 in the Bonferroni test (See ANOVA table 5,6,7,8 in ANNEX 1).

Table 1. Hydrophobic fractions (%) in plant treatments and controls at different sampling dates, per species. Dark coloured cells refer to the hydrophobic fractions in the samples selected for the Equilibrium Dialysis Method. Light coloured cells are the controls of these selected samples, to make visual analysis of possible plant or algae contribution the hydrophobic fraction. Hydrophobic fraction includes humic acids, fulvic acids and neutral fractions.

	Sampling date (days)	Hyd. fraction mean (%)	Control hyd. fraction mean (%)
Myriophyllum	11	41±5	24±2
	18	33±3	24±1
	24	36±4	25±2
	29	46±15	28±5
Glyceria			
	11	36±8	39±16
	18	33±3	36±4
	24	30±3	33±5
	29	31±2	34±8
Lemna			
	11	44±3	29±6
	18	52±3	30±4
	24	52±2	39±3
	29	56±3	67±9
Algae			
	3	< LOD	< LOD
	7	< LOD	< LOD
	10	< LOD	< LOD

In line with the results from UV- absorption at 260 nm, DOC fractions analysed at the ECN showed a slightly larger hydrophobic fraction in *Myriophyllum* and *Lemna* plant treatments than in *Glyceria*. However, in general not much difference is observed among plant treatments. The lowest value was found in the algae treatment. Hydrophilic fraction was higher in *Glyceria* and algae samples, which had the higher DOC concentration values (Table 2).

Table 2. DOC hydrophobic (HA, FA and HON) and hydrophilic (Hy) fractions (mgC/L). HA = Humic Acids fraction, FA = Fulvic Acids fraction, HON = Neutral fraction. DOC is the sum of all fractions. Absolute hydrophobic fraction is the sum of HA, FA and HON. Relative hydrophobic fraction is the percentage that these three fractions represent with respect to the total DOC concentration (Data available on the excel file `parameters and results´)

Sample	DOC (mg C/L)	Concentration of hydrophilic DOC (mg C/L)	Concentration of hydrophobic DOC (mg C/L)	Relative Hydrophobic fraction (%)	Relative Hydrophilic fraction (%)
Myriophyllum	13,12	6,32	6,80	52	47,99
Glyceria	38,40	24,30	14,10	37	63,16
Lemna	12,71	6,62	6,10	48	50,73
Algae	31,74	27,92	3,83	12	97,93

Hydrophobic fractions showed no consistent correlation with DOC concentration or total mass, nor with Chl-a. R^2 values were below 0.5 when all sampling points from all days were plotted per species. The same pattern was observed when plotting the hydrophobic fraction against DOC or Chl-a per sampling date. However, some exceptions were observed in the *Lemna* experiment where significant correlation was found on days 11, 18 and 24 with respect to the Chl-a (See ANNEX 2). Also, on day 24 a $R^2 > 0.5$ was found when correlation was applied with respect to the DOC concentration or total mass. A positive correlation was observed in these exceptions. *Glyceria* showed only one value over 0.5 in the correlation of hydrophobic fraction with DOC on day 11 ($R^2 = 0.85$). In this case, the correlation was negative.

Pesticide interaction with DOC

When the pesticide was applied to water samples coming from the different treatments, calculated Koc values were similar (Table 3). Differences among values were not significant (See ANOVA table 10 in ANNEX 1). Extractions were made after 72h, when the equilibrium was not reached completely. As explained before, corrections were applied to the concentrations outside and inside the membrane to estimate Koc values when equilibrium takes place. Observed Koc values are the corrected ones.

Table 3. Koc values for Λ-cyhalothrin when applied to water samples coming from the different plant species and from algae.

	Log Koc Myriophyllum (L/kg)	Log Koc Glyceria (L/kg)	Log Koc Lemna (L/kg)	Log Koc Algae (L/kg)
	4,54	4,63	4,66	4,13
	4,94	4,54	4,32	4,82
	5,02	4,94	4,92	
mean	4.8±0.2	4.7±0.2	4.6±0.2	4.5±0.3

Conclusions and Discussion

Net DOC release by aquatic macrophytes, algae and sediments

Myriophyllum and *Lemna* plant treatments showed higher DOC concentration than their controls (Figure 2 a and c) at most of the sampling dates. However, we observed that plant contribution to the total DOC concentration was a small part from the net DOC released by sediments. *Myriophyllum* and *Lemna* plant experiments seemed to have reached maximum values at day 24. Afterwards, different processes could be affecting DOC release and variation increases. We did not observe differences in DOC concentration between plant treatment and control in the *Glyceria* experiment at any sampling date (Figure 2 b). This indicates that *Glyceria* plants were not releasing DOC at all. Also in Figure 2 d low DOC values can be observed, generated in treatments without sediments but with algae. In general, values observed in Figure 2 could indicate a high net DOC release coming from sediments. Differences among plants and the low contribution of algae will be discussed at a later point.

In respect to our first research question, we could state that despite plants can contribute to the DOC pool, sediments seem to be a relevant compartment in terms of net DOC release.

This first conclusion is supported by Thomas (1997), stating that sediments could be the major source of *in-situ* DOM in the water column and sediment pore water. Also, Thomas & Eaton (1996a) found that DOC concentration in sediment pore water was strongly related to the concentration in the water column. They observed that DOC concentration in the water column always increased as a result of the concentration increase in the sediment pore water. When DOC in pore water reached its maximum, also the concentration was the highest in the water column throughout the whole measurement period. We also observed a relation between DOC in pore water and the concentration in the water column in our experiment. Final DOC concentration (t = 29) in pore water in the controls from *Myriophyllum* and *Glyceria* experiments (Figure 9) showed values of 49.2±7.7 mg C/L and 117.5±32.5 mg C/L. The concentrations of DOC in the water column of these controls at day 29 were 12.3±2.1 mg C/L and 24.5±6.5 mg C/L respectively. Therefore, these results are in line with the findings of the previous studies.

Thomas & Eaton (1996a) also found amino acids composition in the sediment and water phase to be very similar, indicating a common origin. We should clarify that DOC mainly consist of free amino acids (FAA), peptides, proteins, sugars, carboxylic acids, nucleic acids and humic substances (HS) (Thomas, 1997). Many articles often analyze DOC based on FAA and HS, as the first ones are important sources of energy and chemical information (Manahan *et al*., 1983; Manahan, 1990) and HS represent most of the DOC in terms of mass (Thurman, 1985) . Recently, HS has been proved to be also important in chemical and biological reactions (Jones, 1992). HS in general is related to high molecular weight compounds (Aiken *et al*. 1985, cited in Jones 1992) and FAA with low molecular weight compounds (Thomas, 1997). This DOC characterization will be useful for further discussion.

We analyzed which biotic compartment had the largest net DOC release comparing DOC concentrations and total mass in the plant and algae treatments. We observed that DOC concentration in *Glyceria* plant treatment was in most of the sampling dates higher than in *Myriophyllum,* in *Lemna* plant treatment and in the algae treatment (Figure 2 a-d and Figure 5). Nevertheless, we should take into account that in the *Glyceria* treatment, most of the DOC came from the sediments. *Myriophyllum* plant treatment showed a higher net DOC release than *Lemna* plant treatment until day 24, when the maximum values seem to be reached. At day 29, the concentrations in these two treatments were similar. This may be explained by processes that will be discussed a bit later. DOC net release in all plant treatments was clearly higher than in the algae treatment. However, we should consider that the water volume was not the same for all experiments. If we focus our analysis on DOC as total mass (Figure 7), the picture changes slightly. In this more realistic case, net DOC release in *Glyceria* plant treatment was smaller than in *Myriophyllum* and *Lemna* plant treatment until day 24, similar in day 29 and always higher than algae treatment. *Myriophyllum* was releasing more DOC than *Lemna* at day 24, but again they became similar at day 29. In conclusion, we may state that *Myriophyllum* was the plant species with higher net DOC release, followed by *Lemna* and algae. Additionally, it seems that *Glyceria* was not releasing DOC. DOC total mass values at day 24 were equal to 18.2±1.3 mg, 13.3±0.7 mg, 12.1±0.7 mg and 1.3±0.3 mg, respectively coming from *Myriophyllum, Lemna, Glyceria* and algae treatments. Despite it is not what we predicted, these results are within the range of total mass values obtained in a mesocosm experiment developed in 2010 by the ERA (Environmental Risk Assessment Group). They found DOC total mass values of 10.09 mg C, 8.98 mg C and 8.38 mg C for *Myriophyllum, Lemna* and *Glyceria* respectively.

Algae were expected to be the major biotic contributors to the DOC pool, as Thomas stated in 1997. However, several studies also proved that excreted extracellular products from algae are usually rapidly consumed by bacteria (Cole *et al*. 1982, cited in Baines & Pace, 1991; Sondergard & Jensen 1986, cited in Thomas, 1997), which could justify our findings. Oppositely, Cole *et al*. (1984) found that the immediate net input of algae in a lake was 8 gC m^{-2} $year^{-1}$. This value can be recalculated as 21.91 mg C m^{-2} day^{-1}. Baker & Farr (1987) estimated a DOC production rate for *Lemna* that would be equal to 130 mg C $m^{-2}day^{-1}$. Hence, this value would be much larger than the recalculated value for algae. Based on this data, it might be accepted that our results are in line with existing literature data.

Furthermore, floating plants (*Lemna minnuscula* and *Lemna gibba*) were thought to release more DOC than the rest of aquatic macrophytes as they are smaller in size and have faster growth (Hillman, 1961). However, *Lemna* population in our experiment did not show an optimal growth, which could be the cause of this lower net DOC release than expected. Therefore, we cannot prove that the growth strategy of *Lemna* could make this plant species release more DOC than the others.

On the other hand, existing data shows a higher Net Primary Production (NNP) by emergent plants (41.6 g DW/m2*day) when compared with submerged and floating plants (1.9 and 1.4 g DW/m2*day respectively) (Robertson *et al*., 1999). We thought that *Glyceria* may be the plant species releasing more DOC as there is relation between NNP and DOC (Godmaire & Nalewaijko, 1989). Also, Mann & Wetzel (1996) found that the emergent plant species *Juncus effusus* and *Thypa latifolia* were releasing DOC within a range of 1.9 to 10.6 mg C L^{-1} cm^{-2} (*J. effusus*) and 1.9 to 5.2 mg C L^{-1} cm^{-2} (*T. latifolia*) in a 24h period. They considered a large surface area as DOC leaching from submerged leaves and culms was taken into account. Based on that, we can expect final values larger than 4.48 mg C/L ($\log_e$DOC=1.5), which is the value found for *Lemna* DOC release after 14 days in Baker & Farr (1987). However, our results were not in line with these findings. We should consider that only one *Glyceria* shoot was planted per pot and the contact surface was much lower than for the rest of the plant species as just one part of the shoot was submerged. This could explain that in our experiment, DOC released by *Glyceria* was negligible.

Existing information about *Myriophyllum* DOC release shows that non-axenic plants can have a photosynthetic rate of 11.7±0.3 mg C/g dry*h, excreting as organic carbon 1.3-3.8% of this photosynthetic production (Godmaire & Nalewaijko, 1988). Thus, after doing some calculations and considering the lower percentage, we got a value of 87.6 mg C/g dry after 24 days. This value is not comparable with data found in literature for the rest of the species, however it indicates that our results with respect to mg DOC/g *Myriophyllum* after 24 days (28±2 mg C/g dry. Figure 4) was relatively low compared to existing data. A possible explanation could be that *Myriophyllum* needed a higher water column in order to show a steady growth and have a larger net release of DOC.

Correlation between plant biomass and DOC showed that *Lemna* and algae were the organisms with stronger association among these two variables. This is in line with existing literature stating that algae may be the most important contributor to the DOC pool (Thomas, 1997) despite they are strongly associated with the bacterial community (Flynn *et al*., 2008). Correlation between *Glyceria* biomass and DOC was high, but no plant contribution was observed and therefore it would not be logical to accept this association. *Myriophyllum* biomass was not highly related with DOC. This could be related with the consuming function of epiphytic algae (Thomas & Eaton, 1996b). In Figure 4 we can see that net DOC release per gram of *Lemna* is higher than that of *Myriophyllum*. This result could support the initial hypothesis about the higher DOC release by floating plants compared with other macrophytes.

Variables affecting DOC concentration

With respect to the biotic variables affecting DOC concentration, the presence of plant species could be considered as a biotic variable (See first paragraph in the discussion on `Net DOC release'). However, other biotic and abiotic variables could be important as well. Another biotic variable that we considered was the presence of algae. In our experiment, algae showed the lowest contribution to the DOC pool in comparison with *Myriophyllum*, *Glyceria* and *Lemna* (See also first paragraph in the discussion on `Net DOC release'). Additionally, when correlation among Chl-a and DOC in plant experiments was analyzed, low R^2 values indicated that there was no direct relation between DOC and Chl-a, except for *Lemna*. Consequently, we cannot confirm that extra DOC in *Lemna* plant treatment compared with control is exclusively coming from *Lemna*, but from algae also. Algae contribution in the *Myriophyllum* experiment could be positive if we look at Chl-a in treatment and control in day 29 (Figure 3). There was no significant difference in Chl-a among plant treatment and control, as for DOC values. However, this correlation was proved to be low, $R^2 < 0.5$ (except for day 24). We should also take into account that epiphytic algae and bacteria were not quantified in these experiments. These algae were more visible in *Myriophyllum*, but they could be also present in *Lemna* (Tomas & Eaton, 1996b). In our *Myriophyllum* experiment an increase in variation among plant treatments was found after day 24 (Figure 2 a), lowering differences in DOC concentration and total mass between plant treatment and control. Also, in the *Lemna* plant treatment (Figure 2 c), no much increase in DOC values was observed after day 24. A reasonable explanation to this can be found in Thomas (1997) and Thomas & Eaton (1996b), stating that DOC coming from macrophytes may be lower than expected when associated with epiphytic bacteria and heterotrophic algae. In the *Glyceria* experiment a clear lack in correlation was proved by a low R^2 value. Moreover, a higher Chl-a concentration was found in the plant treatment at day 29, while there was no difference in net DOC release with the control. Therefore, apart from the *Lemna* experiment, in general variations in net DOC release due to floating algae could be neglected. However, there may be an effect on DOC levels coming from epiphytic algae.

Emergent plants can change redox conditions in sediments (Mann & Wetzel, 2000). Therefore they could be related with abiotic variables as well. However, similar values were found in *Glyceria* plant and control treatments when DOC concentrations in pore water were analyzed (Figure 9). Thus, we did not find a contribution of emergent plants (*Glyceria*) to DOC concentration in sediments pore water. Therefore, our data does not fit with what was stated by Mann & Wetzel (2000). We cannot prove the positive effect of emergent plants on DOC concentration in pore water and consequently, on DOC release from sediments. However, a critical mass could be needed to observe some effect of plants in interstitial water.

From the DOC values observed in Figure 7 and 8, an abiotic effect on DOC release could also be observed. At day 29, treatments with higher temperature and light intensity, but no plants contribution (*Glyceria* plant treatment), were reaching similar total mass values than treatments with lower temperature and light. This could be also due to the epiphytic DOC consumption in *Myriophyllum* and *Lemna* plant treatments (as explained in previously). However, in our opinion it seemed that light and temperature could have some effect on DOC final concentrations as well. Furthermore, among controls with only sediments, we observed that DOC in the *Myriophyllum* control tended to be larger than in the *Glyceria* control until day 24. They both always had higher total mass values than in the dark *Lemna* control. First, this could indicate a positive effect on DOC concentration coming from light and temperature. We may expect higher values in the *Glyceria* control, where light intensity was higher and temperature (in water and sediments) is expected to be higher as well. However, the higher values in the *Myriophyllum* control until day 24 and the non-observed difference at day 29 among *Myriophyllum* and *Glyceria* controls could be indicating a possible negative effect of light or temperature. In relation to this, controls with only sediments showed that pore water DOC concentrations were higher in the *Glyceria* experiment than in the *Myriophyllum* experiment (Figure 9). An abiotic positive effect coming from temperature could be the reason of this difference. The control in the *Glyceria* experiment was covered by a lower water layer and higher light intensity was applied. Therefore, a higher temperature in the sediments could be expected. Hence, this could introduce the idea that higher temperatures increase DOC concentration in sediments pore water. This could fit with the idea that higher temperature could be related with higher concentration of FAA and HS in pore water and in the water column (Thomas & Eaton, 1996a). Additionally, it is remarkable that DOC concentrations in the *Glyceria* experiment were increasing until the end (Figure 2 b). This was not observed in the totally dark *Lemna* control, nor in *Myriophyllum* and *Lemna* plant treatments (See first paragraph in the Results section). Hence, this may indicate that there is a steady increase in net DOC release from sediments related with higher temperature.

Several studies found that DOC concentration at higher temperatures are consistent with observed summer DOC maxima (Grieve 1990; Vance & David 1991; cited in Christ & David (1996)). Also, increase in temperature could be related with more bacterial metabolism in sediments, leading to faster decomposition of large compounds into low molecular weight free amino acids (FAA) (Thomas & Eaton, 1996a). In addition to that, it has been proven by several authors that HS could be formed by polymerization of low molecular compounds (Haslam 1989, cited in Serrano 1992; De Haan, 1992). Since HS are the most important DOC compound in terms of mass (Thurman, 1985), we could say that the increase in temperature may be causing an increase in DOC concentration. Conversely, Allard *et al*. (1994) found that UV radiation was degrading effectively HS (high molecular weight compounds) in water, leading to low molecular weight photoproducts. Moreover, Strome & Miller (1978, cited in Reche *et al*. 1998), discovered that UV-photoproducts were more easily degraded by bacteria. Hence, we could state that photodegradation under high light intensities and consequent consumption by bacteria could be decreasing gross DOC levels. However, existing studies identified UV light as the main photodegrading factor, with no effect from wavelengths larger than 365 nm (Kieber *et al*., 1990). Therefore, as the lamps used in our research were emitting light on the PAR range (Photosynthetically Active Radiation), we cannot accept the direct effect of UV-light on DOC in this case. More research is needed to clarify whether it is UV, photosyntetically active light or other factor what could make DOC concentrations decrease.

DOC hydrophobic fractions

Analyses of DOC hydrophobic fractions calculated from spectrophotometric absorption values (Table 1) showed that a stable value was reached after 11 days approximately. *Myriophyllum* and *Lemna* experiments showed larger hydrophobic fractions in plant treatments than in controls until day 24. Afterwards, no differences were observed among plant treatment and control for the *Lemna* experiment. These results could indicate that plants could produce extra hydrophobic compounds compared to sediments. With respect to the Lemna experiment, we could explain this lack in difference among treatments at day 29 by the large increase in hydrophobicity in the control treatment. It has been proven that a nitrate concentration above 25 mg/L can disturb the absorbance signal analysis significantly (Dilling and Kaiser, 2002). The final nitrate concentrations measured in the *Lemna* plant treatment were above 400 mg/L. This higher hydrophobicity could be coming from nutrient addition and no consumption by plants in the control treatment. Corrections were applied when we made the calibration curves for hydrophobic fractions. However, the continuous accumulation of nutrients could lead to higher disturbances than expected.

Furthermore, the results in Table 1 showed not much variation in hydrophobic fractions among plant experiments. Therefore, we could expect that also here the sediments were important contributors to the concentration of hydrophobic DOC. Allard *et al.* (1994) identified low molecular weight compounds (FAA) as hydrophilic acids, based on reduction in absorbance at 254 nm. High molecular weight compounds (HS) showed higher absorption at this wavelength. Therefore, they can be considered hydrophobic. In relation to this, it has been stated that FAA concentrations in the water column could be coming mainly from the sediments (Jorgensen *et al*. 1980, cited in Thomas 1997). Additionally, humic substances (HS) in the water column can be formed by polymerization of low molecular weight compounds (FAA) (Haslam 1989, cited in Serrano, 1992). Thus, this could yield to a high concentration of hydrophobic DOC coming from sediments. On the other hand, several authors also stated that macrophytes are implicated in the release of FAA and HS (Jorgensen *et al*. 1981; Poule & Martin-Jezenkel 1983; cited in Thomas & Eaton (1996a)). Algae experiment showed values below the detection limit, hence, the analysis of these results is not reliable.

Fractionation analysis developed at the ECN (Table 2) gave us values that fitted with previous conclusions. The largest DOC hydrophobic fractions were represented by *Myriophyllum* and *Lemna* plant treatments, differing not much and followed by the *Glyceria* plant treatment. Here a larger hydrophilic fraction was found. This can be explained by the positive effect of temperature on FAA (hydrophilic fraction) concentrations in the sediment pore water, with diffusion into the water column (Thomas and Eaton, 1996a). Algae treatment showed a significantly higher hydrophilic fraction that represents almost the total DOC concentration measured in this treatment with this analysis. Hence, this can be again related with the disturbance coming from EDTA (See Materials and Methods section).

In conclusion, our research showed that plants could increase aqueous concentration of hydrophobic DOC, despite sediments also having a strong contribution.

Pesticide interaction with DOC

Based on our previous findings, with not much difference in DOC hydrophobic fractions between treatments, Log Koc in water samples was also expected to be similar. That is related with existing literature stating that organic compounds as PAHs were correlated with the proportion of hydrophobic acids in DOC (Kukkonen *et al*., 1990). Calculated Log Koc values from λ-cyhalothrin did not differ among samples with DOC from different plants and algae (Table 3). Thus, we it could be said that results fitted with our expectations. Moreover, it has been also found that different organic compounds were associated with different hydrophobic fractions (Akkanen *et al*., 2004; Kukkonen *et al*., 1990). In our case, we did not differentiate among Fulvic (FA), Humic (HA) and Neutral acids (HON). However, ECN results (Table 2) showed that there was not much difference for any hydrophobic fraction among the samples. Therefore, this could also explain the lack in difference among Koc values for different samples. Since it seems that sediments were important contributors to the DOC hydrophobic fraction, this compartment could be influencing strongly the interaction of DOC with pesticides.

Existing data estimates Koc value from λ-cyhalothrin in $1.8*10^5$ L/kg (USDA ARS Pesticide Properties Database, 2005), from which we could calculate a log Koc value equal to 5.26 L/kg. Log Koc in our experiment were ranging between 4.5-4.8 L/kg. The deviation from the estimated value can be explained by experimental errors, pesticide loses (sorption to membranes and glass walls, volatilization due to not perfectly closed flasks) or sampling after 72h when the equilibrium was not reached. Corrections were made to estimate the possible values at equilibrium. However, our calculations cannot represent all processes that can take place until the pesticide equilibrates. Also, the amount of pesticide that we recovered from the applied concentration, after 72h was around 13±5 %. This proves that pesticide lost occurred. Furthermore, we think that our results still show certain affinity between the pesticide and DOC. This assumption is based on the ratios Ca/Cb > 1.2 that we got in most of the samples. This relation is considered to be logical looking at Eq.1 (page 8), where the numerator can be transformed into (Ca/Cb)-1. We assumed that if the amount of pesticide binding to DOC is more than 20 % higher than what is bound to pure water, we could say that there is affinity between the pesticide and DOC.

General conclusions

Regarding our experimental conditions, sediments are one of the most important compartments in the net release of DOC. *Myriophyllum* was the plant species that contributed more to the DOC pool, followed by *Lemna* in terms of DOC concentration and total mass. However, *Lemna* was the most productive organism related to its biomass. Algae net DOC release was very low and it could be directly influenced by bacterial consumption. *Glyceria* biomass could be too low to show any effect.

Our study showed that the distribution between hydrophobic and hydrophilic fractions was constant over time. In *Myriophyllum* and *Lemna* plant treatments we observed that the plants can contribute to the pool of hydrophobic compounds. However, sediments showed the highest contribution to the concentration of hydrophobic DOC. The *Glyceria* plant treatment, with only sediment contribution to DOC, showed slightly larger hydrophilic fraction than the rest of the plant treatments.

Pesticide sorption experiments for the different plant treatments did not generate different log Koc values. Therefore, the similar Koc values that we found are close to our expectations, as the concentrations of hydrophobic DOC were also similar among plant treatments. Our results showed affinity between the pesticide and DOC, despite the calculated log Koc values were slightly lower than the estimated value. We conclude that in our experiments, sediments seem to be highly important in respect to pesticide interaction with DOC.

Recommendations

Shorter period of time would be advisable to measure DOC release from plants without disturbances that could be coming from epiphytic algae. Larger *Glyceria* biomass could be used to observe effect of this specie. With respect to *Glyceria*, only the submerged part should be accounted as contributing biomass.

More research should be done monitoring sediment temperature and using the same water volume for different treatments and experiments.

It would be advisable to reproduce a pore water analysis over a longer period of time, monitoring at shorter time intervals DOC in sediments and water, to analyze the possibility of DOC diffusion from sediments. In this way, also FAA and HS could be analyzed, as well as their accumulation or photodegradation.

With respect to the pesticide experiment, we would advise to use more replicates to reduce variability. Also, it is recommendable to think about more measures that prevent volatilization or sorption to different surfaces. Additionally, the initial experiment to calculate the equilibration time should reproduce as much as possible the final experiment (e.g. using the same number of membranes).

Acknowledgements

My grateful thanks to my supervisors Paul van den Brink and Gertie Arts for the opportunity of working with them and all the support received. Also, I would like to give especial thanks to John Deneer, Bert Jan Groenenberg for all the advices and help received during this months and to the technicians Frits Gillissen, Laura Buijse for the help and information received in the lab. Many thanks also to all the people in the Environmental Risk Assessment Group and the Aquatic Ecology department.

Supporting Information Available

All data from this research is available as a digital version including excel files and results from the statistical analysis with SPSS.

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ANNEX 1. ANOVA tables.

Table 1. One-way ANOVA to test significant differences in DOC concentration, DOC total mass and hydrophobic fraction. Chl-a is tested for values on last day sampling, when ethanol extraction was applied for all experiment. Comparison is done among *Myriophyllum* plant treatment and control.

Plant treatment vs control			
		Myriophyllum	
	F	Sig.	relation*
Day 11			
DOC concentration (mg C/L)	1,083	0,328	
DOC total mass (mg C)	1,083	0,328	
Hydrophobic fraction (%)	67,755	**0,000**	+
Day 18			
DOC concentration (mg C/L)	29,165	**0,001**	+
DOC total mass (mg C)	29,165	**0,001**	+
Hydrophobic fraction (%)	27,596	**0,001**	+
Day 24			
DOC concentration (mg C/L)	9,675	**0,014**	+
DOC total mass (mg C)	9,675	**0,014**	+
Hydrophobic fraction (%)	22,097	**0,002**	+
Day 29			
DOC concentration (mg C/L)	0,819	0,392	
DOC total mass (mg C)	0,819	0,392	
Hydrophobic fraction (%)	9,560	**0,015**	+
Chl-a (µg/L)	1,820	0,214	

* += p. treatment >control; -= p. treatment<control

Table 2. One-way ANOVA to test significant differences in DOC concentration, DOC total mass and hydrophobic fraction. Chl-a is tested for values on last day sampling, when ethanol extraction was applied for all experiment. Comparison is done among *Glyceria* plant treatment and control.

Plant treatment vs control			
	Glyceria		
	F	Sig.	relation*
Day 11			
DOC concentration (mg C/L)	0,128	0,73	
DOC total mass (mg C)	0,128	0,73	
Hydrophobic fraction (%)	0,026	0,875	
Day 18			
DOC concentration (mg C/L)	1,195	0,316	
DOC total mass (mg C)	1,195	0,316	
Hydrophobic fraction (%)	1,133	0,318	
Day 24			
DOC concentration (mg C/L)	1,643	0,236	
DOC total mass (mg C)	1,643	0,236	
Hydrophobic fraction (%)	0,954	0,357	
Day 29			
DOC concentration (mg C/L)	0,086	0,778	
DOC total mass (mg C)	0,086	0,778	
Hydrophobic fraction (%)	0,358	0,569	
Chl-a (µg/L)	9,662	**0,017**	+

* += p. treatment >control; - = p. treatment<control

Table 3. One-way ANOVA to test significant differences in DOC concentration, DOC total mass and hydrophobic fraction. Chl-a is tested for values on last day sampling, when ethanol extraction was applied for all experiment. Comparison is done among *Lemna* plant treatment and control.

Plant treatment vs control			
		Lemna	
	F	Sig.	relation*
Day 11			
DOC concentration (mg C/L)	7,027	**0,029**	+
DOC total mass (mg C)	7,027	**0,029**	+
Hydrophobic fraction (%)		**0.008/0.008**	+
Day 18			
DOC concentration (mg C/L)	10,019	**0,013**	+
DOC total mass (mg C)	10,019	**0,013**	+
Hydrophobic fraction (%)	38,610	**0,000**	+
Day 24			
DOC concentration (mg C/L)	124,939	**0,000**	+
DOC total mass (mg C)	124,939	**0,000**	+
Hydrophobic fraction (%)	51,183	**0,000**	+
Day 29			
DOC concentration (mg C/L)	197,859	**0,000**	+
DOC total mass (mg C)	197,859	**0,000**	+
Hydrophobic fraction (%)	5,350	(1)0.05	
Chl-a (µg/L)		**0.008/0.008**	+

* += p. treatment >control; - = p. treatment<control

(1) Non-parametic test = 0.151 -non transformed-/ 0.151 -log 10 transformed-

non-parametric

non transf./log10 transf.

Table 4. One-way ANOVA to test significant differences in DOC concentration, DOC total mass and hydrophobic fraction. Chl-a is tested for values on last day sampling, when ethanol extraction was applied for all experiment. Comparison is done among algae treatment and control.

Algae treatment vs control			
		Algae	
	F	Sig.	relation*
Day 3			
DOC concentration (mg C/L)	1,239	0,298	
DOC total mass (mg C)	1,239	0,298	
Chl-a (µg/L)	6.789,986	**0,000**	+
Day 7			
DOC concentration (mg C/L)	0,000	0,990	
DOC total mass (mg C)	0,000	0,990	
Chl-a (µg/L)	12.649,310	**0,000**	+
Day 10			
DOC concentration (mg C/L)	8,988	**(1)0,017**	+
DOC total mass (mg C)	8,988	**0,017**	+
Chl-a (µg/L)	12.613,841	**0,000**	+

* += a. treatment >control; - = a. treatment<control

(1) Homog. DOC 0.286, log DOC 0.011

Table 5. One-way ANOVA to test significant differences among algae and plant experiments, treatments and controls separately. DOC concentration, DOC total mass and hydrophobic fraction are compared at t=29 for plants and t=10 for algae, when all organisms are assumed to be at maximum production rate.

Algae and plant experiments comparison				
Plant treatments	F (ANOVA)	Sign. (ANOVA)	Sign.(Bonferroni)	Relation*
Algae (A) - Myriophyllum (M)				
DOC conc.(mg C/L)	58,942	0,000	**0,000**	-
DOC total mass(mg C)	197,946	0,000	**0,000**	-
mg C/g plant or µg Chl-a	3.045,687	0,000	**0,000**	-
Algae (A) - Glyceria (G)				
DOC conc.(mg C/L)	58,942	0,000	**0,000**	-
DOC total mass(mg C)	197,946	0,000	**0,000**	-
Algae (A) - Lemna (L)				
DOC conc.(mg C/L)	58,942	0,000	**0,000**	-
DOC total mass(mg C)	197,946	0,000	**0,000**	-
mg C/g plant or µg Chl-a	3.045,687	0,000	**0,000**	-
Control treatments				
Algae (A) - Myriophyllum (M)				
DOC conc.(mg C/L)	48,179	0,000	**0,000**	-
DOC total mass(mg C)	77,778	0,000	**0,000**	-
Algae (A) - Glyceria (G)				
DOC conc.(mg C/L)	48,179	**0,000**	**0,000**	-
DOC total mass(mg C)	77,778	**0,000**	**0,000**	-
Algae (A) - Lemna (L)				
DOC conc.(mg C/L)	48,179	**0,000**	**0,003**	-
DOC total mass(mg C)	77,778	**0,000**	**0,000**	-

* + = A>M, G or L; - = A<M, G or L

**Assuming homogeneity to get more specific info when comparing groups. (Non-parametric gives a value of 0.001 for all, but that is due to the influence of the strongly different algae)

Table 6. One-way ANOVA to test significant differences in DOC concentration, DOC total mass and hydrophobic fraction, among *Myriophyllum* and *Glyceria* experiments. Treatments and controls are compared separately.

3 plant experiment comparison					
		Myriophyllum (M) - Glyceria (G)			
Plant treatments		F (ANOVA)	Sign. (ANOVA)	Sign.(Bonferroni)	Relation*
Day 11					
	DOC conc.(mg C/L)	5,065	0,025	**0,048**	-
	DOC total mass(mg C)	18,456	18,456	**0,001**	+
	Hydrophobic fraction(%)			0,330	
Day 18					
	DOC conc.(mg C/L)	51,797	0,000	**0,022**	-
	DOC total mass(mg C)	52,391	0,000	**0,000**	+
	Hydrophobic fraction(%)	46,152	0,000	1,000	
Day 24					
	DOC conc.(mg C/L)	124,142	0,000	**0,000**	-
	DOC total mass(mg C)	51,207	0,000	**0,000**	+
	Hydrophobic fraction(%)	40,506	0,000	**0,037**	+
Day 29					
	DOC conc.(mg C/L)	18,051	0,000	**0,003**	-
	DOC total mass(mg C)	2,996	0,088	0,099	
	Hydrophobic fraction(%)	12,803	0,001	**0,028**	+
Control treatments					
Day 11					
	DOC conc.(mg C/L)	12,752	0,001	0,342	
	DOC total mass(mg C)	21,768	0,000	**0,000**	+
	Hydrophobic fraction(%)	3,067	0,084	0,090	
Day 18					
	DOC conc.(mg C/L)	63,507	0,000	**0,000**	-
	DOC total mass(mg C)	9,991	0,003	0,055	
	Hydrophobic fraction(%)	9,105	0,004	**0,003**	-
Day 24					
	DOC conc.(mg C/L)	175,791	0,000	**0,000**	-
	DOC total mass(mg C)	46,444	0,000	**0,000**	+
	Hydrophobic fraction(%)	17,051	0,000	**0,016**	-
Day 29					
	DOC conc.(mg C/L)	74,486	0,000	**0,001**	-
	DOC total mass(mg C)	31,787	0,000	1,000	
-	**Hydrophobic fraction(%)**	28,063	0,000	0,474	

* + = M>G; - = M<G

non-parametric

Table 7. One-way ANOVA to test significant differences in DOC concentration, DOC total mass and hydrophobic fraction, among *Myriophyllum* and *Lemna* experiments. Treatments and controls are compared separately.

3 plant experiment comparison		Myriophyllum (M) - Lemna (L)			
Plant treatments		F (ANOVA)	Sign. (ANOVA)	Sign.(Bonferroni)	Relation**
Day 11					
	DOC conc.(mg C/L)	5,065	0,025	1,000	
	DOC total mass(mg C)	18,456	18,456	1,000	
	mg C/g plant	141.494	0,000	**0,000**	-
	Hydrophobic fraction(%)			0,330	
Day 18					
	DOC conc.(mg C/L)	51,797	0,000	**0,000**	+
	DOC total mass(mg C)	52,391	0,000	**0,000**	+
	mg C/g plant	242,337	0,000	**0,000**	-
	Hydrophobic fraction(%)	46,152	0,000	**0,000**	-
Day 24					
	DOC conc.(mg C/L)	124,142	0,000	**0,000**	+
	DOC total mass(mg C)	51,207	0,000	**0,000**	+
	mg C/g plant	385,400	0,000	**0,000**	-
	Hydrophobic fraction(%)	40,506	0,000	**0,000**	-
Day 29					
	DOC conc.(mg C/L)	18,051	0,000	0,429	
	DOC total mass(mg C)	2,996	0,088	0,429	
	mg C/g plant			**0.003/0.003**	-
	Hydrophobic fraction(%)	12,803	0,001	0,220	
Control treatments					
Day 11					
	DOC conc.(mg C/L)	12,752	0,001	**0,020**	+
	DOC total mass(mg C)	21,768	0,000	**0,020**	+
	Hydrophobic fraction(%)	3,067	0,084	1,000	
Day 18					
	DOC conc.(mg C/L)	63,507	0,000	**0,002**	+
	DOC total mass(mg C)	9,991	0,003	**0,002**	+
	Hydrophobic fraction(%)	9,105	0,004	0,137	
Day 24					
	DOC conc.(mg C/L)	175,791	0,000	**0,000**	+
	DOC total mass(mg C)	46,444	0,000	**0,000**	+
	Hydrophobic fraction(%)	17,051	0,000	**0,000**	-
Day 29					
	DOC conc.(mg C/L)	74,486	0,000	**0,000**	+
	DOC total mass(mg C)	31,787	0,000	**0,000**	+
	Hydrophobic fraction(%)	28,063	0,000	**0,000**	-

** + = M>L; - = M<L

non-parametric

non transf./log10 transf.

Table 8. One-way ANOVA to test significant differences in DOC concentration, DOC total mass and hydrophobic fraction, among *Glyceria* and *Lemna* experiments. Treatments and controls are compared separately.

3 plant experiment comparison						
		Glyceria (G) - Lemna (L)				
Plant treatments		F (ANOVA)	Sign. (ANOVA)	Sign.(Bonferroni)	Relation***
Day 11					
	DOC conc.(mg C/L)	5,065	0,025	0,057	
	DOC total mass(mg C)	18,456	18,456	**0,001**	-
	Hydrophobic fraction(%)			0,330	
Day 18					
	DOC conc.(mg C/L)	51,797	0,000	**0,000**	+
	DOC total mass(mg C)	52,391	0,000	**0,025**	-
	Hydrophobic fraction(%)	46,152	0,000	**0,000**	-
Day 24					
	DOC conc.(mg C/L)	124,142	0,000	**0,000**	+
	DOC total mass(mg C)	51,207	0,000	0,101	
	Hydrophobic fraction(%)	40,506	0,000	**0,000**	-
Day 29					
	DOC conc.(mg C/L)	18,051	0,000	**0,000**	+
	DOC total mass(mg C)	2,996	0,088	0,996	
	Hydrophobic fraction(%)	12,803	0,001	**0,001**	-
Control treatments					
Day 11					
	DOC conc.(mg C/L)	12,752	0,001	**0,001**	+
	DOC total mass(mg C)	21,768	0,000	**0,018**	-
	Hydrophobic fraction(%)	3,067	0,084	0,505	
Day 18					
	DOC conc.(mg C/L)	63,507	0,000	**0,000**	+
	DOC total mass(mg C)	9,991	0,003	0,343	
	Hydrophobic fraction(%)	9,105	0,004	0,193	
Day 24					
	DOC conc.(mg C/L)	175,791	0,000	**0,000**	+
	DOC total mass(mg C)	46,444	0,000	**0,000**	+
	Hydrophobic fraction(%)	17,051	0,000	0,097	
Day 29					
	DOC conc.(mg C/L)	74,486	0,000	**0,000**	+

DOC total mass(mg C)	31,787	0,000	**0,000**	+
Hydrophobic fraction(%)	28,063	0,000	**0,000**	-

*** + = L>G; - = L<G

Table 9. One-way ANOVA to test significant differences among DOC in sediment pore water in plant treatments and controls, in *Myriophyllum* and *Glyceria* experiments. Differences among plant species are also tested, comparing plant treatments and controls separately.

	PW DOC		
	F	Sign.	Relation*
Myriophyllum (M) - Myr.control (MC)	3,162	0,113	
Glyceria (G) - Glyc. control (GC)	2,350	0,164	
Myriophyllum (M) - Glyceria (G)	65,682	**0,002**	-
Myr. Control (MG) - Glyc. Control (GC)	20,068	**0,000**	-

* + = M>MG or G>GC or M>G or MG>GC; - = M<MG, G<GC, M<G, MG<GC

Table 10. One-way ANOVA to test significant differences among Log Koc calculated for λ-cyhalothrin when applied to water samples from *Myriophyllum, Glyceria*, *Lemna* and algae treatments.

Koc				
	F (ANOVA)	Sign. (ANOVA)	Sign.(Bonferroni)	*relation
Myriophyllum (M) - Glyceria (G)	0,276	0,841	1,000	
Myriophyllum (M) - Lemna (L)	0,276	0,841	1,000	
Glyceria (G) - Lemna (L)	0,276	0,841	1,000	
Algae (A) - Myriophyllum (M)	0,276	0,841	1,000	
Algae (A) - Glyceria (G)	0,276	0,841	1,000	
Algae (A) - Lemna (L)	0,276	0,841	1,000	

* + M>G or M>L or G>L or A>M or A>G or A>L

ANNEX 2. Coefficients of determination (R^2).

	DOC_Chl-a	Hydrop._Chl-a	Hydrop._DOC
Myriophyllum			
All sampling points*	0,49	0,08	0,00
Day 11	0,00	0,36	0,32
Day 18	0,07	0,02	0,33
Day 24	0,67	0,40	0,10
Day 29	0,03	0,22	0,17
Glyceria			
All sampling points*	0,13	0,00	0,10
Day 11	0,06	0,04	0,85
Day 18	0,00	0,00	0,11
Day 24	0,00	0,01	0,13
Day 29	0,05	0,01	0,48
Lemna			
All sampling points*	0,66	0,58	0,03
Day 11	0,44	0,76	0,25
Day 18	0,64	0,80	0,30
Day 24	0,81	0,79	0,79
Day 29	0,86	0,39	0,48
Algae			
All sampling points	0,14	-	-
Day 3	0,05	-	-
Day 7	0,03	-	-
Day 10	0,48	-	-

* Only Chl-a measurements until day 24 were considered. At day 29, different method was used to determine Chl-a concentration.
*DOC refers to DOC concentration as well as DOC total mass. R^2 values were the same for both measurements.