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Leaf litter ecological fate in the Schelde Estuary in Belgium

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Abstract: Two dominant species of Willow (*Salix triandra*) and Reed (*Phragmites australis*) along the Schelde Estuary (in Belgium) were selected in this research. The pigments of higher plant was used as biomarkers, the decomposition process of the two species were studied after they fall into the Schelde Estuary. After statistical analysis (Spearman rank order correlation, $P < 0.05$), the results has shown the decomposition dynamics pattern of the pigments, and the willow showed different pattern in comparing with the reed, e.g. Chlorophyll-a decomposition dynamics for willow is: $y_1 = 12196x^2 - 175895x + 1E+06 + k$, $R^2 = 0.5706$ while for reed is: $y_2 = -37878x^2 + 229782x + 734282 + k$, $R^2 = 0.9065$. The precise time of the leaf litter spent in the water was also calculated as were less than 24 days, 24 – 37 days, longer than 37 days (willow) and less than 24 days, longer than 24 days (reed), the leaf litter fate of the two dominant species in the Schelde Estuary was also compared.

Keywords: leaf litter ecologcal fate; *Salix triandra*; *Phragmites australis*; Schelde Estuary

Introduction

The Schelde River originates in Saint-Quentin (Northern France), passes through Belgium and the Netherlands and then flows into the North Sea in Vlissingen. It is the only true estuary left in the Delta area (Herman, 1999).

The Belgian government approved the so-called SIGMA plan following dike burst along the Zeeschelde, caused by a large scale of storm surge in 1976. In 1995, The OMES project was set up to study the environmental effects of the SIGMA plan. Data were collected for the construction of an integrated ecosystem model. Dominant vegetation of marshes like reeds, willows grow along the bank of the Schelde, detritus derived from leaf litter of these vegetation could be important source of organic and inorganic matters input to the Schelde (Tam, 1998). This detritus material, both as particulate organic matter and dissolved organic matter (POM and DOM) could play an important role in providing nutrients and energy to the aquatic food webs in the Schelde (Whiles, 1997; 1999; Latter, 1998; Twilley, 1997; Kuehn, 1998). As a result of the extension of intertidal area, the impact of these vegetation on the shore ecosystem had increased. Therefore the modeling of macrophytic vegetation succession and litter production and decomposition are also included in OMES.

One of the question posted in relation to the fate of the leaf litter entering into the water of the Schelde, is how long it remains within the estuarine water phase. A possibility to determine the "age" of leaf litter collected from the estuarine water, could be to compare its chemical composition to the pattern of changing occurring in the decomposition process. The possibility to look into pigments as "biomarkers" to determine the retention time of the leaf litter in the Schelde was the scope of this study.

1 Materials and method

1.1 Materials

Two dominant species of vegetation along the Schelde, willow (*Salix triandra*) and reed (*Phragmites australis*) were used.

1.2 Sampling

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Leaves of standing shoots of willow (*Salix triandra*) and reed (*Phragmites australis*) were collected in October 1998 from the Schelde Estuary near Dendermonde. They were put into the nets whose mesh size is 500 μm and submerged into the water. During 3 months, leaves were collected from the nets at certain periods(October 12, 19, 26; November 5, 18; December 8 in 1998 and January 14 in 1999).

1.3 HPLC measurement

1.3.1 Pigment extraction

Pieces with an average surface of 24.52 mm^2 (± 10%) were cut out of the leaves with a perforator. For each sampling date, 3 leaves were analyzed, and 3 replicate pieces taken out of each leave. Each piece of leaf was brought into a test tube with 1.8 ml of 90% acetone. The leaf was crushed with a glass rod, and 0.2 ml of filtered(0.45 mm), double distilled water added. The sample was then further ground. The test tube was cooled during the procedure by putting it in ice. When completely homogenized, the sample was transferred to a centrifuging tube and centrifuged during 3 minutes. The supernatants was decanted and suck up in a syringe covered with a 0.5 mm filter(Millipore).

1.3.2 HPLC analysis

The analysis was done using a Waters HPLC, equipped with a Waters 600 Controller, an autosampler and an absorbance detector of a fluorimeter. The column used was a reversed phased column(Spherisorb ODS2). The following solvents were used in a gradient protocol:(1)80 units methanol/20 units 0.5 mol/L ammonium acetate;(2)90 units acetonitrile/10 units water;(3)ethyl acetate.

2 Results

2.1 Statistical analysis(Spearman rank order correlation)

As shown in Table 1, the pigments which has a similar decomposition pattern (after Spearman rank order correlation analysis, $P < 0.05$) were grouped, the pigments group decomposition dynamics pattern is shown in Fig. 1c. In the same way, several groups of pigments decomposition dynamics patterns were achieved, as shown in Fig.1 and Fig.2.

Table 1 Analysis for pigments group 1(willow)

Spearman rank order correlations $N = 8$, $P < 0.01$		
Nonpar stats	MD pairwise deleted	
pair of variables	Spearman R	p-level
W1.61 & W3.03	0.9730	4.8360E-05
W1.61 & W10.29	0.9459	3.7901E-04
W1.61 & W2.42	0.8919	2.9082E-03
W3.03 & W10.29	0.9730	4.8360E-05
W3.03 & W2.42	0.9189	1.2529E-03
W10.29 & W2.42	0.8378	9.4062E-03

2.2 Pigments decomposition dynamics pattern

Pigments decomposition dynamics pattern for willow as shown in Fig.1. Fig.1 shows how the pigments concentration(ABS) changed with the incubation time(day), since no pigment identification was done, each pigment was represented by their different retention time, eg, in Fig.1c, "1.61" represents the pigment whose retention time from HPLC is 1.61, others are so on. Fig.1a and Fig.1b gave us information that whether the leaf litter stayed in the water longer than 37 days or not.

The pigments group in Fig.1a, we can see that all of the pigments concentration can be detected within 37 days of incubation, and with a trend of decreasing with the time. After 37 days, the concentration decreased under the limit of detection of HPLC(except for RT 21.15).

The pigment in Fig.1b, its concentration showed a sharp decrease within the first week, then it remained more less constant in the following two weeks, when incubation time reached 37 days, the pigment decomposed completely, and its concentration is under the limit of HPLC detection.

From the decomposition dynamics pattern a and b, we can get the time the litter stayed in the water. For example, if the pigments in Fig.1 a or b is present, the "age" of the litter is less than 37 days, if

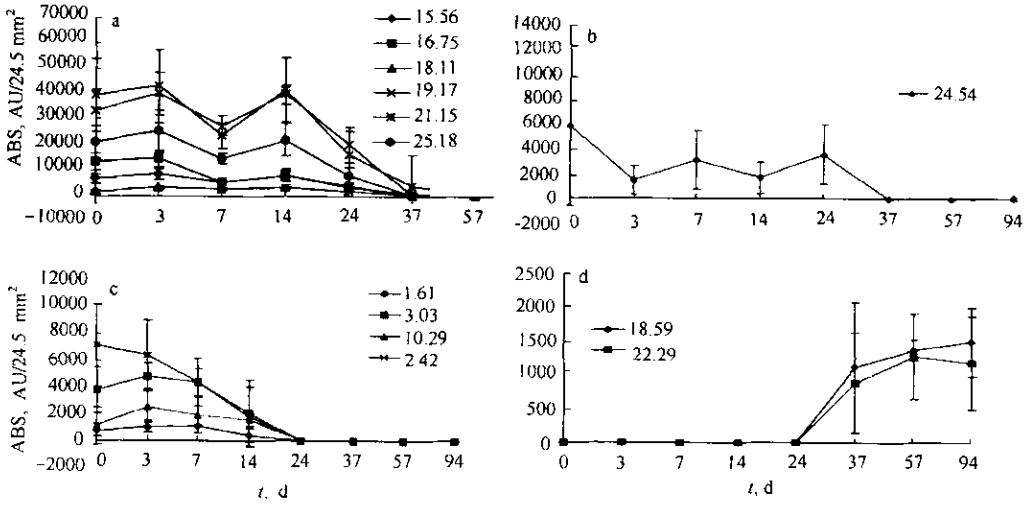

Fig.1 Decomposition dynamics pattern of pigments for different groups for willow

group 3 or group 7 is absent, the "age" of the litter is longer than 37 days.

In Fig.1c, we can see clearly that within the first 24 days of decomposition there is a sharp decrease in concentration for pigments(RT 1.61, 3.03, 10.29, 2.42), after 24 days, the concentration remained zero(below detection limit).

In Fig.1d, pigments were only detected after 24 days of incubation. The concentration increased reaching a quasi-plateau from 57 days onwards for both pigments.

Combining Fig.1c and d, we can infer whether the leaf litter stayed in the water longer than 24 days (three weeks) or within 24 days(three weeks). In this way, the determination of the "age" of the litter (willow) in the water can be used by using Fig.1c and d. eg. if the pigments group in Fig.1c is present while the pigments group in Fig.1d is absent on the other hand, then we can infer the "age" of the litter is longer than 24 days. Pigments decomposition dynamics pattern for reed as shown in Fig.2.

The decomposition dynamics pattern in Fig.2 shows that the peak of the concentration of pigment(RT 10.13) at incubation time of 3 days, after 3 days, it decreased fast until under the limit of detection of HPLC at 24 days of incubation. From 24 days onward, it can not be detected anymore over the whole decomposition time range.

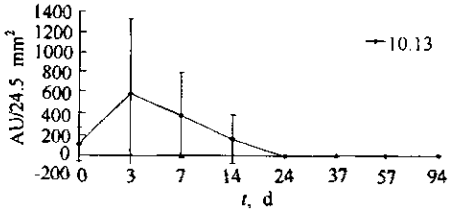

Fig.2 Decomposition dynamics pattern of pigments for reed

We can infer the "age" of the litter from the pattern in Fig.2. For example, if the pigment(RT 10.13) is present, the "age" of the litter is less than 24 days, if it is absent, the "age" of the litter is longer than 24 days.

Chlorophyll-a decomposition dynamics pattern for willow and reed as shown in Fig.3.

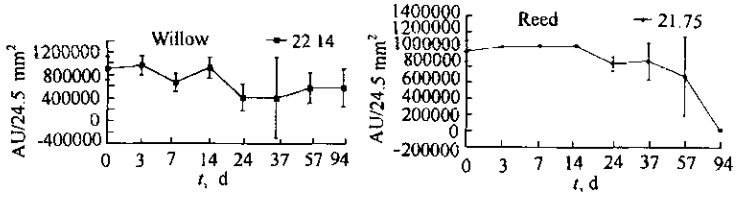

Fig.3 Chlorophyll-a decomposition dynamics pattern

Differences in decomposition dynamics of chlorophyll-a for willow and reed can be seen from Fig.3. A fast decaying phase of chlorophyll-a within 24 days for willow compared with chlorophyll-a remaining relatively constant till after 57 days of incubation for reed.

2.3 Determination of the time the litter stayed in the water

Combining the pigments decomposition dynamics pattern in Fig.1(willow), more precise information about the "age" of the litter can be got, as shown in Table 2. In this way, we can detect the "age" of litter for willow is from less than 24 days, 24 – 37 days and longer than 37 days. "age" of litter for reed can also be detected from Fig.2, which is from less than 24 days and longer than 24 days.

Table 2 Determination of the "age" of the litter(willow)by using Fig.1

	c		d		a	
b	+	–	+	–	+	–
+	< 24days	24—37days	24—37days	< 24days	< 37days	—
–	—	> 37days	> 37days	—	—	> 37days

Notes: a represents the pigments group in Fig.1 a; b represents the pigments group in Fig.1 b; c represents the pigments group in Fig.1 c; d represents pigments group in Fig.1 d; + . represents pigments detected from HPLC; – . represents pigments undetected from HPLC

3 Discussion and conclusion

Leaf senescence is accompanied by the metabolism of chlorophyll to nonfluorescent catabolites (Hoertensteiner, 1999). The pathway of chlorophyll degradation comprises several reactions and includes the occurrence of intermediary catabolites. After removal of phytol and the central Mg atom from chlorophyll by chlorophyllase and Mg dechelatase, respectively, the porphyrin macrocycle of pheophorbide a is cleaved(Hoertensteiner, 1999).

Unfortunately, no pigment identification was done in this study, so it is to some extent difficult to discuss the chlorophyll-a degradation pathway in this paper. Our recommendation is that further identification of the pigments will be useful to study the chlorophyll-a degradation pathway and chlorophyll-a degradation products of the riparian leaf litter in the Schelde.

Nevertheless, we still can get some information from the decomposition dynamics pattern of pigments. Combining the decomposition dynamics pattern of chlorophyll-a for willow (Fig. 3) and pigments group decomposition dynamics pattern in Fig.1d, we can see that with the fast decaying of chlorophyll-a within 24 days, accompany with the appearance of the pigments in Fig.1d after 24 days. This could imply that chlorophyll-a converted to the pigments in Fig.1d. The pigments in Fig.1d(RT 18.59 and RT 22.29) probably were the products(chlorophyllides, pheophorbides, and pheophytins)of chlorophyll-a degradation (Hoertensteiner,1999). The delayed appearance of pigments in Fig. 1d is probably due to leaching (Gessner, 1989), leaching out of the leaves and the incubation bags. The fast decaying of chlorophyll-a within 24 days may not only due to the degradation of the pigments, chlorophyll-a as a big molecule and its leaching into the water could be quite considerable. So further investigation on how much chlorophyll-a leaching from the leaf litter into the water column becomes very necessary.

The decomposition dynamics pattern of chlorophyll-a degradation is different between willow and reed (Fig.3). Chlorophyll-a can be detected during the whole time range of the experiment for willow. Two phases of decomposition with a fast phase within 37 days and slow phase after that. While for reed, chlorophyll-a decomposed very slowly within 57 days of incubation, and from 57 days onward there is a sharp decrease of the concentration of chlorophyll-a. The different decomposition pattern of chlorophyll-a in willow and reed probably due to the differences in chemical makeup of the leaf litter, in particular the nitrogen and tannin content(Twilley, 1986).

The decomposition calibration pattern for chlorophyll-a is quite different between willow and reed, the

species differentiation may cause the difference as one of the possible explanation (Osterofsky, 1997). Another possible causes may be the effect of phytoplankton association, due to the attachment of the phytoplankton on the leaves, may lead to an overestimation of the chlorophyll-a concentration from HPLC detection.

All these elements lead us to draw this conclusion that the "age" of the leaf litter(willow & reed)can be inferred relatively precise by using HPLC data. The precision of reed may seem crud, but is relevant in the context of litter transport dynamics within the estuary.

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