Decomposition of macrophytes and dynamics of enzyme activities in subalpine marshes in Lake Tahoe basin, U.S.A.

Jae Geun Kim1,3 & Eliška Rejmánková2
1Department of Biology Education, Seoul National University, Seoul 151-742, Korea. 2Department of Environmental Science and Policy, University of California, Davis, CA 95616, U.S.A. 3Corresponding author∗

Received 19 November 2002. Accepted in revised form 27 February 2004

Key words: Carex, decomposition, enzyme activity, Nuphar, Ranunculus, subalpine marsh

Abstract

To reveal the environmental and substrate quality effects on decomposition process and enzyme activities, litterbag experiments containing Nuphar and Carex leaves, Nuphar rhizome, and Ranunculus shoot, were carried in five-subalpine marshes in Lake Tahoe basin, USA. Alkaline phosphatase, β-glucosidase, and β-xylosidase activities were determined by a fluorogenic method using methyumbelliferyl substrates. Carex leaves, Nuphar rhizome and leaves, and Ranunculus shoots lost, respectively, 33, 67, 82 and 93% of original dry weight over 268 days. Decay rates were different among substrates but not among marshes. Nitrogen and carbon contents increased during the first 58 days and subsequently remained stable. Phosphorus content was stable during the experimental period except for a decrease in the first 16 days in Nuphar shoots. Enzyme activities in decomposing Carex and Nuphar leaves in four marshes were not significantly affected by environmental conditions. β-glucosidase and β-xylosidase activities in decomposing Carex leaves increased with time, but in other plant tissue these enzyme activities remained stable during experimental period. Enzyme activities were significantly different among decomposing substrates. Alkaline phosphatase activity was highest in Nuphar leaves (ca. 1286 µ-mole h−1 g DW−1) but lower and similar in other plant tissues (ca. 100 and 10 µ-mole h−1 g DW−1, respectively). This study showed differences in decay rates and enzyme activities rely on substrate and not the environment conditions of the study area. Decomposition rates in the early stage of decomposition were related to cumulative enzyme activities.

Introduction

The decomposition of plant litter in wetlands is important to ecosystem functions such as soil formation and nutrient cycling and wastewater treatment systems. A small part of macrophyte plant material produced is directly consumed by herbivores (Wetzel, 1975). Therefore the process of decomposition is one of the key processes in the recycling of nutrients, and it is one of the major factors in the functioning of the wetland ecosystem. Decomposition processes include nearly all changes in organic matter; senescence or death, fragmentation, leaching, feeding by detritivores, changes of component chemistry, etc. and Melillo et al. (1989, p. 189) stated ‘Decay processes in an ecosystem can be thought of as a continuum beginning with fresh plant litter and leading to the formation of refractory soil organic matter’. In general, decomposition rates are determined by the substrate quality, climate, and site factors such as soil type and water quality in wetlands (Godshalk and Wetzel, 1978; Kim, 2001; Swift et al. 1979). Key factors affecting decomposition are litter quality, especially C/N ratio, lignin/N ratio (Koukoura et al., 2003; Moretto et al., 2001; Ross et al., 2002) and the physical and chemical characteristics of the environment (Kalburtji et al., 1998; Vitousek et al., 1994). Effects on elevated CO2 and temperature on decomposition were dependent on litter quality and environmental characteristics such as N availability (De Angelis et al., 2000; Kandeler et al., 1998; Loiseau and Soussana, 1999).
Litterbag experiments have been used for the determination of litter decay rates but have the limitation of losing fine particle less than the mesh size. Saprophytic microbes cause about 60–70% of the mass loss of macrophyte detritus during decomposition by means of hydrolytic exoenzymes (Brock, 1984; Harrison and Mann, 1975). Thus, the activity of exoenzymes in the decomposing material is a very important factor determining the rate of decay (Kok and Van der Velde, 1991; Sinsabaugh and Findley, 1995). The patterns of enzyme activities of different classes have been shown to vary among litters of different plant species (Linkins et al., 1990; Fioretto et al., 2000). Also, results of Carreiro et al. (2000) indicate that changes in soil pH, nutrient availability (such as \( \text{NO}_3^- \) and \( \text{NH}_4^+ \)) and litter quality can result in different enzyme activities. However, few authors tried to relate decay rate and exoenzyme activity (Kok and Van der Velde, 1991; Sinsabaugh, 1994; Sinsabaugh et al., 1994a, b) and there is little information on the time course of the activity of exoenzymes during early decay process of the plant material in subalpine wetlands.

This study was aimed to determine the dynamics of nutrient cycling of wetland plant materials and the changes in enzyme activities in decomposing materials in representative subalpine marshes in Lake Tahoe basin. The investigation assessed if decomposition and enzyme activities differed between the dominant species or between sites, and whether the nutrient contents in water affect litter decomposition rates and enzyme activities.

Materials and methods

Study sites

Five subalpine marshes were selected for this study in Lake Tahoe basin, California in USA: Upper Grass Lake, Lily Lake, Frog Pond, Miller Meadow, and Snow Creek (see Kim et al., 2001 for detailed information of marsh characteristics). According to previous study (Kim et al., 2001), dry mass accumulation rates were low in Upper Grass Lake and Lily Lake, middle in Frog Pond and Miller Meadow, and high in Snow Creek: 0.081, 0.143, 0.350, 0.425, and 0.778 kg m\(^{-2}\) yr\(^{-1}\), respectively. The permanently flooded area of Upper Grass Lake and Lily Lake was covered with \textit{Nuphar luteum} ssp. \textit{polysepalum} and the moist area was covered with \textit{Carex rostrata}. The flooded area of Miller Meadow was covered with \textit{N. luteum} ssp. \textit{polysepalum} and \textit{Scirpus subterminalis} and the moist area was covered with \textit{C. rostrata}. The flooded area of Frog Pond was covered with \textit{N. luteum} ssp. \textit{polysepalum} and some patches of \textit{C. rostrata} and moist area were covered with \textit{C. rostrata}. \textit{Ranunculus aquatilis} var. \textit{capillaceus} dominates the flooded area of Snow Creek and the saturated soggy area was covered with \textit{Sparganium angustifolium}.

Litterbag preparation, recovering, and analyses

Leaves of \textit{N. luteum} ssp. \textit{polysepalum} and \textit{C. rostrata} were collected in four subalpine marshes for marsh effects on decomposition and enzyme activities in August 21–23, 1999; Upper Grass Lake, Lily Lake, Frog Pond, and Miller Meadow. In addition to above collection of \textit{Nuphar} and \textit{Carex} leaves, rhizomes of \textit{N. luteum} ssp. \textit{polysepalum} were collected in Miller Meadow and shoots of \textit{Ranunculus aquatilis} var. \textit{capillaceus} were collected in Snow Creek for substrate effects on decomposition and enzyme activities. \textit{Nuphar} rhizome was cut into 1 cm thickness. \textit{Ranunculus} shoots were killed in hot water (60 °C) for 3 min. Litterbags (mesh size 1 mm) were filled with 50–100 g of entire fresh plant samples (ca. 10 g dry weight) at the sampling sites because pre-drying of plant material affects weight loss and nutrient release (Brock et al., 1982, 1985; Rogers and Breen, 1982) and kept in a water column. Five litterbags of each litter type at each site were retrieved on 16, 31, 58, 107, and 268 days. Retrieved litterbags were rinsed in slow running tap water. The contents of the litterbag were divided into two subsamples: one for dry weight and element analyses and the other for enzyme assay.

For the enzyme assays, Methylumbelliferyl (MUF)-phosphate, MUF-β-glucopyranoside, and MUF-β-xyloside were used as model substrates for alkaline phosphatase, β-glucosidase, and β-xylosidase, respectively. The assays were done by modification of a fluorogenic method (Freeman et al., 1995). A 0.4 g of fresh subsample was homogenized for 30 s with 40 mL of distilled water by a BIOSPEC PRODUCTS INC. model M133/1281-O Bio-homogenizer. Two mL of slurry was added with 2 mL of 2 mM MUF substrate (final conc. 1000 μM) and incubated at 30 °C for 60 mins (sample amount and substrate concentration were decided from the enzyme saturation curves). One and half mL of incubation solution was taken into 1.5 mL Eppendorf centrifuge tube and centrifuged at 7200 g for 5 min. 0.5 mL of the supernatant was
### Table 1. Surface water characteristics at five marshes during study period (from August 1999 to May 2000)

<table>
<thead>
<tr>
<th></th>
<th>Upper Grass Lake</th>
<th>Lily Lake</th>
<th>Frog Pond</th>
<th>Miller Meadow</th>
<th>Snow Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water Temp. (°C)</strong></td>
<td>6.4–16.3</td>
<td>5.4–16.1</td>
<td>5.9–17.0</td>
<td>1.6–18.0</td>
<td>4.5–13.0</td>
</tr>
<tr>
<td><strong>Conductivity (µS cm⁻¹)</strong></td>
<td>23–40</td>
<td>8–17</td>
<td>10–20</td>
<td>13–50</td>
<td>114–186</td>
</tr>
<tr>
<td><strong>Na (mg L⁻¹)</strong></td>
<td>1.31–4.33</td>
<td>0.98–2.75</td>
<td>2.90–3.99</td>
<td>1.55–4.95</td>
<td>6.64–12.37</td>
</tr>
<tr>
<td><strong>Ca (mg L⁻¹)</strong></td>
<td>1.83–5.33</td>
<td>1.17–2.15</td>
<td>1.36–2.52</td>
<td>1.22–5.68</td>
<td>13.15–17.38</td>
</tr>
<tr>
<td><strong>Mg (mg L⁻¹)</strong></td>
<td>0.22–0.84</td>
<td>0.14–0.34</td>
<td>0.30–0.48</td>
<td>0.18–0.78</td>
<td>5.93–10.80</td>
</tr>
<tr>
<td><strong>SRP (mg L⁻¹)</strong></td>
<td>&lt;0.10–4.03</td>
<td>&lt;0.10–4.56</td>
<td>&lt;0.10–6.58</td>
<td>0.55–53.27</td>
<td>3.01–22.38</td>
</tr>
<tr>
<td><strong>NO₃ (mg L⁻¹)</strong></td>
<td>4.49–14.56</td>
<td>3.01–10.58</td>
<td>7.56–11.94</td>
<td>5.90–53.63</td>
<td>4.07–23.57</td>
</tr>
<tr>
<td><strong>NH₄ (mg L⁻¹)</strong></td>
<td>5.37–16.12</td>
<td>2.95–24.72</td>
<td>6.51–33.84</td>
<td>2.67–36.74</td>
<td>12.55–34.84</td>
</tr>
</tbody>
</table>

Transferred into 13 mm (4) × 100 mm (length) borosilicate glass tube and 2 mL of pH11 buffer (0.05 M glycine/0.2 M NaOH buffer) was added. Fluorescence was determined with a Turner Quantech FM109525 filter fluorometer at 430 nm emission and 365 nm excitation wavelength. For each treatment, a calibration was made using 25 µM of MUF free acid solution (instead of substrates) in the sample slurry to correct interference of phenolics and adsorption to slurry. Six replicates of each sample were analyzed. Integration of enzyme activity over time was done by multiplying the mean activity over each interval by the length of the interval (Jackson et al., 1995).

Air-dried sub-samples were ground in a mortar with a pestle and sieved with a standard #60 sieve (mesh size 250 µm). Total carbon and nitrogen were determined on a Carlo-Erba series 5000 CHN-S analyzer. Total phosphorus was determined by ICP-AES after microwave acid digestion (Sah and Miller, 1992).

Surface water samples were collected at the same time as litterbag recovery and brought to the laboratory in a cooler where they were filtered through Fisher Scientific G4 glass fiber filter. Ammonium, nitrate and soluble reactive phosphorus (SRP) were analyzed with indophenol method, hydrazine method and colorimetric method, respectively (Hunter et al., 1993). Water conductivity and pH were measured with an Oaktron conductivity meter and a Fisher Scientific pH meter. Calcium (Ca), magnesium (Mg), and sodium (Na) concentrations were determined by a Perkin-Elmer 2380 Atomic Absorption Spectroscopy following the methods described in Allen (1989).

**Data analyses**

Data for Carex leaves and Nuphar leaves in four marshes were averaged or all data were included when these data were used for the analyses of substrate effects on decomposition constant and enzyme activities. The changes (X) in the undecomposed litter as percentages of their initial quantity (X₀) at time T (days), were described following the equation of Olson (1963): \[ X = X₀e^{-kT}, \]

where X₀ is the initial % weight, e the natural log constant and k (d⁻¹) the decomposition constant (Olson, 1963; Swift et al., 1979). Increasing values of k indicate increasing rates of litter mass loss or nutrient release or other parameter changes. Decomposition constants were calculated twice because decomposition data during 0–268 days in some marshes did not fit well in the model: one is for 0–268 days and the other is for 0–58 days. StatView for Windows (Abacus Concepts, Version 4.57) was used for the statistical analyses.

**Results**

**Water characteristics**

Surface water temperature ranged from 1.6 °C (Dec. 4, Miller Meadow) to 18.0 °C (Sept. 5, Miller Meadow) during study period (Table 1). Surface water characteristics in Upper Grass Lake and Lily Lake were similar. Frog Pond and Miller Meadow had large range of ammonium concentration. Miller Meadow had large range of SRP and nitrate and Snow Creek had high conductivity and cation and ammonium concentrations. SRP, nitrate, and ammonium concentrations in surface water among marshes showed a similar trend to sedimentation rate but did not have a high correlation.
Table 2. Decomposition constant k (per day) of the best fitted curve \( X = X_0 e^{-kT} \) during 268 days incubation for four substrates in five subalpine marshes. Parentheses indicate k and \( r^2 \) values during first 57 days incubation.

<table>
<thead>
<tr>
<th></th>
<th>C. leaves</th>
<th>N. leaves</th>
<th>N. rhizome</th>
<th>R. shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( k )</td>
<td>( r^2 )</td>
<td>( k )</td>
<td>( r^2 )</td>
</tr>
<tr>
<td>Upper</td>
<td>0.0010*</td>
<td>0.755</td>
<td>0.0069*</td>
<td>0.702</td>
</tr>
<tr>
<td>Grass Lake</td>
<td>(0.0015)</td>
<td>(0.263)</td>
<td>(0.0280*)</td>
<td>(0.981)</td>
</tr>
<tr>
<td>Lily Lake</td>
<td>0.0009</td>
<td>0.393</td>
<td>0.0057*</td>
<td>0.703</td>
</tr>
<tr>
<td>Frog Pond</td>
<td>(0.0063*)</td>
<td>(0.993)</td>
<td>(0.0238*)</td>
<td>(0.981)</td>
</tr>
<tr>
<td>Miller</td>
<td>0.0015</td>
<td>0.554</td>
<td>0.0059</td>
<td>0.623</td>
</tr>
<tr>
<td>Meadow</td>
<td>(0.0018)</td>
<td>(0.756)</td>
<td>(0.0128)</td>
<td>(0.734)</td>
</tr>
<tr>
<td>Bless Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*means that k value is significant at the level of \( P < 0.05 \).

Comparison of decomposition process of Carex and Nuphar leaves in four marshes

Decomposition constants of Carex leaves over 268 days were similar in Upper Grass Lake and Lily Lake as 0.001 day\(^{-1}\) (ca. 28% of original) and in Miller Meadow and Frog Pond as 0.002 day\(^{-1}\) (ca. 40% of original) (Table 2). However, this difference was not statistically significant. Decomposition data over 268 days of Carex leaves in Lily Lake and Frog Pond did not fit the model but those over 58 days fitted well. Decomposition constants of Nuphar leaves over 268 days were not statistically significant among marshes and average \( k \) value was 0.006 day\(^{-1}\) (83% of original).

Decomposition constants over 58 days in Miller Meadow were relatively lower compared to others. Sampling site in Miller Meadow was covered with shallow water over 31 days but it was exposed to air between incubation times of 31 and 58 days. When litterbags were recovered in this marsh on 58 days, they were covered with clay and litters were dried. Litterbags were covered with thick ice at 107 days.

Carbon contents of Carex leaves in four marshes increased over the study period and there was no significant difference among sites (Figure 1). Overall carbon content of Nuphar leaves increased to 50% at 58 days and remained at the same level. However, C content of Nuphar leaves in Miller Meadow decreased to 38% at 58 days and increased over the remaining study period. Carbon contents of Nuphar leaves among marshes except Miller Meadow did not show significant difference during study period. Nitrogen contents of Carex leaves in four marshes increased slightly during 31–58 days and maintained at similar level and those of Nuphar leaves increased from 1.9% to 3.4% over 58 days and maintained at the same level. Phosphorus contents of Carex and Nuphar leaves in four marshes decreased a little and maintained at the same level.

Comparison of decomposition process of four substrates

Table 2 shows that decomposition constants were significantly different among substrates. Average \( k \) values over 268 days of Carex and Nuphar leaves were 0.0013 day\(^{-1}\) (33% of original) and 0.0059 day\(^{-1}\) (82% of original) and \( k \) values of Nuphar rhizome and Ranunculus shoot were 0.0033 day\(^{-1}\) (67% of original) and 0.00089 day\(^{-1}\) (93% of original), respectively.

Figure 2 shows the C, N, and P dynamics of these substrates during 268 days decomposition period. Carbon contents were slightly increased over the study period except in Ranunculus shoot; C content in Ranunculus shoot increased to 36% at 58 days and decreased continuously. Nitrogen contents were slightly increased to 16 days and remained constant in Carex leaves, increased to 3.7% over the study period in Nuphar leaves, increased to 2.3% and remained constant in Nuphar rhizome, and increased to 3.6% and decreased to 2.7% in Ranunculus shoot. Phosphorus
contents were relatively constant except in *Ranunculus*
shoot; P content was highest in time 0 as 4.7 g kg\(^{-1}\),
decreased to 2.41 g kg\(^{-1}\), and remained at this level.

Decomposition constant and original N and P con-
tents were highest in *Ranunculus* shoot and those were
lowest in *Carex* leaves. Nitrogen and P contents in
*Nuphar* leaves were continuously increased and de-
composition rate was higher than *Carex* and *Nuphar*
rhizome.

Comparison of enzyme activities of decomposing
*Nuphar* and *Carex* leaves in four marshes

Alkaline phosphatase activity in decomposing *Carex*
leaves maintained at the same level (no statistically
significant difference) with some fluctuation but \(\beta\)-
glucosidase and \(\beta\)-xylosidase activities were increased
with incubation time (Figure 3). \(\beta\)-glucosidase activity
was similar in early incubation period. However, there
was a big difference at 286 days; highest in Lily Lake
and lowest in Upper Grass Lake. \(\beta\)-xylosidase activity
was significantly lower in Upper Grass Lake than
in others. Alkaline phosphatase activity in decompos-
ing *Nuphar* leaves increased with time until 58 (in
Frog Pond) or 107 days (Lily Lake and Upper Grass
Lake) and decreased. \(\beta\)-glucosidase and \(\beta\)-xylosidase
activities increased in 16 days and decreased slowly
with time. Alkaline phosphatase, \(\beta\)-glucosidase, and
\(\beta\)-xylosidase activities were lower in Miller Meadow
than in others since 58 days. Litterbags in Miller
Meadow were exposed to air at 58 days and submerged
again between 58 and 106 days.

Relationship between enzyme activities and marshes
depends on substrate and enzyme type (Table 3). Al-
kaline phosphatase activities in *Carex* and *Nuphar*
leaves were significantly different among marshes. β-glucosidase activities were significantly different among marshes only in Carex leaves. Marsh type did not give any significant effect on β-xylanase activities of both leaves.

**Comparison of enzyme activities in four decomposing substrates**

Patterns of three enzyme activities were similar in all substrates except β-glucosidase and β-xylanase in Carex leaves (continuously increased) (Figure 4). Alkaline phosphatase activity was highest in Nuphar leaves (average ca. 1286 µ-mole h⁻¹ g DW⁻¹) and similar in others (average ca. 150 µ-mole h⁻¹ g DW⁻¹). β-glucosidase and β-xylanase activities were high in Nuphar leaves and rhizome (average ca. 1000 and 65 µ-mole h⁻¹ g DW⁻¹, respectively) and low in others (average ca. 100 and 10 µ-mole h⁻¹ g DW⁻¹, respectively).

Enzyme activities were significantly related to substrate types (Table 3). Enzyme activities in decomposing Ranunculus shoot were lowest (Figure 4) but decay rate was highest (Table 2), which implies that decomposition of Ranunculus shoot relies on other factors such as leaching and fragmentation rather than enzyme activities. Enzyme activities were related with decay rate in Nuphar leaves and rhizome and Carex leaves (Figure 4 and Table 2). Also, enzyme activities were related with the element contents such as C (including starch) and P (Figures 2 and 4).

The correlation between mass loss and cumulative enzyme activities. Mass loss was well correlated with logarithm of cumulative enzyme activity.

**Discussion**

**Comparison of decomposition process of Carex and Nuphar leaves in four marshes**

Even though sedimentation rates were different among the four marshes, decomposition constants of Carex and Nuphar leaves were not significantly different
among marshes. However, decay rates of Carex leaves in four marshes (average 33% loss) were low in comparison with other studies in different environments; 64% loss of *C. rostrata* over 113 days in Pope Marsh of Lake Tahoe basin (Kim, 2001), 60% loss of *C. diandra*, 51% loss of *C. rostrata*, 39% loss of *C. lasiocarpa*, and 32% loss of *C. acutiformis* over 12 months in the Netherlands (Aerts and de Caluwe, 1997), 58% loss of *Carex* over 1 year in peatlands of central Alberta, Canada (Szumigalski and Barley, 1996). These study areas include cooler areas than the four marshes and also a similar environment (Pope Marsh). Even though our study area is more favorable to microorganisms due to high temperature, decay rates were lower than others. This can be explained by following reasons: *Carex* leaves in this study were not killed, not segmented, and not dried.

Decay rate (82% loss) of *Nuphar* leaves in four marshes was little lower than in other studies; 90% loss of *Nuphar* over 113 days (Kim, 2001), ~90% loss of *Nuphar* sp. in 200 days (Fogel and Tuross, 1999), 55% loss of *Nymphaea alba* during 50 days in a laboratory (Kok and Van der Velde, 1991), almost loss of fresh *N. alba* leaves in summer in Netherlands (Brock et al., 1985), 96% loss of *Nasturtium officinale* in 27 days in summer in New Zealand (Howard-Williams 1982).
Figure 4. Enzyme activities in decomposing Carex leaves, Nuphar rhizome and leaves, and Ranunculus soot in subalpine marshes. Average and 1 S.E. of 12 samples for leaves in four marshes and 3 samples for others in one marsh per substrate type (6 replicates per 1 sample).

Table 4. Regression coefficients of mass loss and cumulative enzyme activities $y = a + b \cdot \log(x)$; $y$: mass loss (%), $x$: cumulative enzyme activity ($\mu$-mole/gDW)

<table>
<thead>
<tr>
<th>Litter type</th>
<th>Enzyme</th>
<th>$n$</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuphar leaves</td>
<td>Phosphatase</td>
<td>20</td>
<td>-512</td>
<td>86</td>
<td>0.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nuphar leaves</td>
<td>Glucosidase</td>
<td>20</td>
<td>-511</td>
<td>86</td>
<td>0.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nuphar leaves</td>
<td>Xylosidase</td>
<td>20</td>
<td>-445</td>
<td>94</td>
<td>0.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carex leaves</td>
<td>Phosphatase</td>
<td>20</td>
<td>-112</td>
<td>23</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carex leaves</td>
<td>Glucosidase</td>
<td>20</td>
<td>-55</td>
<td>14</td>
<td>0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carex leaves</td>
<td>Xylosidase</td>
<td>20</td>
<td>-51</td>
<td>16</td>
<td>0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nuphar rhizome</td>
<td>Phosphatase</td>
<td>5</td>
<td>-246</td>
<td>51</td>
<td>0.95</td>
<td>0.005</td>
</tr>
<tr>
<td>Nuphar rhizome</td>
<td>Glucosidase</td>
<td>5</td>
<td>-130</td>
<td>28</td>
<td>0.94</td>
<td>0.007</td>
</tr>
<tr>
<td>Nuphar rhizome</td>
<td>Xylosidase</td>
<td>5</td>
<td>-102</td>
<td>29</td>
<td>0.94</td>
<td>0.006</td>
</tr>
<tr>
<td>Ranunculus shoot</td>
<td>Phosphatase</td>
<td>5</td>
<td>-224</td>
<td>51</td>
<td>0.86</td>
<td>0.024</td>
</tr>
<tr>
<td>Ranunculus shoot</td>
<td>Glucosidase</td>
<td>5</td>
<td>-403</td>
<td>85</td>
<td>0.82</td>
<td>0.035</td>
</tr>
<tr>
<td>Ranunculus shoot</td>
<td>Xylosidase</td>
<td>5</td>
<td>-156</td>
<td>51</td>
<td>0.83</td>
<td>0.032</td>
</tr>
</tbody>
</table>

*All data for enzyme activities in Nuphar leaves and Carex leaves in four marshes were included in this analysis.

et al., 1983). Kim (2001) used the same setting in the same area except different sampling and starting time (May 9) and got high decay rate. Also, other studies were started in summer. There are two possible explanations: high concentration of nitrogen and phosphorus in early sampled materials or high water temperature is responsible for high decay rate. Nitrogen and P contents of Nuphar leaves in Kim (2001) were 3.2% and 3.95 g kg$^{-1}$ compared to 1.2% and 1.49 g kg$^{-1}$ in this study and water temperature ranged from 11.7 to 16.3 $\degree$C in Kim (2001) compared to from 1.6 to 18.0 $\degree$C in this study.

This study implies that environmental characteristics of four marshes are not different enough to show different decay constants of Carex and Nuphar leaves.

Comparison of decomposition process of four substrates

Four substrates in this study have different C, N, P composition and their decay rates were significantly different. The initial C/N or C/P ratio among other factors affecting decomposition is important in the decomposition process because there is nutrient accumulation in many litter types through microbial immobilization during early decay (Taylor et al., 1991; Verhoeven et al., 1990). C/N ratio and P contents were related to decomposition in this study and this result shows again that substrate quality is an important factor for determining decay rate (see Swift et al., 1979 for detail). Phosphorus content in Ranunculus shoot decreased during 15 days and remained
at the same level. This might be a leaching effect because only *Ranunculus* shoots were killed to prevent regeneration during study period. Even though alkaline phosphatase activity in *Ranunculus* shoots was low, 40% of original mass was lost during 15 days. This means that enzyme was not responsible for this mass loss and supports leaching effect in *Ranunculus* shoots.

Comparison of enzyme activities of decomposing *Nuphar* and *Carex* leaves in four marshes

Alkaline phosphatase activities in *Carex* leaves remained constant and there is no significant difference. β-glucosidase and β-xylosidase activities gradually increased and differences of those enzyme activities among marshes increased with time. Increase of enzyme activity means the increase of microorganism activities or population (Chang and Yoo, 1986). *Carex* leaves were not good for microorganisms at first and became favorable for microorganisms with time.

Alkaline phosphatase activities in *Nuphar* leaves divided into two groups: one of Lily Lake and Upper Grass Lake and the other of Frog Pond and Miller Meadow. Water characteristics were similar between in-group and different between groups and this might be responsible for this difference. β-glucosidase and β-xylosidase activities were not affected by environmental differences among Lily Lake, Upper Grass Lake and Frog Pond. Enzyme activity in *Nuphar* leaves at Miller Meadow was strongly affected by dryness (at 57 days) and maintained the low level even though substrates were submerged into water again. This implies that dryness can change some characteristics of substrate related to enzyme activities.

Comparison of enzyme activities in four decomposing substrates

Alkaline phosphatase activity was highest in *Nuphar* leaves from time 0 to 268 days and was similar to other substrates. Also, the other two enzyme activities were high in *Nuphar* leaves. This means that *Nuphar* leaves are favorable for microorganisms from time 0. β-glucosidase and β-xylosidase activities in *Nuphar* rhizome were high and similar to those in *Nuphar* leaves. This might come from high content of starch or non-structural carbon source in *Nuphar* rhizome.

It would be suggested that different enzyme activities come from the difference of membrane and internal structure and nutrient contents among plant species. Our results show the enzyme activities in live plant materials (at time 0) and enzymes from microorganisms substituted enzymes from plant itself with time.

Enzyme activities in this study were much higher than other studies; Sinsabaugh et al. (1994a) reported β-glucosidase and β-xylosidase activities in particulate organic materials of less than 40 and 10 μ-mole h⁻¹ g OM⁻¹, respectively during 180 days in a stream in New York and Jackson et al. (1995) reported β-glucosidase and β-xylosidase activities of max. 40 and 50 μ-mole h⁻¹ g OM⁻¹, respectively on around 50 day of incubation in a coastal *Typha* marsh in Ohio. Phosphatase activity in sediment was reported by Freeman et al. (1995) in a peatland, UK and Kang and Freeman (1999) in wetlands, UK, as max. 170 and 11 μ-mole h⁻¹ g DW⁻¹, respectively. These studies were done with organic materials that were already in late phase of decomposition. This comparison supports the substrate effect on enzyme activities.

Freeman et al. (1998) reported the seasonal change of enzyme activities in peatland soil but our results did not show this trend. Even though water temperature ranged from 1.6 to 18.0 °C, enzyme activities were not affected by temperature change in this study. Our results suggest that the decay process of plant materials in subalpine marshes based on enzyme activities is not affected by temperature in early stage of decomposition.

Sinsabaugh et al. (1994a) collected and analyzed published exoenzyme data and suggested cumulative lignocellulase activity is well correlated with % mass loss. Also, they tried several regression models to reveal the relationship between enzyme activities and mass loss and suggested single or multiple regressions for this relationship. As a suggestion of Sinsabaugh et al. (1994a), mass loss was well correlated with cumulative activities of three enzymes in this study. This study shows that it may be a good way to make models appropriate for each decomposition stage for the understanding of ecosystem process and simulation of ecosystem responses to disturbance.

Conclusion

*Carex* and *Nuphar* leaves in subalpine marshes decomposed exponentially and there is no significant difference in decay rate of *Nuphar* leaves among marshes. However, four different plant materials showed significantly different decay rates. In subalpine marshes in
Lake Tahoe basin, USA, decay rate was determined by substrate quality, not by environmental factors. Alkaline phosphatase activity was stable during decomposition experiment in all materials but $\beta$-glucosidase and $\beta$-xyllosidase activities were increased with time in Carex leaves but stable in others. Enzyme activities were not affected by low temperature in winter but affected by dryness. This study showed high decay rate in early stage is related with high enzyme activities in decomposing macrophytes and implied that there is alternative decay process for Ranunculus shoot rather than enzymatic degradation.

Acknowledgements

This study was supported by US EPA (R819658 & R825433) Center for Ecological Health Research at UC Davis. Although the information in this document has been funded by the United States Environmental Protection Agency, it may not necessarily reflect the views of the Agency and no official endorsement should be inferred.

References

Chang N K and Yoo J H 1986 Annual fluctuations and vertical distribution with time in Carex leaves but stable in others. Enzyme activities were not affected by low temperature in winter but affected by dryness. This study showed high decay rate in early stage is related with high enzyme activities in decomposing macrophytes and implied that there is alternative decay process for Ranunculus shoot rather than enzymatic degradation.

References


Section editor: J.M. Boddy