Initial carbon and nitrogen contents and sugar release characteristics in stream water during initial leaching of Quercus glauca leaves

Hirokazu Kitamura
Laboratory of Landscape Conservation, Minami-Kyushu University, Miyakonojo, Miyazaki 885-0035, Japan
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To clarify the release characteristics of sugars in relation to initial carbon and nitrogen contents of evergreen Quercus glauca leaves during initial leaching period, which dominate and contribute considerable quantities of allochthonous material to streams in the region, well washed and dried new and old leaves of Q. glauca (sun leaves and shade leaves) were submerged in invertebrate-free stream water after measuring the carbon and nitrogen contents of each leaf. 1, 5, 10, 20 and 30 days after submersion, sugar concentrations of the water were measured. The mean initial carbon contents ranged from 45.75 to 46.86 (%), indicating higher values than the deciduous leaves in references. The mean initial nitrogen content ranged from 1.83 to 2.16 (%) and significant difference was observed between shade-exposed old leaves and leaves from the other groups (P<0.0001). The initial carbon and nitrogen contents of leaves did not influence the release rates of carbon and nitrogen from the leaves. Sucrose, glucose and fructose were detected. Sucrose accounted for more than 60% of the total sugars. The average concentration of total sugar in the water treatment containing new leaves without aeration reached a maximum concentration of 26 (mg/l) after 30 days of immersion. Q. glauca leaves have a longer sugar-leaching period and leached sugars less rapidly than the desiduous leaves. The release rate and subsequent decomposition into water and carbon dioxide were increased by abrasion of leaf surfaces. These differences between deciduous and evergreen leaves might arise due to differences in leaf composition and structure. Sugars are derived from soluble carbohydrates that are lost through the cuticle of evergreen leaves; however, the relatively thicker cuticle of the evergreen leaves than deciduous leaves reduces the rate of sugar release. The tough outer surfaces of evergreen leaves may also slow rate at which the mass of the leaf decreases, particularly until such time as the outer layer of the epidermis, which contains cutin, breaks down in water.

Kye wards: carbon and nitrogen contents, leaching, Quercus glauca leaves, stream water quality, sugar.

1. INTRODUCTION

Leaf litter in streams, also referred to as allochthonous material, is the primary source of energy for the secondary consumers in the streams. This organic matter is decomposed through a combination of physical, chemical and biological processes. In leaves, the process of decomposition can be divided into leaching, conditioning, and fragmentation. Consequently, shortly after entering a stream, the soluble chemicals within the leaf are leached from the plant tissue, which is then colonized by microbes and fragmented by mechanical abrasion and invertebrate activity. The instream processing of this allochthonous material results in the production of dissolved organic matter (DOM) and the release of inorganic nutrients into the water. Since considerable quantities of allochthonous material enter lotic ecosystems from riparian forests, substances derived from the decomposition of leaf litter may have an important effect on stream water quality.

Several studies have suggested that the leachates from the leaf litter of different tree species are markedly different, and that they have different effects on the chemical characteristics of stream water. Studies have also examined the physical degradation of leaf litter, including decreases in the mass of allochthonous material over time and the chemical changes associated with the degradation of deciduous leaf litter in streams. The dependence of woodland stream ecosystems on allochthonous organic matter, primarily autumn-shed leaf litter, is well documented. However, relatively few studies have examined the leachates of evergreen leaves or their effect on stream water quality during initial decomposition. Indeed, the nutrient contents of evergreen leaves and their contribution to the dissolved organic substances in lotic systems is still largely unknown.

In a previous paper, we clarified the release characteristics of total organic carbon (TC), total nitrogen (TN), and specific ions from evergreen Quercus glauca leaves
were considered to be new leaves. The leaves had been growing since March 2010 and still appeared yellow-green in color due to the relatively short time that had elapsed since leaf expansion. Leaves that were picked from the older branches of the tree were also considered to be old leaves and were collected immediately before abscission, which means that they had been growing since the previous spring and could be distinguished from new leaves by their dark-green color. All the leaves collected were whole leaves and retained their original shape without any skeletonization. Any visually abnormal or injured leaves were not harvested at the time of sample collection on May 20, 2010. Leaf emergence in Quercus glauca at the study site started at the end of March and expansion of the leaves occurred from the beginning of April to the end of May every year. Stream water (100 L) was collected from the stream at the same site for all leaching experiments.

2-2. Measurement of initial carbon and nitrogen leaf contents
The harvested leaves described above were washed with distilled water to remove dust and aerosol, then air-dried for 1 week and oven-dried at 80°C for 12 h. Ten leaves of each group were then selected, and tissue samples measuring approximately 2 mg were removed from each leaf. Samples were always taken from an area 5 mm to the right of the midrib in the center of the leaf. Each fragment was analyzed using an ash-free CHNS/O Analyzer (2400 Series II, Perkin Elmer, Inc.,) to measure the initial carbon and nitrogen leaf contents.

2-3. Measurement of sugar concentrations leached from leaves
Sugar concentrations released from sun leaves, in which no significant differences in initial carbon and nitrogen contents of old and new leaves were observed, were analyzed. The harvested leaves described above were washed with distilled water to remove dust and aerosol, then air-dried for 1 week and oven-dried at 80°C for 12 h. Old and new leaves of sun leaves (i.e. SUN and SUO) were submerged in invertebrate-free stream water with or without aeration as described previously. The four water treatments included old leaves with aeration, old leaves without aeration, new leaves with aeration, and new leaves without aeration. Water was aerated to simulate reaeration in natural streams. In each treatment, 25 subsamples of five leaves were then randomly selected, weighed and submerged in 1 (l) of stream water in a beaker. All subsamples were then allowed to stand at ambient temperature (20-25°C) for 1, 5, 10, 20 and 30 days when five subsamples from each treatment were randomly selected for analysis. In addition, each water subsample was then filtered through a 0.20 (μm) filter and sugar concentrations in the water were measured by high performance liquid chromatography and a Shodex column (NH2P-50 4D, Hitachi K.K.). (The rate of the decrease in leaf mass and the concentrations of TC and TN leached from leaves in each subsample during the initial leaching period have been published previously).

3. RESULTS

3-1. Initial carbon and nitrogen contents of leaves
The initial carbon and nitrogen contents of the leaves in each group are shown in Fig.2. A relatively wide variety
Initial carbon and nitrogen contents and sugar release characteristics in stream water during initial leaching of *Quercus glauca* leaves

of compounds were observed. The mean initial carbon content and standard error in the SUN, SUO, SHN and SHO groups was 45.75 ± 0.75, 46.19 ± 0.18, 46.41 ± 0.20 and 46.86 ± 0.06 (%), respectively. The mean initial nitrogen content and standard error in the SUN, SUO, SHN and SHO groups was 1.86 ± 0.04, 1.83 ± 0.03, 1.97 ± 0.04 and 2.16 ± 0.04 (%), respectively.

Differences among the groups of leaves were analyzed by one-way ANOVA (Table 1), which showed that there were no significant differences in initial carbon content among the groups. Conversely, the initial nitrogen contents of the leaves in the different groups were significantly different (*P* < 0.0001). Since the results for nitrogen contents among groups were significantly different, differences among individual groups were estimated using Scheffe’s multiple range test (Fig.3).

For initial nitrogen content, significant differences were observed between the SHO group and the other three groups of leaves (*P* < 0.05). No significant differences in the initial nitrogen content were observed in the other groups.

### 3-2. Sugar

Sugar concentrations released from sun leaves, in which no significant differences in initial carbon and nitrogen contents of old and new leaves were observed, were analyzed. No fragmentation of leaves was observed in any of the samples at the end of the experiment.

The mean sugar concentrations in the leaves subjected to each of the water treatments over the course of the experimental period are shown in Fig.4. To avoid errors in the estimated sugar concentrations of leachates obtained from leaves due to the differences in the mass of leaves submerged in the water, sugar concentrations in the figure are given per 1 (g) of leaf tissue.

In the present measurements, sucrose, glucose and fructose were detected. Initial values for sucrose, glucose and fructose in stream water were 2.5, 0.0, and 0.5 (mg/l), respectively. The total sugar concentration was obtained by adding the concentrations of sucrose, glucose and fructose.

For sucrose, glucose and fructose, only slight differences were apparent among treatments before day 5 and the concentrations of these sugars in each treatment was less than 4 (mg/l). However, marked differences appeared among leaf groups 10 days after immersion. After day 10, old leaves without aeration released considerably more total sugar than the leaves in the other water treatments; the mean concentration of total sugar in the old leaves without aeration was 13 (mg/l) after 10 days of immersion, which gradually decreased thereafter. Conversely, the average concentration of total sugar in the water treatment containing new leaves without aeration reached a maximum concentration of 26 (mg/l) after 30 days of immersion. In the leaves exposed to aeration, the mean concentration of total sugar reached a maximum after 5 to 10 days before gradually decreasing thereafter. Thus, sugar concentrations in water treatments without aeration

<table>
<thead>
<tr>
<th>Table 1. Result of one-way ANOVA</th>
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<tbody>
<tr>
<td>dependence variable</td>
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<tr>
<td>carbon content</td>
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<td>nitrogen content</td>
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Fig. 2. Initial carbon (a) and nitrogen (b) contents in leaves from each leaf-type group.

Fig. 3. Results of Scheffe’s multipul range test. Letters above columns indicate statistically significant differences (*P* < 0.05).

![Image of initial carbon and nitrogen contents in leaves from each leaf-type group.](image1.png)

![Image of mean original nitrogen content of a leaf.](image2.png)
Initial carbon and nitrogen contents and sugar release characteristics in stream water during initial leaching of *Quercus glauca* leaves were considerably higher than they were in water treatments with aeration, and maximum sugar concentrations observed in water treatments without aeration after 10 days. In all treatments, the fluctuations observed in the sucrose concentration were similar to those observed for total sugar.

Glucose was seldom detected in the leachate during the experimental period, except in the treatment of old leaves without aeration when the leached glucose concentration ranged from 1.5 to 3.5 (mg/l) after 10 days. Similarly, fructose was only detected in the leachates from old leaves without aeration and new leaves without aeration, reaching maximum concentrations of 3 and 7 (mg/l), after 10 and 30 days after immersion, respectively.

The ratios of the mean concentrations of sucrose, glucose and fructose to the total sugar concentration on each sampling day are shown in Fig. 5. As shown by the figure, sucrose accounts for more than 60% of the total sugars that are leached from leaves after 30 days in all treatments, except for the water treatment containing old leaves with aeration, which had the lowest sugar concentrations of all treatments.

4. DISCUSSION

4-1. Overall comparison of the decrease in leaf mass, and TC and TN release between deciduous and evergreen leaves

In deciduous leaves, most leaching occurs within a few days of immersion in water. For example, autumn shade leaves in water can lose up to 40 (%) of their dry mass over several days [11]; the ash-free dry mass of sugar maple, yellow birch, and beech was 85.4 (%) of the initial mass after 2 days of being submerged in the laboratory [25]. Webster *et al.* (1986) [32] demonstrated that up to 25 (%) of the initial dry mass of certain riparian, deciduous tree leaves (*e.g.*, *Alnus* *sp.*, *Salix* *sp.*) was lost by leaching in the first 24 h of immersion [30]. Contrary to the leaves of deciduous species, the evergreen leaves of *Q. glauca* examined, lost 4~7 (%) of their initial mass within 24 h [27]. The overall decrease in leaf mass of evergreen leaves at 30 days is thus less than a quarter that of deciduous leaves. In addition, the rate of the decrease in leaf mass rates is slower than that observed in deciduous leaves.

A carbon loss ratio, using total carbon (TC) or total organic carbon (TOC) and dissolved organic carbon (DOC), has often used to infer the amount of carbon leached from leaves. In the leaves of the deciduous *Salix gracilistyla*, considerable leaching of DOC from leaf litter occurred within 24 h after immersion in water; indeed, over a 14 day period, approximately 65 (%) of the total DOC in senescent leaves had been leached within 24 h of immer-
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In the previous study, TC concentrations from *Q. glauca* leaves in different water treatments increased for 5 to 10 days after immersion [17]. Taken together, the findings of these previous studies indicated that the rate of DOC leaching from leaves of *S. gracilistyla* seemed to be similar to the rate of TC leaching from the leaves of *Q. glauca*. However, the total concentration of TC leached from *Q. glauca* leaves was higher than the DOC concentrations from *S. gracilistyla* leaves.

These differences in the characteristics of carbon release between *S. gracilistyla* and *Q. glauca* leaves may be due to differences in the cuticular surfaces of these deciduous and evergreen leaves. In submerged leaves, the outer layer of the epidermis, which is covered by cutin, degrades and leaches water-soluble materials as it becomes more permeable. Given the anatomical and morphological differences between evergreen leaves and deciduous leaves, leaching from these leaves is also expected to differ between species.

According to the results of the previous study [17], as with TC concentrations, TN concentrations leached from *Q. glauca* leaves increased for the first 5 to 10. Thereafter, TN concentrations in the leachate of the non-aerated treatments generally remained constant, while those in the aerated treatments continued to rise after the 10th day. The TN concentration in the leachate from old leaves after 10 days was slightly higher than that leached from new leaves. The increases in the TN concentration in leachate of each water treatment can be attributed to the increase in the nitrogen content of the decomposing leaves resulting from the increase in microbial activity [3, 7].

### 4-2. Initial carbon and nitrogen contents of *Q. glauca* leaves

Table 2 shows the values for the overall comparison of carbon and nitrogen contents in tree leaves reported previously. In sun leaves of *Q. glauca*, the mean initial carbon contents range from 45.75 to 46.86 (%) and no significant differences were observed among the four leaf groups (SUN, SUO, SHN and SHO). The mean initial nitrogen content in the leaves of the four groups ranged from 1.83 to 2.16 (%) and significant difference was observed between SHO leaves and leaves from the other groups. Compared to the previous studies listed in Table 2, the values obtained for initial carbon content in *Q. glauca* in this study are relatively high. These relatively high carbon values in *Q. glauca* could potentially be attributed to cutin covering the surface of the evergreen leaves. However, these values are expected to differ between species, particularly with respect to the timing of leaf emergence,
when the leaves were harvested, and the availability of sunlight. For example, the organic carbon content of the macrophytic tissue remained relatively constant throughout the growing season, declining near the end of the experiment in August \(^{26}\). It is thus possible that, during the growth period, the carbohydrates produced in leaves by photosynthesis are locked within the cuticles and epicuticular waxes, increasing the carbon content of the leaves.

In addition, in the present study, significant differences were observed in the initial nitrogen content of sun leaves and shade leaves (\(P < 0.0001\)). Because the initial carbon content of leaves depends on weather conditions (temperature and precipitation) of the spot in which plants have been growing and physiological processes of the tree as well as sunlight availability \(^{27, 28}\), further measurements and analysis on initial nitrogen contents of leaves under several conditions must be required. The finding that the initial nitrogen content is greater in shade leaves than it was in sun leaves could possibly be, in part, attributed to the immobilization of nitrogen in microorganisms on the surface of the shade leaves. However, further research is required to clarify this issue.

Figure 6 shows the relationship between mean TC and TN concentrations leached from sun leaves of Quercus glauca in water without aeration during the initial leaching period for 15 days in a previous study \(^{17}\). In the same figure, the mean initial carbon and nitrogen contents in the Quercus glauca leaves harvested in the present study are shown; peak TC and TN concentrations were observed in the initial leaching period, i.e. 5 to 10 days after immersion.

In the present study, TC and TN contents were not determined from the same leaves that were used to determine the initial carbon and nitrogen contents. This is because removing a portion of the leaf for sampling to measure carbon and nitrogen would affect both the quantity of leachate and the rate of leaching from the leaf in subsequent experiments. For example, the amount of water-soluble organic substances leached from needle leaf litter during one day increases from about 1 (%) to 10-12 (%) if leaves are cut beforehand \(^{26}\). Similarly, the initial carbon and nitrogen contents in the present study were not measured using the same leaves that were used for the TC and TN leaching experiments. Nonetheless, based on the data shown in Fig 6, it does not seem that the initial carbon and nitrogen contents of leaves do not influence the release rates of carbon and nitrogen from the leaves.

However, future studies should verify the leaching rates and the contents of carbon and nitrogen of the leaves indicated by the present study through further statistically significant measures considering the timing of leaf emergence, when the leaves were harvested (growing season and non-growing season). In addition, it is necessary to clear the chemical contents of soils in which plants have been growing, because materials absorbed from their roots tend to be accumulated in the plants, leaching when submerged.

### 4.3. Sugar-leaching characteristics of Q. glauca leaves

Although leachates from leaves contain a variety of chemicals, sugars are one of the major abundant forms of DOM leached from leaves. In Salix gracilistyla leaves, the total amount of sugar leached within 24 h after leaf immersion was accounted for approximately 40 to 70 (%) of the total amount of sugar that leached from the leaves after 14 days. In addition, peak levels of sugar in the leachate were observed after 5 to 10 days \(^{28}\). Similarly, 70 to 80 (%) of the sugars in leaves of white oak and hickory leaves are leached during the first two weeks of immersion \(^{26}\). Compared to these rapid sugar-leaching rates in deciduous leaves, the rates from the evergreen leaves of Quercus glauca were markedly slower and relatively little sugar leached within the first few days after immersion. Peak sugar concentrations in water containing either old or new leaves were observed after 10 and 30 days, respectively (Fig 4). These findings show that Quercus glauca leaves have a longer sugar-leaching period than Salix gracilistyla. In addition, Quercus glauca leaves leached sugars less rapidly than Salix gracilistyla leaves.

In Salix gracilistyla leaves, carbohydrates produced by photosynthesis are considered to be locked in the cuticles and epicuticular waxes of the leaves during the growth period. However, in the senescence period, carbohydrates in leaves are broken down into simple sugars, such as sucrose, and the biomembranes become permeable to soluble carbohydrates \(^{29}\). Contrary to this finding, the present results show that, in new and old leaves of Quercus glauca and initial nitrogen content against TN (b).

![Fig. 6. Comparison of initial carbon content against TC (a) and initial nitrogen content against TN (b).](image-url)
harvested during the growing period, more than 60 (%) of total sugar leached from the leaves by the 30 day after immersion in water was sucrose (Fig.5).

During the experimental period, the TC in each treatment increased to 50 (mg/l) within the first 5 days, as carbon was leached from the leaves of all samples 17. After 5 days, TC concentrations remained constant in non-aerated water and increased after day 10 in aerated water, regardless of whether the leaves were new or old. However, considerable differences in the amount of TC that had leached into the water were observed among treatments by the end of the experiment (old leaves with aeration, 71.2 (mg/l); old leaves without aeration, (51.6 mg/l); new leaves with aeration, 75.2 (mg/l); new leaves without aeration, 56.6 (mg/l)). The total sugar concentration/TC ratios that were measured over the course of the experiments are shown in Table 3. The table shows that after 30 days, the total sugar/TC ratios in the leachate ranged from 0.5 and 2.4 (%), and 17 and 46 (%), with and without aeration, respectively. These findings indicate that the ratio of total sugars to TC in Q. glauca leaves fluctuates depending on whether or not the water is aerated.

The major agents for removal of sugars from the water may be water temperature, microbial activity in the water, and oxygenation. In the present experiments, only very low concentrations of sugars were detected in each sample after 10 days of immersion under aerated conditions. The reason for this may be because the sucrose that leached from Q. glauca leaves may have been oxidized and converted into water and carbon dioxide (see reaction (1) below). Consequently, the aerated condition may not prevent sugars from leaching from the leaves, but rather because the sucrose in the leachate was oxidized by aeration. The reactions showing how water-soluble sucrose (disaccharide) was broken down into glucose and fructose (simple sugars) by hydrolysis is shown in equations (2) and (3) below. However, detailed analysis is required in order to better understand the release of sugar from Q. glauca leaves.

\[
\text{C}_{12}H_{22}O_{11} + 12O_2 \rightarrow 11H_2O + 12CO_2 \quad (1)
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + \text{C}_6\text{H}_2\text{O}_6 \quad (2)
\]

\[
\text{C}_6\text{H}_12\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{H}_2\text{O} + 6\text{CO}_2 \quad (3)
\]

As mentioned above, the rate of sugar release from Q. glauca during the first 5 days was relatively slow compared to rates of leaching in S. gracilistyla leaves. The time taken for total sugar concentrations to peak after starting the experiment was approximately 5 days in leaves of S. gracilistyla, and from 10 to 30 days in Q. glauca 26. In the deciduous leaves of the vine maple and alder, a rapid decrease in leaf mass occurs primarily by decomposition of the most labile constituents, including soluble carbohydrates (such as sugars), soluble protein, hemicellulose, and additional soluble organic matter 18, while the decrease in leaf mass proceeds more slowly in evergreen leaves. Differences in the release patterns of total sugar between deciduous and evergreen leaves might arise due to differences in leaf composition and structure. Sugars are derived from soluble carbohydrates that are lost through the cuticle of evergreen leaves; however, the relative thickness of the evergreen leaf cuticle reduces the rate of sugar release. The tough outer surfaces of evergreen leaves (e.g., cuticle) may also slow rate at which the mass of the leaf decreases 19, particularly until such time as the outer layer of the epidermis, which contains cutin, breaks down in water. The release rate of total sugars from leaf litter submerged in water and subsequent decomposition into water and carbon dioxide may be increased by abrasion of leaf surfaces.

5. CONCLUSIONS

The initial carbon content of evergreen Q. glauca leaves appears to be relatively higher than that of deciduous leaves because of the tough outer surfaces of evergreen leaves (e.g., cuticle). After leaves of Q. glauca enter streams in runoff, water soluble carbohydrates such as sugars, which consist of cutin, are leached over a relatively longer period than in deciduous leaves. Thus, the sugars in the tougher evergreen Q. glauca leaves take longer to leach than they do from the softer deciduous leaves. Similarly, the decrease in the mass of submerged Q. glauca leaves is slower than it is in the softer evergreen leaves of deciduous trees.

In Q. glauca, the amounts and rates of carbon and nitrogen components leached from the leaves are not influenced by the initial carbon and nitrogen contents of the leaves. This implies that more components are leached from leaves after leaf immersion during the initial leaching period of two weeks regardless of the initial carbon and nitrogen contents of the leaves. In this study, no significant difference was observed in the initial carbon content between new and old Q. glauca leaves, or in sun and shade leaves.

Although numerous studies have been conducted on submerged leaves in decouisous species to estimate the nitrogen dynamics in the leaves during leaching and decomposing period[10, 21, 20], few of these studies have considered the possible role of sunlight exposure of the leaves and how this impacts leaching. However, in Q. glauca leaves, the initial nitrogen content of the leaves appears to be influenced by the extent to which the leaves

<p>| Table 3. Ratios of total sugar concentrations and TC over time for different water treatment groups |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Submerged period (day)</th>
<th>Total sugar concentrations (ppm)</th>
<th>TC concentrations (ppm)</th>
<th>Total sugar / TC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>old leaves with aeration</td>
<td>0.03</td>
<td>3.36</td>
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<td>old leaves without aeration</td>
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<td>0.24</td>
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<td>new leaves with aeration</td>
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<tr>
<td>new leaves without aeration</td>
<td>2.20</td>
<td>2.21</td>
<td>1.75</td>
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were exposed to sunlight. Further experiments considering the potential contribution of abiotic factors at the growing sites should therefore be undertaken.

DOM is leached from any canopy leaves and leaf litter on the forest floor, as well as from leaves that have been transported to streams in runoff; all of these sources of DOM affect stream water quality. It is therefore that further experiments be undertaken to more accurately clarify the release and uptake of nutrients in leaching and decomposing process of leaves.

ACKNOWLEDGMENTS

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REFERENCES

ているが，常緑樹リーフリターの溶出特性については不明な点が多い，そこで本研究は，南九州河畔域に広く分布する常緑広葉樹のアラカシリーフリターの溶出初期における重量損失と炭素・窒素放出特性を考察した前年度研究に引き続き，溶出初期におけるアラカシリーフリターの炭素・窒素含有量と糖類溶出特性を明らかにすることを目的とした。

2010年5月，一ツ瀬川支流竹尾川下流（宮崎県西都市）の河畔域に生育するアラカシの当年葉（2010年春以降に形成された葉）と一年葉の陰葉と陽葉を採取し，十分な洗浄・乾燥と重量測定の後に，各グループの葉10枚を無作為に選定し，葉央部の主脈脇から2 mgの破片に含まれる炭素および窒素の含有量を測定した。この測定と並行して，1,000（CC）の現地渓流を満たした50個のビーカーに5枚ずつ投入してサブサンプルとし，温度調整をしない実験室に置いた。このうち25個のビーカーには市販ポンプにより酸素を供給し続けた。葉を投入してから1, 5, 10, 20, 30日後に，各実験区から無作為にサブサンプルを5個づつ回収し，葉の乾燥重量を計測し投入水に含まれる糖類濃度を測定した。

炭素含有量は45.75～46.86（％），窒素含有量は1.83～2.16（％）で，これらは落葉樹リーフリターでの既往報告値よりも高い数値であった。炭素含有量は葉のグループ間で有意な違いはなかったが，窒素含有量は一年生陰葉と他のグループとの間に有意な違いが認められた。葉の炭素および窒素含有量の多寡は，葉からの炭素および窒素溶出量に影響しないことが推測された。糖類については，スクロース，グルコース，フルクトースが検出され，全糖類の60%はスクロースであった。全糖類濃度は投入から30日後に最大値26（mg/l）を示し，アラカシリーフリターからの糖類溶出期間は，落葉樹の既往報告結果と比較して長いことが判明した。また，糖類の溶出とその後の分解は酸素供給により促進された。

アラカシリーフリターと落葉樹リターとの違いは，葉の構造と組成の違い，とりわけアラカシリーフリターの表面に厚く覆うクチン質に起因する可能性があると推論した。