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APPLICATION OF A QUINONE BIOMARKER FOR THE ANALYSIS OF HILLSLOPE RUNOFF

by

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SYNOPSIS

The applicability of a quinone biomarker for the analysis of hillslope runoff was investigated. First, quinone profiles of three streams as well as a hillslope runoff in a forested headwater catchment were compared. The quinone composition of hillslope runoff differed from others. Moreover, remarkable differences in quinone profile of hillslope runoff under different rainfall conditions were found. Then, the behavior of the quinone biomarker during the increase and decrease of hillslope runoff after a period of rainfall was examined. The fractional changes in Q-9 (H_2), Q-10 (H_2), Q-11, MK-6 and MK-10 indicated the effect of interflow.

INTRODUCTION

Hillslope runoff is often a topic of research in hydrological studies. Water qualities such as nitrate (1) and environmental isotopes (2) have helped as tracers to analyze hydrological processes in hillslopes. In these analyses, mass balance equations of water quantities and tracers were made between runoff at a spring and its components. The number of unknown parameters used in the both equations is larger than that of equations. Therefore, various hypotheses are introduced to reduce the number of the unknown parameters and then to solve the equations. However, sometimes these hypotheses do not agree with the actual phenomena (3). Hence, it is probable that the methodology for the analysis of hillslope runoff has not been established yet. In order to develop a more advanced methodology, it is necessary to develop a new tracer that can reflect the underground environment in hillslope and has much information that is able to make the hypothesis reduced.

In this study, we focused on a quinone biomarker (4) as a new tracer to analyze hillslope runoff. The following matters were examined to investigate its applicability:

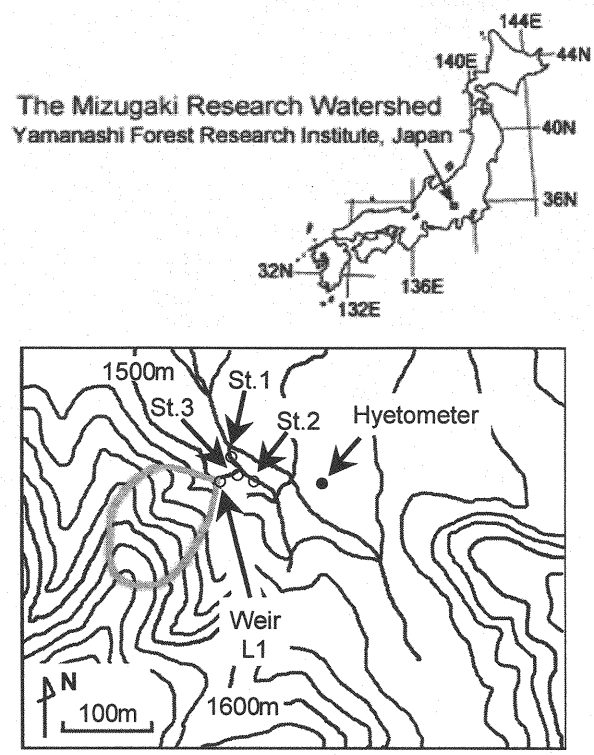

Fig. 1 Experimental catchment

1) Quinone profiles of three streams as well as a hillslope runoff in a forested headwater catchment were compared. The differences in quinone profile of the hillslope runoff under different rainfall conditions were evaluated quantitatively.
2) The behavior of each quinone species was examined during the increase and decrease of hillslope runoff after a period of rainfall.

MATERIALS AND METHODS

Experimental catchment

Field observations were performed at The Mizugaki Research Watershed located in northern parts of Yamanashi prefecture, Japan (Fig. 1).

Observation and sampling

Precipitation was monitored continuously by a hyetometer. Water levels were measured every 5min automatically by a water gage installed into a downstream weir (L1) from a spring. Then, the amount of runoff was estimated from the monitored water levels.

In order to achieve the above objective, water samples of around 20L were taken from four

sampling points on June 11th and 25th, July 2nd and August 6th, 2003. They were the weir (L1), a down stream from wetland (St.2), that from L1 (St.3) and a confluence of both the streams (St.1). Furthermore, the run off caused by a rainfall of 73.4mm from August 14th to 16th was examined. Then, water samples were taken from near the spring on August 15th, 17th and 23rd, 2003. The runoff on August 15th was included in the increase part of the hydrograph. On the other hand, that on August 23rd was in the decrease part. August 17th was just after the top peak.

Quinone profile method

Quinone biomarker

Quinone is a coenzyme used as proton carrier in electric transport chain of bacteria (4). Quinone structure is divided into four components: ubiquinone (Q-n (H_x)) which is used at aerobic and anoxic respiration, menaquinone (MK-n (H_x)) at anaerobic respiration, plastquinone (PQ-n) and vitamin K1 (VK1) at photosynthesis, where n and Hx represent the length of the isoprene unit of the side chain and the number of hydrogen atoms saturating the double bonds of the isoprene unit, respectively. Basically, a bacterium has a predominant quinone species, which is stable even though environmental conditions change. Moreover, the quinone content corresponds to that of biomass. Quinone can be analyzed quantitatively by using only chemical method without knowledge of microbiology. Therefore, it can be applied as biomarker to complex microbial community such as activated sludge (5) and soil (6).

Quinone analysis

The weight of water sample was measured and then the water sample was filtrated by means of a glass fiver filter with 0.3μm of pore size (GF-75, ADVANTEC). In order to extract lipid including quinone from the filtration residue, a chloroform-methanol mixture (2:1, v/v) and n-hexane were used in turn. Thereafter, the crude quinone extract in n-hexane was made concentrated by Sep-Pak Plus Silica Cartridge (WATERS) and separated to MK and Q with 2 % and 10 % diethylether-hexane, respectively. Quinone species were analyzed by high performance liquid chromatography and then identified by the spectrum and the equivalent number of isoprene unit (4) calculated from their retention time. The molar concentration of quinone species was estimated from the water sample volume converted from its weight. Furthermore, quinone profile defined as the molar fraction of each quinone species was also determined.

Dissimilarity index

In order to investigate the difference in quinone profile of two water samples quantitatively, dissimilarity index value (D-value) was calculated according to the Eq. (1).

$$D(i,j) = 0.5\sum_{k=1}^{m}\left|x_{i,k} - x_{j,k}\right| \qquad (1)$$

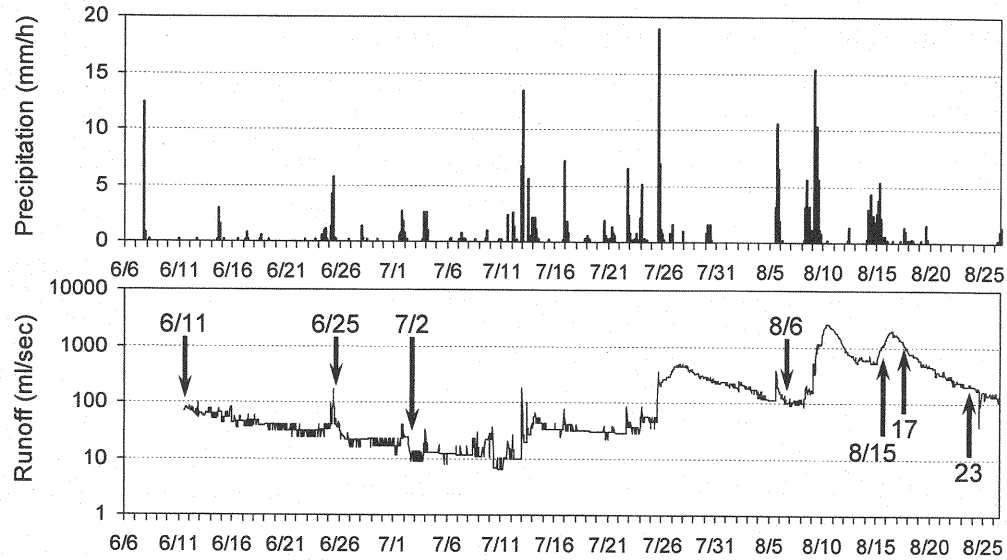

Fig. 2 Precipitation and hillslope runoff

where m is the number of quinone species and $x_{i,k}$ and $x_{j,k}$ are the molar fractions of the k quinone species for the i and j samples, respectively. D-value is in the range of 0 to 1. A value less than 0.1 indicates that microbial communities of two samples are similar. On the other hand, more than 0.2 means that both are significantly different.

RESULTS AND DISCUSSION

Precipitation and hillslope runoff

The precipitation observed from June 6th to August 26th is shown in Fig. 2. In addition, the runoff at L1 is also shown in the same figure. The hillslope runoff on June 11th was larger than those on June 25th and July 2nd. In spite of this, it is likely that the runoff on June 11th was dominated by base flow because 90hrs had passed from the last rainfall event. Conversely, direct flow would be predominant in those on June 25th, July 2nd and August 6th since only 3, 12 and 10hrs had passed, respectively. However, the precipitation of the last period of rainfall before July 2nd was only 10.8mm and the hillslope runoff was relatively low. For these reasons, it is possible that the runoff on July 2nd was regarded as base flow. On the other hand, the runoff on August 6th was relatively large. That is why the hydrological processes in hillslope on June 25th, July 2nd and August 6th were probably different.

The runoff on August 15th and 17th was around 10 times as large as that on June 11th. Although 80hrs had passed from the last rainfall event, the runoff on August 23rd was around twice as large as that on June 11th.

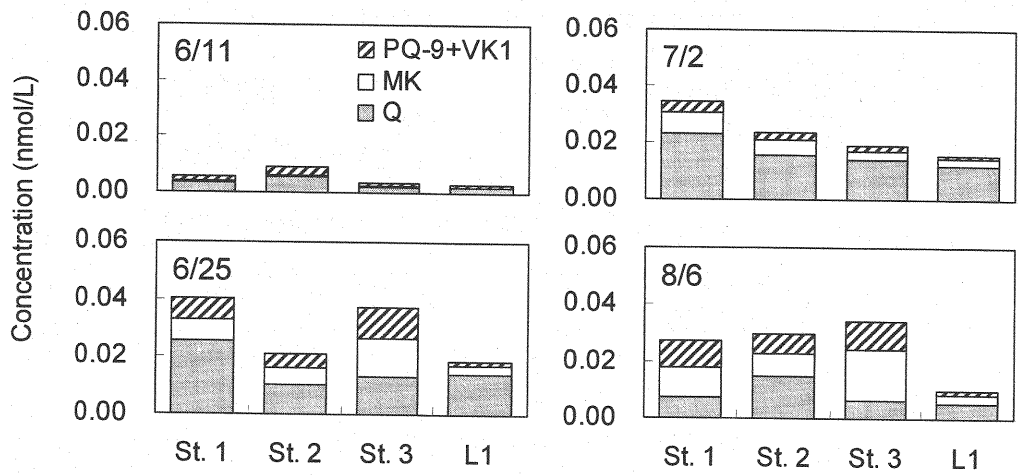

Fig.3 Quinone concentration of the runoff and streams

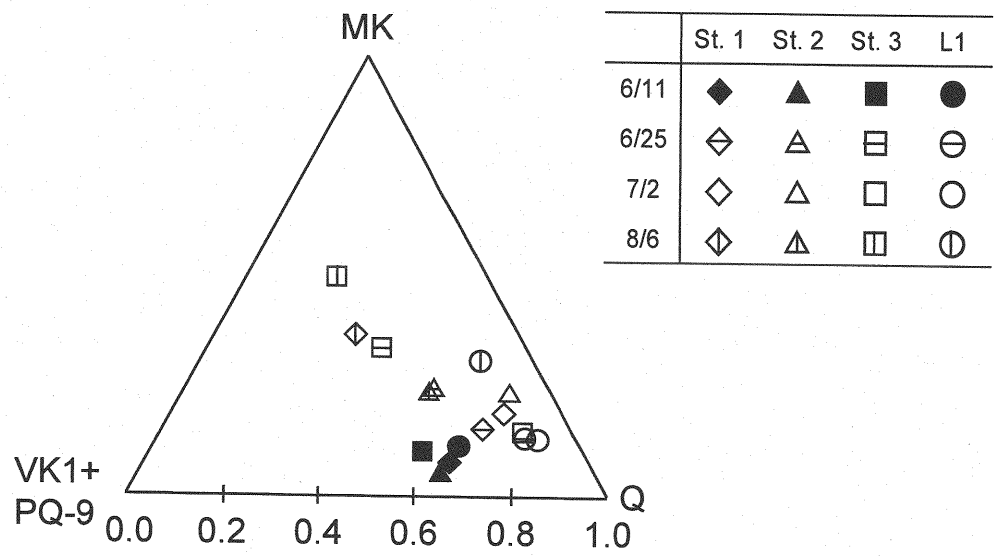

Fig. 4 Triangle diagram based on Q, MK and PQ-9+VK1

Quinone profile of the runoff and streams around the headwater catchment

Fig. 3 shows the quinone concentration of the runoff and streams on June 11th, 25th, July 2nd and August 6th. Q, MK, PQ and VK1 were detected on every sampling date. The quinone concentration on June 25th, July 2nd and August 6th was higher than that on June 11th. That is to say, the runoff dominated by direct flow contained more bacteria than that by base flow. We surmised that bacteria ran off from the wetland that existed in the upstream at St.2 and St.3. On the other hand, it seems that the infiltrating rainwater would run off though other pathway in the hillslope where the base flow had not passed at L1. Consequently, we inferred that bacteria that existed in the pathway ran off.

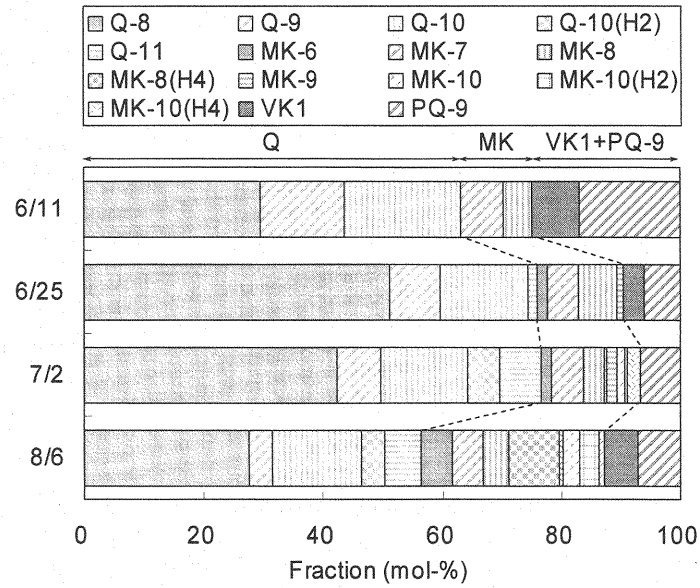

Fig. 5 Quinone profile of the hillslope runoff

Table 1 Dissimilarity index

	6/11	6/25	7/2	8/6
6/11				
6/25	27.9			
7/2	32.9	16.4		
8/6	31.4	30.6	23.3	

The composition of Q, MK and PQ+VK1 is shown as a triangle diagram in Fig. 4. Four marks representing the quinone composition of different sampling points on June 11th were almost converged. July 2nd also showed the same tendency. On the other hand, the marks of samples on June 25th and August 6th were scattered due to their high MK composition at St.2 and St.3. The runoff discharged from the wetland probably included MK-containing bacteria. St.1 did not indicate the middle composition between St.2 and St.3. Other runoff might have flowed into St.1. Different rainfall conditions brought about various quinone compositions.

Then, the molar fractions of Q, MK, PQ and VK1 species at L1 were examined (Fig. 5). On every sampling date, Q was found as the major fraction of quinone species. On June 11th, five quinone species were detected, which was three Q, two MK and two PQ+VK1. The quinone profile indicated that Q-8 was present as most predominant, Q-10 was the second and Q-9 was the third, and that the most predominant MK was MK-7 and MK-8 was second. On another sampling date, in addition to the above three Q species, Q-10 (H_2) and Q-11 were also detected, although the

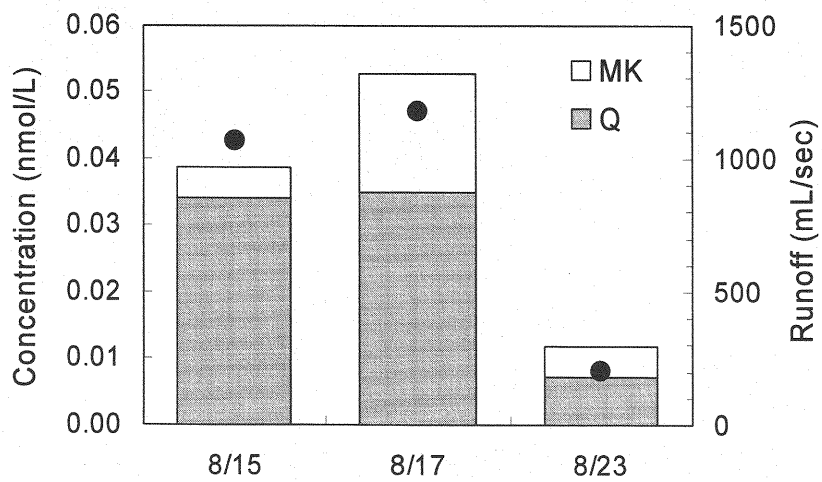

Fig. 6 The Change of quinone concentration during the increase and decrease of hillslope runoff

order of major three Q did not change. On the other hand, in addition to the above two MK species, MK-6 and MK-9 were also detected on June 25th. Furthermore, MK-8 (H_4), MK-10, MK-10 (H_2) and MK-10 (H_4) were also detected on July 2nd and August 6th. The order of major two MK on July 2nd was the same as on June 11th. However, June 25th and August 6th showed MK-8>MK-7 and MK-8 (H_4)>MK-7, respectively. Different rainfall conditions caused different bacteria to run off.

In order to evaluate these differences in quinone profile, D-value was calculated (Table 1). All D-values were more than 10%. It is concluded that the microbial community of every sample was significantly different (4). In particular, June 25th and July 2nd had 32.9% of the highest value. Namely, their runoff pathway in the hillslope was significantly different in spite of the fact both the runoff characteristics were similar.

Behavior of quinone biomarker during the increase and decrease of hillslope runoff

In this work, the temporal change in quinone species was examined during the increase and decrease of hillslope runoff after a rainfall event. Quinone concentration of August 15th, 17th and 23rd is shown in Fig. 6. Since quinone profile of runoff was affected by surface conditions as mentioned above, water samples were taken near the spring. As a result, only Q and MK were detected. The change in quinone concentration corresponded to that in the hillslope runoff.

Fig. 7 shows the fractional change in detected twelve quinone species. In order to discuss the relationship between quinone species and interflow, Q-9 (H_2), Q-10 (H_2), Q-11, MK-6, MK-9, MK-10 and MK-10 (H_4) we focused on the twelve quinone species, because the other five quinone species, that were detected on June 11th when base flow dominated, could not obviously become the indices for interflow. The fraction of Q-10 (H_2) and Q-11, which were major two Q on August 15th as shown in Fig. 2, decreased as the hillslope runoff increased. They could increase from the

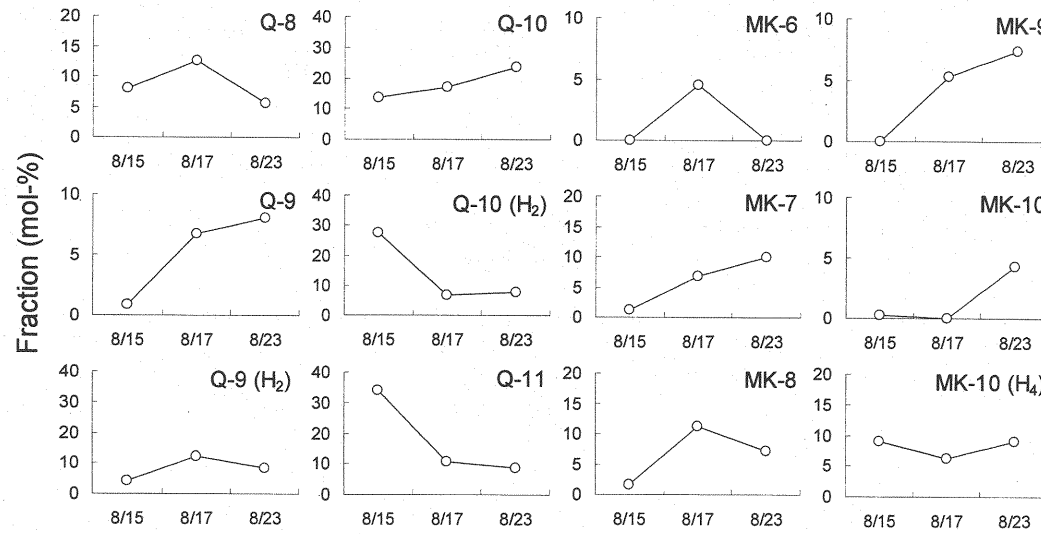

Fig. 7 The behavior of quinone species

initial rising stage of the runoff before August 15th because they have never been major species at the above four sampling date. In other words, they corresponded to early interflow. On the other hand, there were two quinone species that increased as the hillslope runoff increased. One is Q-9 (H_2), which corresponded to decreasing part of the runoff. The other is MK-6, which was detected for the first time on August 17th. Both species could reflect interflow. Furthermore, MK-10 that had higher fraction compared with on August 15th and 17th could correspond to late interflow. While the above quinone species showed a close correlation with the change of hillslope runoff, the following quinone species also existed. MK-10 (H_4) showed the opposite trends with the change of runoff. MK-9 made its fraction increase during the observation period. Therefore, it was difficult to explain the relationship between them and the runoff components.

As mentioned above, quinone species detected from runoff differed in accordance with rainfall conditions. It is probable that different rainfall conditions brought different runoff pathway. Hence, the above quinone species that have good correspondence with the runoff components are not nessesarily unique. Further investigations of the quinone profile of hillslope runoff and soil simultaneously are important in the future.

CONCLUSIONS

Quinone profile of hillslope runoff was obviously different from that of streams in the headwater catchment. In addition, findings showed significant differences under different rainfall conditions. In other words, it is possible that quinone profile of hillslope runoff reflects runoff pathway. Then, the behavior of quinone species was examined during the increase and decrease of hillslope runoff after a rainfall event. Consequently, the fractional changes in Q-9 (H_2), Q-10 (H_2), Q-11, MK-6 and MK-10 are evidence that they reflect the effect of interflow.

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