Evaluation of hydrological processes in a mountainous small basin using a quinone biomarker

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Abstract An applicability of quinone biomarker to the analysis of hillslope runoff was investigated. At 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, there were remarkable differences in quinone profile of hillslope runoff under different rainfall conditions. Then, the behavior of quinone biomarker during the increase and decrease of hillslope runoff after a rainfall event was examined. The fractional changes in Q-9 (H2), Q-10 (H2), Q-11, MK-6 and MK-10 suggested the effect of interflow.

Keywords Biomarker; hillslope; interflow; quinone; rainfall-runoff

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Introduction

It is recognized that rainfall events cause pollutants such as organic matters and nitrogen compounds to run off from the mountainous basin. In order to understand the runoff mechanism of pollutants, it is fundamentally necessary to elucidate the hydrological processes, especially the identification of the runoff pathway in the hillslope.

Hillslope runoff is one of the classical topics in hydrological studies. Water qualities such as nitrate (Takeuchi *et al.*, 1984) and environmental isotopes (Sklash, 1990) have helped as tracers to analyze hydrological processes in the hillslope. 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 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 (McDonnel, 1990). Hence, it is likely to say that the methodology for the analysis of hillslope runoff has not been established yet. In order to develop the advanced methodology, it would be necessary to focus on the new tracer that reflects the underground environment in the hillslope and has much information that is able to make the hypothesis reduced.

In this study, we focused on quinone biomarker (Hiraishi *et al.*, 1989) as a new tracer to analyze hillslope runoff. The following were examined to investigate its applicability.

(1) Quinone profiles of three streams as well as a hillslope runoff in a forested headwater catchment were compared. Especially, 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 rainfall event.

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Materials and methods

Experimental catchment

Field observation was performed at a forested headwater catchment of approximately 0.65 ha located in the northern parts of Yamanashi prefecture, Japan (Figure 1).

Observation and sampling

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

In order to achieve the above objective 1), water samples of around 20 L were taken from four sampling points on June 11 and 25, July 2 and August 6, 2003. They were the weir (L1), down stream from a wetland (St.2), that from L1 (St.3) and a confluence of both the streams (St.1). To achieve the objective 2), the run off caused by a rainfall of 73.4 mm from August 14 to 16 was focused on. Then, water samples were taken from near the spring on August 15, 17 and 23, 2003. The runoff on August 15 consisted in the increase part of the hydrograph. On the other hand, that on August 23 was in the decrease part. August 17 was just after the top peak.

Quinone profile method

Quinone biomarker. Quinone is a coenzyme employed as proton carrier in electric transport chain of bacteria (Hiraishi *et al.*, 1989). Quinone structure is divided into four components: ubiquinone (Q-n (Hx)) which is used in aerobic and anoxic respiration, menaquinone (MK-n (Hx)) in anaerobic respiration, plastoquinone (PQ-n) and vitamin K1 (VK1) in photosynthesis, where n and Hx represent the length of the isoprene unit of the side chain and the number of hydrogens 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, quinone content corresponds to that of biomass. Quinone can be analyzed quantitatively by using only chemical methods without knowledge of microbiology. Therefore, it has been applied as a biomarker to complex microbial communities such as activated sludge (Furumai *et al.*, 2001) and soil (Fujie *et al.*, 1998).

Quinone analysis. The weight of the water sample was measured and then the water sample was filtrated using a glass fibre filter with 0.3 μm of pore size (GF-75,

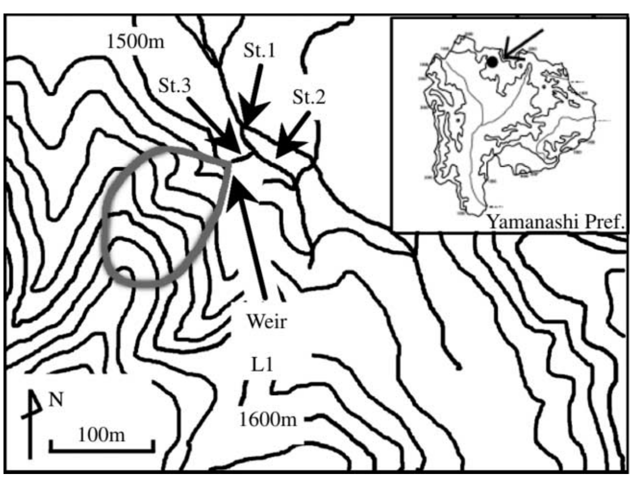

Figure 1 Experimental catchment

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 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 units (Hiraishi *et al.*, 1989) calculated from their retention time. The molar concentration of quinone species was estimated from the water sample volume converted from its weight. Furthermore, the 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 Equation 1.

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

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 contrary, more than 0.2 means that both are significantly different.

Results and discussion

Precipitation and hillslope runoff

The precipitation observed from June 6 to August 26 is shown in Figure 2. In addition, the runoff at L1 is also shown in the same figure. The hillslope runoff on June 11 was larger than those on June 25 and July 2. In spite of this, it is likely that the runoff on June 11 was dominated by base flow because 90 hrs had passed from the last rainfall event. Conversely, direct flow would be predominant in those on June 25, July 2 and August 6 since only 3, 12 and 10 hrs had passed, respectively. However, the precipitation of the last rainfall event before July 2 was only 10.8 mm and the hillslope runoff was relatively low. For this reason, it is possible that the runoff on July 2 was regarded as base flow.

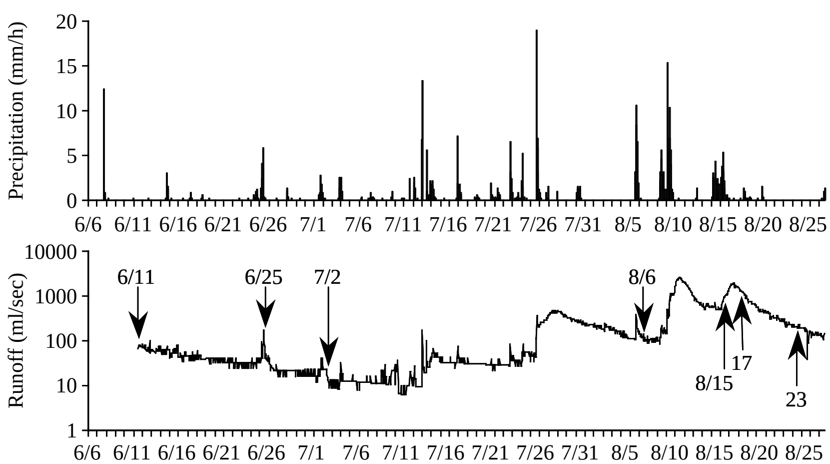

Figure 2 Precipitation and hillslope runoff

On the other hand, that on August 6 received the rainfall event under the condition that the level of original runoff was relatively high. That is why the hydrological processes in hillslope on June 25, July 2 and August 6 were probably different.

The runoff after August 14 occurred following the rainfall event of 96.2 mm on August 8 and 9. The runoff on August 15 and 17 was around 10 times as large as that on June 11. Although 80 h had passed from the last rainfall event, the runoff on August 23 was around twice as large as that on June 11.

Quinone profile of the runoff and streams around the headwater catchment

Figure 3 shows quinone concentration of the runoff and streams on June 11, 25, July 2 and August 6. Q, MK, PQ and VK1 were detected on every sampling date. The quinone concentration on June 25, July 2 and August 6 was higher than that on June 11. That is to say, the runoff dominated by direct flow contained more bacteria than that by base flow. It is suggested 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 through other pathways in the hillslope where the base flow had not passed at L1. Consequently, it is inferred that bacteria that existed in the pathway ran off.

The composition of Q, MK and PQ + VK1 is shown as a triangular diagram in Figure 4. Four marks representing quinone composition of different sampling points on

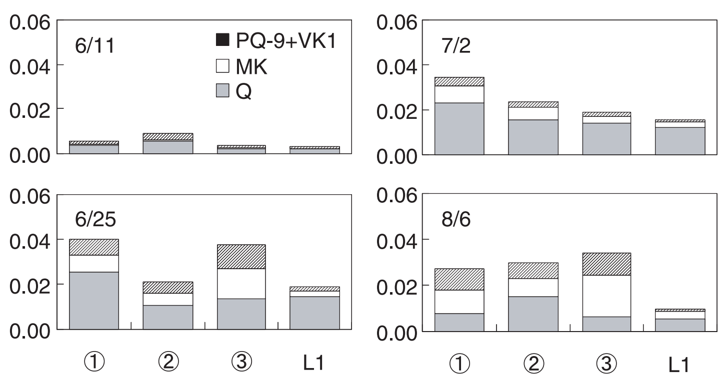

Figure 3 Quinone concentration of the runoff and streams

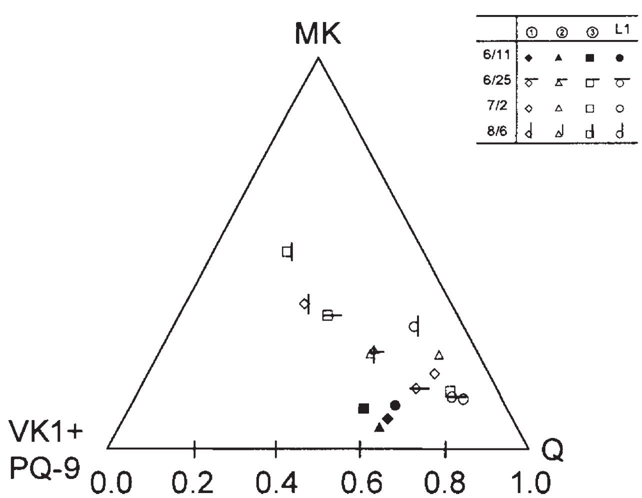

Figure 4 Triangle diagram based on Q, MK and PQ-9 + VK1

June 11 were almost converged. July 2 also showed the same tendency. On the other hand, the marks of samples on June 25 and August 6 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 besides these might have flowed into St.1. Different rainfall conditions brought various quinone compositions.

Then, the molar fractions of Q, MK, PQ and VK1 species at L1 were examined (Figure 5). In every sampling date, Q was found as the major fraction of quinone species. On June 11, five quinone species were detected, which were three Q, two MK and two PQ + VK1. The quinone profile indicated that Q-8 was present as the most predominant, Q-10 was the second and Q-9 was the third, and that the most predominant MK was MK-7; MK-8 was second. On other sampling dates, in addition to the above three Q species, Q-10 (H2) and Q-11 were also detected, although the order of the major three Qs was not changed. On the other hand, in addition to the above two MK species, MK-6 and MK-9 were also detected on June 25. Furthermore, MK-8 (H4), MK-10, MK-10 (H2) and MK-10 (H4) were also detected on July 2 and August 6. The order of the major two MKs on July 2 was the same as on June 11. However, June 25 and August 6 showed MK-8 > MK-7 and MK-8 (H4) > MK-7, respectively. Different rainfall conditions caused different bacteria to run off.

In order to evaluate these differences in quinone profile, the D-value was calculated (Table 1). All D-values were more than 10%. It is interpreted that the microbial community of every sample was significantly different (Hiraishi *et al.*, 1989). In particular, June 25 and July 2 had 32.9% of the highest value. Namely, their runoff pathway in the

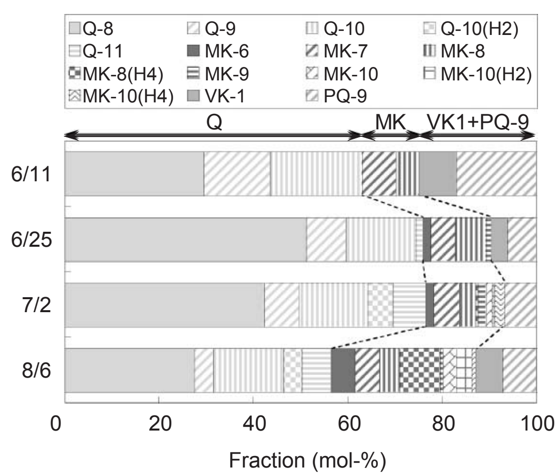

Figure 5 Quinone profile of the hillslope runoff

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	

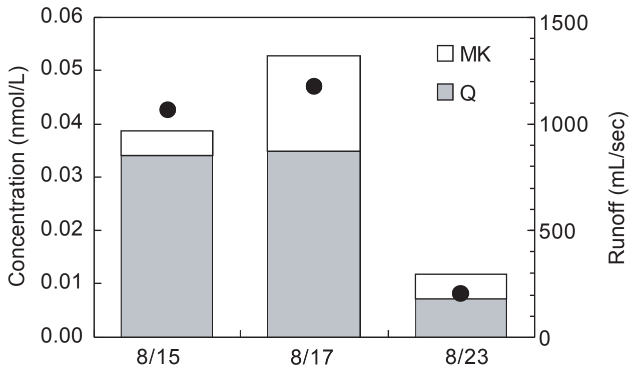

Figure 6 Change in quinone concentration during the increase and decrease of hillslope runoff

hillslope would be significantly different although both the runoff characteristics were similar.

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

Here, the temporal change in quinone species was examined during the increase and decrease of hillslope runoff after a rainfall event. Quinone concentration of August 15, 17 and 23 is shown in Figure 6. Since the 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.

Figure 7 shows the fractional change in twelve detected quinone species. In order to discuss the relationship between quinone species and interflow, Q-9 (H2), Q-10 (H2), Q-11, MK-6, MK-9, MK-10 and MK-10 (H4) were focused on among the twelve quinone species, because the other five quinone species, that were detected on June 11 when base flow dominated, cannot obviously become the indices for interflow. The fraction of Q-10 (H2) and Q-11, which were the major two Q on August 15 as shown in Figure 2, decreased as the hillslope runoff increased. They could increase from the initial rising stage of the runoff before August 15 because they have never been major species at the above four sampling dates. In other words, they could be corresponding to early interflow. On the other hand, there were two quinone species that increased as the hillslope runoff increased. One is Q-9 (H2), which corresponded to decreasing part of the runoff. The other is MK-6, which was detected for the first time on August 17. Both species could reflect interflow. Furthermore, MK-10 that had a higher fraction compared with on August 15 and 17 could correspond to late interflow. While the above quinone species showed good correlation with the change in hillslope runoff, the following quinone species also existed. MK-10 (H4) showed the opposite trends with the change in 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 seems that different rainfall conditions brought a different runoff pathway. Hence, the above quinone species that has good correspondence with the runoff components would not be always unique. It will be important to investigate the quinone profile of hillslope runoff and soil simultaneously in future.

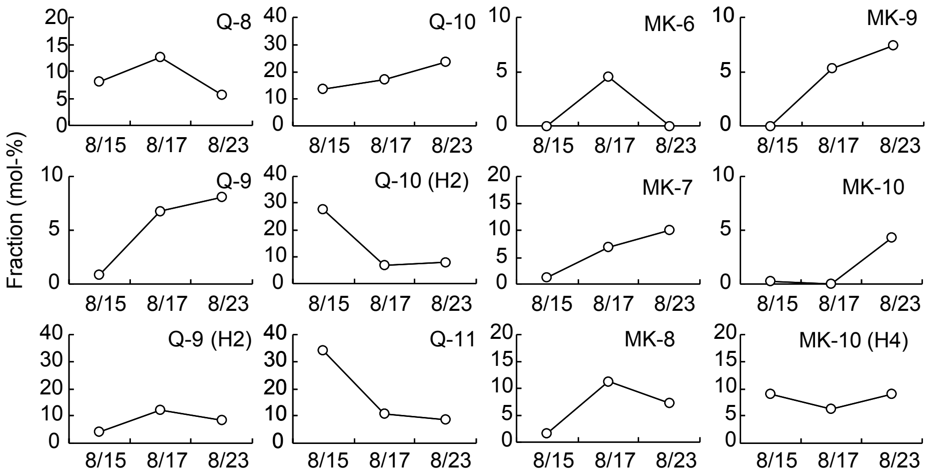

Figure 7 The behavior of quinone species

Conclusions

The quinone profile of hillslope runoff was different from that of streams in the headwater catchment. It showed significant differences under different rainfall conditions. Therefore, the behavior of quinone species was examined during the increase and decrease of hillslope runoff after a rainfall event. As a result, the fractional changes in Q-9 (H2), Q-10 (H2), Q-11, MK-6 and MK-10 suggested the effect of interflow.

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