

## Record

## Effect of storing stream water samples at low temperature on microbial functional diversity analysis by Biolog EcoPlate™\*1

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Taiki Mori, Kei Suzuki, Hiromi Yamagawa: **Effect of storing stream water samples at low temperature on microbial functional diversity analysis by Biolog EcoPlate™**. *Kyushu J. For. Res.* 76 : 133 – 134, 2023 Biolog EcoPlate™ is widely used for determining metabolic diversity of heterotrophic microorganisms present in the environmental samples including stream water. Stream water samples are sometimes stored at low temperature before the Biolog EcoPlate™ analysis, but the cold storage of the samples may alter the microbial activity during the assay. Here, we tested the effect of storing stream water samples at low temperature on microbial functional diversity analysis by Biolog EcoPlate™, using stream water samples. Contrary to our initial hypothesis, we did not observe statistical differences in average well color development (AWCD) between water sample measured at the sampling day and those stored at low temperature (4°C) for 3 days. However, the lack of statistical significance does not justify the cold storage, and further tests are required to justify the cold storage of water samples before Biolog EcoPlate™ assay.

**Key words:** microbial functional diversity

### I. Introduction

Biolog EcoPlate™ is widely used for determining metabolic diversity of heterotrophic microorganisms present in the environmental samples (Tiquia, 2010; Schafer *et al.* 2012). Biolog EcoPlate™ is composed of three sets of 31 different carbon substrates and a control (in total 96 wells) with tetrazolium dye in each well, which turns violet when the substrate is respired by microbial communities in the well. This method can provide a multifunctional index of microbial community in natural environment including streams (Nakamura *et al.* 2022).

Stream water samples are sometimes stored at low temperature before the Biolog EcoPlate™ analysis (Zhao *et al.* 2021). However, the cold storage of the samples may alter the microbial activity, affecting the results. For example, it was demonstrated that cold storage and subsequent laboratory incubation of soils taken from tropical forests substantially suppressed nitrogen mineralization, by altering soil microbial activity (Arnold *et al.* 2008). Since microbial functional diversity analysis by Biolog EcoPlate™ is based on microbial heterotrophic respiration, the results may also be impacted by cold storage. Here, we tested the effect of storing stream water samples at low temperature on microbial functional diversity analysis by Biolog EcoPlate™, using stream water samples. We hypothesized that cold storage negatively influences microbial activity, affecting the analysis of Biolog EcoPlate™.

### II. Materials and Methods

#### Water sampling

Water samples were collected from four streams located in

Kumamoto Prefecture (Site 1; N 32.818996, E 130.908235, Site 2; N 32.774364, E 130.938539, Site 3; N 32.632906, E 130.783309, and Site 4; N 32.678521, E 130.638877). Water temperature and dissolved oxygen (DO) were measured at the time of sampling (Table 1). We also measured pH and electrical conductivity (EC) with a pH/ION meter (HM-42 X, DKK-TOA CORPORATION, Japan) and an EC meter (CM-41 X, DKK-TOA CORPORATION, Japan) using the samples stored at 4°C (Table 1).

#### Ecoplate assays

Ecoplates were incubated for 5 days at 25°C under the dark condition after inoculated with 100 μL of the stream water samples at the sampling day and those stored at low temperature (4°C) for 3 days (n = 3 for each sample). Optical density (OD) at 590 nm were measured immediately after the end of the incubation using the microplate reader (MPR-A 100, ASONE, Osaka, Japan). We calculated the average well color development (AWCD) as follows:

$$AWCD = \sum (OD_i - c) / 31$$

, where  $OD_i$  indicates the OD value measured at wavelength of 590 nm in substrate  $i$  in Ecoplates, and  $c$  indicates OD values of control well.

#### Statistics

Linear mixed effects model with treatment (cold-storage vs control) as a fixed effect and site as a random effect was used to test the statistical significance of the effect of cold storage on AWCD. Non-metric multidimensional scaling (NMDS) was used to visualize the distribution pattern of the microbial metabolic

\*1 森大喜, 鈴木圭, 山川博美: 渓流水サンプルの低温保存がバイオログエコプレートによる微生物機能の多様性解析に及ぼす影響.

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profiles obtained by Biolog EcoPlate™. Statistical analyzes were performed using R ver. 4.1.1. (The R Foundation for Statistical Computing, Vienna, Austria) with *lme4*, *lmerTest*, and *vegan* package (R Core Team, 2021).

Table 1.

site	temperature °C	DO mg L-1	pH	EC mS m <sup>-1</sup>
1	17.6	9.17	7.43	8.63
2	14.4	9.10	7.62	8.47
3	16.3	9.13	7.18	30.0
4	17.4	9.27	7.21	11.38

### III. Results and Discussion

Contrary to our initial hypothesis, we did not observe statistical differences in average well color development (AWCD) between water sample measured at the sampling day and those stored at low temperature (4°C) for 3 days (Fig. 1). This was partly due to high variability of the microbial metabolic profiles (Fig. 1), which were substantially different within samples (Fig. 2). Thus, the present result did not show the impact of cold storage of stream water samples on microbial functional diversity analysis by Biolog EcoPlate™. However, the lack of statistical significance does not justify the cold storage. Further tests, using more samples with larger number of replicates, are required to justify the cold storage of water samples before Biolog EcoPlate™ assay.

### References

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 (2022年11月12日受付；2023年1月5日受理)

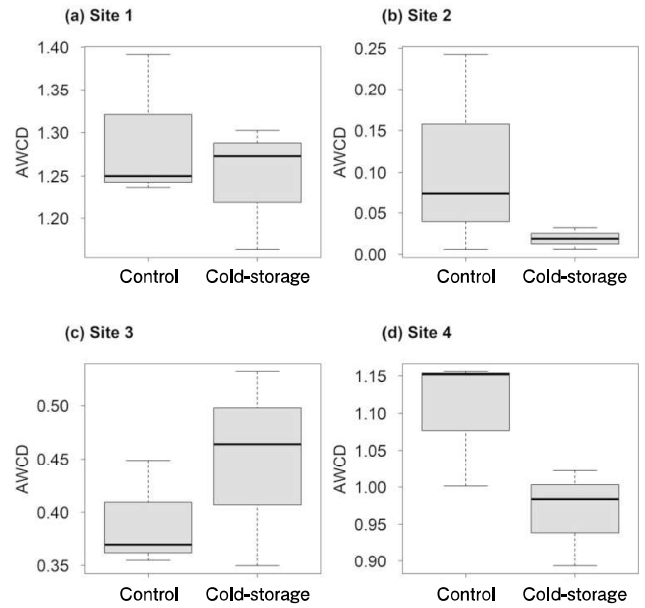


Fig. 1. Effects of storing stream water samples at low temperature on average well color development (AWCD). No statistical significance was obtained using mixed linear effect analysis ( $P > 0.05$ ).

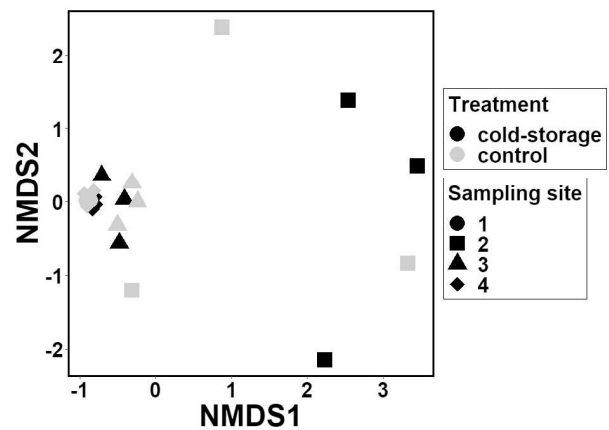


Fig. 2. Non-metric multidimensional scaling (NMDS) ordination plots according to 31 substrates of Biolog EcoPlate™ after 5 day-incubation. Water samples at the sampling day (control, black-colored) and those stored at low temperature (4°C) for 3 days (cold-storage, gray-colored) were assayed.