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### Data Article

# Data of coenzyme activities in throughfall and rainfall samples taken at five subtropical forests in southern China



Taiki Mori <sup>a, b</sup>, Senhao Wang <sup>a, c</sup>, Wei Zhang <sup>a, \*</sup>, Jiangming Mo <sup>a</sup>

<sup>a</sup> Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden and Guangdong Provincial Key Laboratory of Applied Botany, Chinese Academy of Sciences, Guangzhou, 510650, China

<sup>b</sup> Department of Forest Site Environment, Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki, 305-8687, Japan

<sup>c</sup> University of Chinese Academy of Sciences, Beijing, 100049, China

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#### ABSTRACT

The data presented in this article are referred to the research article "A potential source of soil coenzymes: From the phyllosphere to soil via throughfall" (Mori et al., 2019). The data included the activities of  $\beta$ -1,4-glucosidase (BG, EC 3.2.1.21),  $\beta$ -D-cellobiosidase (CBH, EC 3.2.1.91),  $\beta$ -1,4-N-acetyl-glucosaminidase (NAG, EC 3.2.1.52), leucine amino peptidase (LAP, EC 3.4.11.1), polyphenol oxidase (PPO, EC 1.10.3.2), and phosphomonoesterase (PME, EC 3.1.3.2). The informatin of study sites and sampling method are shown in Fig. 1 and 2.

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\* Corresponding author. South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Guangzhou, 510650, China.

E-mail address: [zhangwei@scbg.ac.cn](mailto:zhangwei@scbg.ac.cn) (W. Zhang).

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## Specifications Table

|                            |   |
|----------------------------|---|
| Subject area               | Ecology   |
| More specific subject area | Ecosystem Ecology   |
| Type of data               | Table and csv file  |
| How data was acquired      | Fluorescence enzyme assay and spectrophotometric assay.   |
| Data format                | Raw, analyzed   |
| Experimental factors       | Throughfall and rainfall samples were taken in five types of subtropical forests located in southern China. The samples were taken after rainfall events as soon as possible.   |
| Experimental features      | A portion of the collected samples was incubated with artificial substrates. After the incubation, we determined the changes in the substrates, which represent the enzyme activity of the collected samples.   |
| Data source location       | The studied area is situated within Dinghushan Biosphere Reserve (DHS) (112° 10' E, 23° 10' N), and Heshan National Field Research Station (HS) (112° 50' E, 22° 34' N) in Guangdong province, China. Data are available in this article.   |
| Data accessibility         |   |
| Related research article   | Taiki Mori, Senhao Wang, Wei Zhang, and Jiangming Mo 2019. A potential source of soil ecoenzymes: From the phyllosphere to soil via throughfall. <i>Applied Soil Ecology</i> . <a href="https://doi.org/10.1016/j.apsoil.2019.02.004">https://doi.org/10.1016/j.apsoil.2019.02.004</a> [1]. |

**Value of the data**

- This data, for the first time, provide information of ecoenzymes transferred from forest canopy into soils via throughfall.
- This data will be available for future works comparing the amount of ecoenzymes in throughfall among different types of forests in different climate zone.
- The present data can be reused in future works looking for the contribution of ecoenzyme in throughfall to soil enzyme activity.

**1. Data**

The six types of ecoenzymes, i.e.,  $\beta$ -1,4-glucosidase (BG, EC 3.2.1.21),  $\beta$ -D-cellobiosidase (CBH, EC 3.2.1.91),  $\beta$ -1,4-N-acetyl-glucosaminidase (NAG, EC 3.2.1.52), leucine amino peptidase (LAP, EC 3.4.11.1), polyphenol oxidase (PPO, EC 1.10.3.2), and phosphomonoesterase (PME, EC 3.1.3.2) are shown in Table 1. Supplementary csv file provides the raw data of the enzyme activities. The activities of BG, CBH, NAG, LAP, and PME were measured by fluorescence enzyme assays. The activity of PPO was determined by spectrophotometric assay.

**2. Experimental design, materials, and methods****2.1. Study site**

This study was conducted in five subtropical forests in two research stations, i.e., Dinghushan Biosphere Reserve (DHS) (112°10'E, 23°10'N) [2] and Heshan National Field Research Station (HS)

**Table 1**

The enzyme activity of throughfall and rainfall in five subtropical forests in southern China.

| TF/RF | site | Forest type | BG ( $\mu\text{mol MUB h}^{-1} \text{L}^{-1}$ ) | CBH ( $\mu\text{mol MUB h}^{-1} \text{L}^{-1}$ ) | LAP ( $\mu\text{mol MUC h}^{-1} \text{L}^{-1}$ ) | NAG ( $\mu\text{mol MUB h}^{-1} \text{L}^{-1}$ ) | PME ( $\mu\text{mol MUB h}^{-1} \text{L}^{-1}$ ) | PPO ( $\mu\text{mol DOPA h}^{-1} \text{L}^{-1}$ ) |
|-------|------|-------------|---|--|--|--|--|---|
|       |      |             | Average SE                                      | Average SE                                       | Average SE                                       | Average SE                                       | Average SE                                       | Average SE  |
| TF    | HS   | AA          | 2.316 0.385                                     | 0.228 0.078                                      | 0.040 0.030                                      | 3.058 1.103                                      | 9.382 2.056                                      | 0.168 0.046                                       |
| TF    | HS   | EU          | 5.809 0.901                                     | 0.751 0.209                                      | 0.051 0.035                                      | 3.534 0.510                                      | 10.873 2.773                                     | 0.251 0.037                                       |
| TF    | DHS  | MF          | 0.573 0.225                                     | 0.039 0.018                                      | 0.047 0.013                                      | 0.275 0.133                                      | 0.437 0.181                                      | 0.042 0.024                                       |
| TF    | DHS  | BF          | 0.268 0.096                                     | 0.015 0.010                                      | 0.043 0.012                                      | 0.089 0.050                                      | 0.353 0.243                                      | 0.009 0.006                                       |
| TF    | DHS  | PM          | 0.342 0.069                                     | 0.003 0.003                                      | 0.056 0.012                                      | 0.074 0.035                                      | 0.067 0.044                                      | 0.021 0.006                                       |
| RF    | DHS  |             | 0.051 0.051                                     | 0.033 0.033                                      | 0.000 0.000                                      | 0.092 0.066                                      | 0.067 0.034                                      | 0.003 0.003                                       |
| RF    | HS   |             | 0.000 0.000                                     | 0.000 0.000                                      | 0.003 0.003                                      | 0.000 0.000                                      | 0.000 0.000                                      | 0.040 0.006                                       |

BF, primary monsoon evergreen broadleaf forest. MF secondary mixed pine/broadleaf forest. PM, planted *Pinus massoniana* forest. AA, planted *Acacia auriculiformis* forest. EU planted *Eucalyptus urophylla* forest. DHS, Dinghushan. HS, Heshan. TF, throughfall. RA, rainfall. BG,  $\beta$ -1,4-glucosidase. CBH,  $\beta$ -D-cellobiosidase. NAG,  $\beta$ -1,4-N-acetyl-glucosaminidase. LAP, leucine amino peptidase. PME, phosphomonoesterase. PPO, polyphenol oxidase.

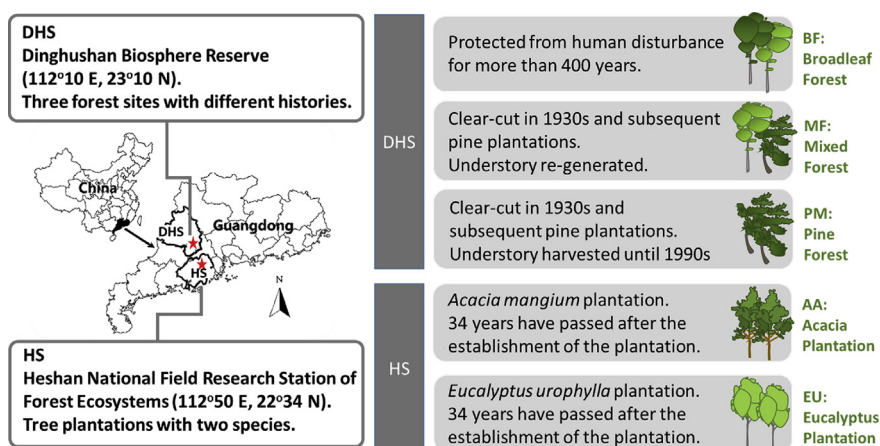
(112°50'E, 22°34'N) (Fig. 1) [3]. Three types of forests, i.e., a primary monsoon evergreen broadleaf forest (BF), a secondary mixed pine/broadleaf forest (MF), a planted *Pinus massoniana* forest (PM) are located in DHS. According to earlier studies, the BF has been protected for more than 400 years [2]. Dominant species of the BF are *Castanopsis chinensis* Hance, *Schima superba* Gardn. & Champ., *Cryptocarya chinensis* (Hance) Hemsl., *Machilus chinensis* (Champ. ex Benth.) Hemsl. and *Syzygium rehderianum* Merr. & Perry in the canopy and subcanopy layer [2,4]. The other two forests in DHS (MF and PF) were clear-cut in the 1930s, and *P. massoniana* plantations were established thereafter. The pine plantation was naturally colonized by broadleaf species to become a mixed forest in MF. Meanwhile, the understory vegetation and litter in PM were harvested constantly until the late 1990s, which resulted in a dominance of *P. massoniana* (more than 90% of the total basal area) in PM. In MF, *Pinus massoniana* and *Schima superba* are the dominant tree species. The soils in the DHS are lateritic red earths (Oxisols) formed from sandstone [2,4]. In HS site, the other two forests, i.e., a planted *Acacia auriculiformis* forest (AA), and a planted *Eucalyptus urophylla* forest, are located. Both AA and EU are 34 years old at the time of sampling. Previous studies reported that the soils in the two plantations are classified as Acrisols [5]. The annual precipitation and mean annual temperature are 1927 mm and 21.0 °C, respectively, in DHS [6], and 1580 mm and 22.5 °C, respectively, in HS [7].

## 2.2. Sampling

In August 2017, throughfall samples were collected in the five subtropical forests. We placed plastic boxes both inside and outside of the five forests (Fig. 2). We prepared 7, 5, 7, 6, and 6 replications of the boxes in BF, MF, PM, AA, and EU, respectively. We also collected rainfall samples in order to determine the background enzyme activity of rainfall. The boxes were placed at the outside of the forests, where there was no vegetation above the boxes. Rainfall samples were collected in triplicate in each research station. In total, 31 throughfall samples and 6 rainfall samples were collected. Those samples were collected as soon as possible after a rainfall event and kept cool/frozen in the research station near the forests.

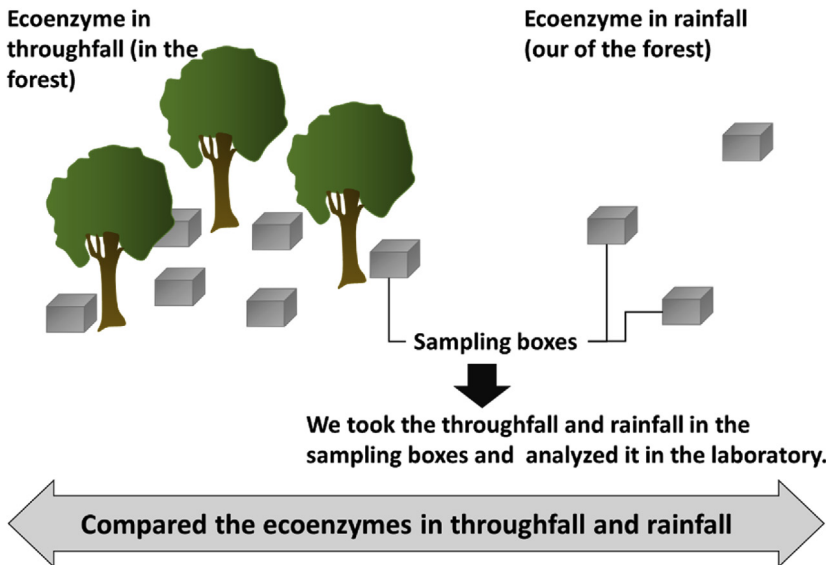
## 2.3. Enzyme assay

We measured six types of ecoenzymes in the throughfall and rainfall samples. The activities of  $\beta$ -1,4-glucosidase (BG, EC 3.2.1.21),  $\beta$ -D-cellobiosidase (CBH, EC 3.2.1.91),  $\beta$ -1,4-N-acetyl-glucosaminidase



**Fig. 1.** Five subtropical forests located in Guangdong province, China. BF, primary monsoon evergreen broadleaf forest. MF secondary mixed pine/broadleaf forest. PM, planted *Pinus massoniana* forest. AA, planted *Acacia auriculiformis* forest. EU planted *Eucalyptus urophylla* forest. DHS, Dinghushan. HS, Heshan.

## 1. Sampling



## 2. Analysis

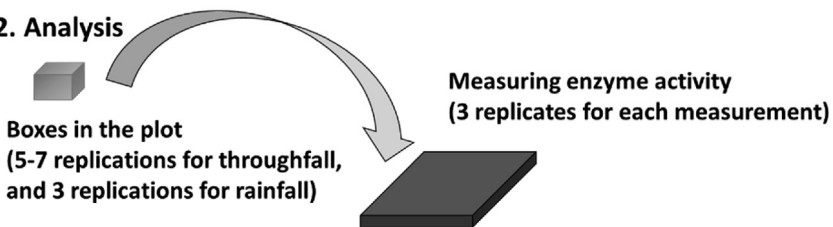


Fig. 2. Research procedures.

(NAG, EC 3.2.1.52), leucine amino peptidase (LAP, EC 3.4.11.1), polyphenol oxidase (PPO, EC 1.10.3.2), and phosphomonoesterase (PME, EC 3.1.3.2) were determined. Fluorescence enzyme assays were used for determining hydrolytic enzyme activities [8,9]. A portion of the collected samples (100  $\mu$ ) was dispensed into 96-deep-well plates. For analyzing BG, CBH, NAG, and PME activities, substrates labeled with 4-methylumbelliferone (MUB) were added. For the analysis of LAP activity, 7-amino-4-methylcoumarin (MUC) were added. The final substrate concentration was 150  $\mu$ M for BG, CBH, NAG, and PME, and 100  $\mu$ M for LAP. The solution volume was 1000  $\mu$ L. After mixed well, the deep plates were incubated for 4 hours at 20  $^{\circ}$ C in the dark. After the incubation, 250  $\mu$ L of the incubated solution was transferred into black 96-well plates. We measured fluorescence (365 nm excitation, 450 nm emission) with a microplate spectrophotometer. Standard lines were prepared for all samples by determining the fluorescence of known concentrations of the MUB or MUC solutions with 100- $\mu$ L aliquots of the collected samples. PPO activity was measured by spectrophotometric assay [9]. A portion of the collected samples (100  $\mu$ ) was dispensed into 96-deep-well plates with 700  $\mu$ L of pure water and 200  $\mu$ L of dihydroxyphenylalanine (DOPA, 25 mM). The deep-well plates were incubated for 65 hours at 20  $^{\circ}$ C in the dark. Absorbance at 450 nm was measured using a microplate spectrophotometer. We prepared negative and blank controls for all enzyme assays. For minimizing "well to well variation" (Bell et al., 2013), three assay replicates in each plate were prepared. The activities of each types of ecoenzymes are represented in units of  $\mu$ g substrates (MUB for BG, CBH, NAG, and PME; MUC for LAP; and DOPA for PPO)  $\text{h}^{-1} \text{L}^{-1}$ .

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## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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