Dynamic measurements of earthworm respiration

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Abstract

Earthworms are critical soil organisms, facilitating biogeochemical cycles in soil ecosystems through the formation of soil aggregates by drilling soil and accelerating microbial activity. However, the dynamic measurements of earthworm respiration have not been updated for several decades, although understanding earthworm respiration is a fundamental step in understanding the role of earthworms in soil biogeochemistry. In the present study, we applied our dynamic measurement system of soil gas exchange to earthworm respiration. Eisenia japonica and Metaphire hilgendorfi, which are typical earthworms in Japan, were used in this study. We continuously measured earthworm respiration in one or two earthworms for the first time. After confirming the possibility of continuous measurement of earthworm respiration in our system, we tested the system’s performance by investigating how earthworm respiration changes in/detached from soil conditions and temperature. Responses to temperature were different between the two species, possibly because of their physiological differences. Earthworm respiration was not different among temporal treatments of in/detached from soil (only 5%–10%). Continuous time-series data of earthworm respiration obtained by the system could be subjected to mathematical time-series analyses. Wavelet analysis showed that various scales of respiration enhancements until 16 min can be observed. Taken together, the application of our system to earthworm studies will considerably enhance our understanding of earthworm sciences in future.

Key words: Eisenia japonica; Flow-through chamber technique; Metaphire hilgendorfi; Temperature; Time-series analysis

1. Introduction

Because of the critical importance of soils in material flow in ecosystems, soil respiration is a continuing research target (Inoue, 1986; Luo and Zhou, 2006; Sun et al., 2017; Yonemura et al., 2017; Katayama et al., 2018; Nakano and Shimoda, 2018). Earthworms are key soil organisms, facilitating soil respiration and material flow in ecosystems through the decomposition of organic matter and formation of soil aggregates through soil cultivation (e.g., Darwin, 1881; Edwards and Bohlen, 1996; Coleman and Grossley, 2003; Lavelle and Spain, 2005). Recently, in connection with global warming, there have been extensive studies investigating earthworms and their association with the emission of greenhouse gases, such as carbon dioxide (CO$_2$) and carbon flows in soil (e.g., Lubbers et al., 2013; Hasegawa et al., 2017; Kaneda et al., 2017). A recent big topic of material flow control by earthworms is whether earthworms activate or suppress microbial soil respiration through the cast formation in connection with global warming (e.g., Lubbers et al., 2013). The soil respiration changes caused by earthworms are very subtle with contradictory results. Basic understanding of earthworms is lacking, particularly earthworm respiration. Technical progress of micro-scale measurements of earthworm respiration, with controlled food supply, temperature, and other soil parameters, are essential for mechanical understanding of carbon dynamics in long-time incubation experiments. To date, recent technical advances in gas analyzers may drastically update methodology to measure earthworm respiration.

Most previous studies on earthworm respiration have focused on the relationships between respiration and temperature because respiration can be considered an index of earthworm activities. Basic earthworm respiration measurement can be traced back to Vernon (1897), who measured respiration in earthworms and other organisms, and to Pomerat and Zarrow (1976), who observed a positive Ahrenius-relationship between respiration rate and temperature ranging 9°C–27°C in Lumbricus terrestris. Saroja (1959) reported the acclimation of earthworm respiration to temperature, whereas Bolton (1970) and Phillipson and Bolton (1976) studied seasonal variations in earthworm respiration in an experimental system based on the flow-through chamber technique and O$_2$ respirometry, showing maxima in summer. They used 20 earthworms for the measurements to match the demand of CO$_2$ difference required in the CO$_2$ analyzer at that time. Sustr and Pižl (2009; 2010) reported that Dendrobaena mrazeki had the lowest respiration rates. In relation to temperature dependence, diurnal rhythms in earthworm respiration rates were investigated using simulated temperatures in the laboratory (Chuang et al., 2004). According to Chuang et al. (2004), Amyntas gracilis crawls to the soil surface following rain owing to higher O$_2$ consumption with decrease in temperature. Uvarov and Scheu (2004a) revealed that fluctuating temperature regimes (0°C–10°C, 5°C–15°C and 10°C–20°C) resulted in higher respiration rates than stable temperature.
regimes (5°C, 10°C, and 15°C), indicating that, at diurnally fluctuating temperatures, earthworms consume more resources via respiratory metabolism.

Respiration rates differ among species (Šustr and Pižl, 2009). The body mass-specific respiration rate decreases with increased body weight because surface area to volume ratio decreases with increased body weight (Šustr and Pižl, 2009). Body mass-specific respiration rates do not differ between adults and juveniles (Šustr and Pižl, 2009). Epigeic species have higher respiration rates than endogeic ones (Phillipson and Bolton, 1976). In some earthworm species, respiration rates may decrease with increased population density (Uvarov and Scheu, 2004b).

The studies conducted by Uvarov and Scheu (2004a) and Šustr and Pižl (2010) facilitated in understanding the other aspects of earthworm respiration because they were generally poorly understood, and further investigations were challenging because of the complexity of earthworm ecology (e.g., Edwards and Bohlen, 1996; Lavelle and Spain, 2005). In addition, the basic method of measuring earthworm respiration is the closed-chamber technique. More technical improvements and studies on other earthworm respiration aspects are critical for further progress in understanding earthworm ecology.

Earthworm respiration rates are estimated by measuring O₂ consumption (Uvarov and Scheu, 2004a; Chuang et al., 2004, 2006; Föster et al., 2006; Chuang and Chen, 2008) or CO₂ emission from earthworms (Phillipson and Bolton, 1976; Uvarov and Scheu, 2004b). Numerous previous studies (Šustr and Pižl, 2009) were conducted using O₂ consumption in combination with the Warburg Apparatus, which was originally designed for observing biological respiration in cells and tumors by Otto Heinrich Warburg.

We developed dynamic systems to measure soil gas exchange (Yonemura et al., 2019) in our laboratory. The dynamic system is based on the flow-through chamber, and its details are reported in Yonemura et al. (2019). The systems can continuously and simultaneously measure exchanges of several gases from various biological specimens while controlling various factors, such as temperature and gas concentrations. Because the authors developed the systems, their tools can be adapted for various applications or integrated into other sub-systems.

In the present study, we applied one of our dynamic measurement systems to soil gas exchange in earthworm respiration, denoted by CO₂ emission, and conducted some basic experiments. From the methodological point of view, this study is an update of Bolton (1970) and Phillipson and Bolton (1976) after 40 years with a more elaborate measurement system. Earthworm species employed in this study are Eisenia fetida and Metaphire hilgendorfi, which are typical earthworm species in Japan (Ishizuka and Minagoshi, 2014). Because CO₂ exchange in earthworms indicates earthworm respiration, we targeted CO₂ exchange in earthworms. Exchanges of other gases, such as CH₄, N₂O, NO, H₂, and CO, were not measured in earthworm experiments in the present study, although previous studies (e.g., Horn et al., 2003; Kernecker et al., 2014) reported N₂O and CH₄ emissions from earthworms.

After we ascertained that continuous measurements of earthworm respiration were possible with our system, we investigated how earthworm respiration shifts along with shifts in soil conditions and temperature. Continuous time-series data of earthworm respiration can be obtained from mathematical time-series analyses. We apply a Wavelet analysis to earthworm respiration data because of the significance of bio-rhythm.

2. Materials and methods

2.1 Application of the system to earthworm respiration

We modified the system (Yonemura et al., 2019) to be appropriate for measuring earthworm respiration. Carrier flow through the system was driven by a BEBICON oil-free air compressor (0.4LE-8S, Hitachi Industrial Equipment Systems Co., Ltd., Tokyo, Japan) that absorbed air from outside. After directing air to the laboratory, CO₂ and humidity in the carrier flow were reduced to undetectable levels (PNEUDRI MiDAS, Domnick Hunter Filter Ltd., Durham, England), enabling more accurate measurement of earthworm respiration through the detection of subtle CO₂ differences across chambers than at atmospheric conditions, with fewer drifts caused by pressure and temperature fluctuation. The total flow rate of carrier gas was maintained at 1100 ml min⁻¹ by a mass flow controller (SEC-E40 range 0–5000 ml min⁻¹, Horiba Stec, Co., Ltd. Kyoto, Japan). The carrier flow was humidified by a water bubbler and divided into five flows, four of which were chamber carriers that flushed the chambers’ inner space and one was used as a base flow. The humidity environment of the air inside the chamber where the earthworms were installed was kept at saturation water vapor to avoid damage to the earthworms by drying and to keep soil water content unchanged to the extent possible. The flow of chamber carriers was maintained at 185 ml min⁻¹ using mass flow controllers (SEC-E40 range 0–500 ml ml⁻¹, Horiba Stec, Co., Ltd., Kyoto, Japan).

The chamber used to measure earthworm respiration had an inner diameter of 9.5 cm and height of 11.8 cm (volume: 850 cm³). A petri dish with an inner diameter of 8.5 cm (57 cm²) and height of 2.3 cm was used. The chambers comprised an upper lid and lower part. The upper lid was detached from the lower part, when the petri dish containing the soil sample was exchanged. The two parts of the chamber were sealed with a Viton ring. A 12V-type fan, attached to the chamber’s top part, was normally driven by a 5V voltage supply except when studying the effects of the fans’ rotations on emissions to make gas concentrations in the inner air of the chamber uniform by stirring. Every time earthworms were exchanged, the chambers’ flow rates and base carriers were checked using a soap-film flowmeter (SF-1100, Horiba Stec, Co., Ltd., Kyoto, Japan).

The chambers were placed in an incubator (IG420, Yamato Scientific Co., Ltd., Tokyo, Japan) whose temperature control could be scheduled. After flushing the chambers, the five flows were dehumidified using cold traps (AF20-01C, SMC Corporation, Tokyo, Japan), placed inside a low-temperature incubator (LTI-601SD, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), and set at 1°C. After dehumidification, one set of carrier flows, including both the chamber and base flow, was selected by a series of magnetic valves (GAB352-6, CKD Corporation, Komaki, Japan) at an interval of 12 min for EX2 and EX3 (see following sections) and was driven to a CO₂ analyzer by a pump.
co2 was analyzed using a LI820 infrared CO2 analyzer (LI820, Licro Inc., Lincoln, NE, USA). Furthermore, the CO2 analyzer’s linearity was checked for lower ranges of CO2 concentrations using CO2 standard cylinders containing 20 and 986.3 ppm of CO2 in N2-base because low CO2 concentrations were expected in our experiments. Linearity was ascertained to be within 1%.

The earthworms’ respiration rates were calculated using a mass balance (Yonemura et al., 2013). The unit of the respiration rate was adjusted to be µl CO2 h−1 at 15°C to be comparable to rates in previous studies. The unit denoted the respiration rate per chamber, which was not divided by the earthworms’ body weight. Nevertheless, approximate levels of respiration rates per body mass of earthworms were mentioned in the text. Conversion from µl CO2 h−1 to µmol CO2 h−1 and µg CO2 h−1 required multiplication with 0.0423 and 1.86, respectively.

2.2 Preparation of earthworms

*M. hilgendorfi* individuals were taken from fields at the Institute for Agro-Environmental Sciences NARO, Tsukuba, Japan, in June 2012. These individuals were collected from the space between the litter layer and mineral soil surface. *E. japonica* individuals were taken from fields at International Nature Farming Research Center, Matsumoto, Japan, in June 2011. These individuals were collected from the mineral soil surface (0–10 cm). After collection from the fields, the earthworms were stocked with soil at 15°C in the laboratory by using soil, where individuals were collected until they were used for experiments during June to July 2012. Rice straw was put into soil to keep *E. japonica*, whereas litter was put on the soil surface to keep *M. hilgendorfi*. The same sets of two *M. hilgendorfi* individuals and four *E. japonica* individuals were used through the following experiments (EX1–EX4).

2.3 Experiments

2.3.1 Experiment 1 (EX1): First test of continuous measurement of earthworm respiration

To check whether earthworm respiration could be measured by the system, we placed one *M. hilgendorfi* and four *E. japonica* individuals without soil in chambers and continuously monitored their respiration rates under dark conditions. Furthermore, all the experiments (EX1–EX4) were conducted under dark conditions without the removal of cast after they were taken out of incubator. To get continuous data, only the carrier flow from one chamber was introduced to the CO2 analyzer.

2.3.2 Experiment 2 (EX2): Earthworm respiration with temporal temperature change

As mentioned in the introduction, temperature is a critical parameter in earthworm respiration. Two sets of one *M. hilgendorfi* individual and two sets of two *E. japonica* individuals were placed in chambers without soil in an incubator with the temperature maintained at 15°C. After more than 10 h at 15°C, temperature was sequentially changed to 20°C, 15°C, 10°C, and finally 15°C every 6 hours.

2.3.3 Experiment 3 (EX3): Earthworm respiration in soil or detached from soil

Earthworms are soil organisms, and soil is essential for earthworms as a food and movement medium. “With soil” or “In soil” and “Without soil” or “Detached from soil” experimental conditions are critical for measuring earthworms’ respiration rates. However, previous experiments, which reported respiration rates in earthworms, differed in the existence of soil. Two sets, No. 1 and 2 of individual *M. hilgendorfi*, and four individuals of *E. japonica*, were placed in chambers. The two conditions, “In soil (IS)” and “Detached from soil (DS)” were alternated every 3 days (Fig. 1, Table 1). Earthworms were removed from the soil and placed in other places in chambers. The respiration of both the earthworms and the soil were measured. The contribution of earthworm respiration was afterwards obtained by subtracting the soil respiration from the total respiration.

The alluvial soil was collected from fields at the Institute for Agro-Environmental Sciences NARO. The soil was sieved through a 1-mm mesh before use. The sieved soil properties were as follows: carbon content, 2.4%; pH (H2O), 5.6; sand, 37.4%; silt, 22.7%; and clay, 39.9%. The soil weight places on each petri was 89 g, whose dry weight was 50 g, where the soil water content was adjusted to be 85% of the water holding capacity (=0.93 as soil water content). The soil water content decreased to 62% at the end of EX3.

2.3.4 Experiment 4 (EX4): Analyses of earthworm respiration by Wavelet analyses

After EX3, this experiment, using *M. hilgendorfi* No 1 and 4 individuals of *E. japonica*, was conducted at 15°C without soil. After getting continuous data from the system, various analyses, such as time-series analyses, were possible. Representative analysis of the time-series analyses is frequency analysis as done by Fourier frequency transfer. We conducted frequency analyses in line with Fast Fourier Transfer (FFT) analyses. The FFT analyses were performed using version 2.6.2 of R software (R Development Core Team 2011).

Another powerful tool for time-series-analysis is Wavelet analysis that obtained scales, magnitude, and timing of the time-series variations. The continuous Wavelet transformation for a discrete time series \( \{x_1, x_2, \ldots, x_n\} = \{x(t_0), x(\Delta + t_0), \ldots, x((N-1) \Delta + t_0)\} \) is defined as

\[
 w(\mu, s) = \frac{1}{\sqrt{s}} \sum_{k=1}^{N} x_k \Psi^*(\frac{\Delta(k-u)}{s}),
\]

where \(s\) and \(\mu\) are scale parameter and translation time, respectively (e.g., Chui, 1992). The scale parameter is specified by an index, \(s = \alpha \times 2^{\text{oct}-1}\). The scale index \(\text{oct} (=1, 2, \ldots, \log_2(N/2))\) represents the number of octaves, where \(\lfloor\cdot\rfloor\) is Gauss symbol. \(\alpha\) denotes the smallest resolvable scale. For each scale, \(s_{\text{oct}}\), the Wavelet coefficients \(\{w(1, s_{\text{oct}}), w(2, s_{\text{oct}}), \ldots, w(N, s_{\text{oct}})\}\)
are obtained by the transformation. The results are presented in a Wavelet scalogram of the Wavelet power spectrum of the target time series. In this analysis, Mexican Hat type function was adopted as Mother Wavelet $\Psi(t)$ in eq. (1). The calculation of continuous Wavelet transformation was conducted using Mathematica 11.0 (Wolfram Research Inc., 2016).

### 3. Results

#### 3.1 EX1

Time series of measured respiration rates are presented in Fig. 2. Respiration rates in earthworms, which are different from gas emissions from soil (Yonemura et al., 2019), exhibited high temporal variations. An individual of *M. hilgendorfi* had higher respiration than the four *E. japonica* individuals. Individual respiration rate in an *E. japonica* individual was approximately 6 µl CO$_2$ h$^{-1}$ and approximately 200 µl CO$_2$ h$^{-1}$ in an *M. hilgendorfi* individual. Even taking body weight into consideration (Table 1), the respiration rate per unit body weight in *M. hilgendorfi* (approximately 100 µl CO$_2$ g$^{-1}$ h$^{-1}$) was higher than that in *E. japonica* (approximately 50 µl CO$_2$ g$^{-1}$ h$^{-1}$).

#### 3.2 EX2

A time series of earthworm respiration with temperature changes are shown in Fig. 3. As explained in EX1, earthworm respirations exhibited high temporal variation, and *M. hilgendorfi* individually had higher respiration rates than *E. japonica* even in consideration of body weight. *M. hilgendorfi* had a lower respiration rate when the temperature was controlled at 20°C and 10°C. The decreased respiration rates were approximately 15% and 45% for 20°C and 10°C to those at 15°C, respectively. *E. japonica* had higher respiration when the temperature was controlled at 20°C, but lower when the temperature was controlled at 10°C. The increase and decrease in respiration rates were approximately 50% and 100%, for 20°C and 10°C, respectively. Because the respiration rates in *E. japonica* increased with temperature, the temperature coefficient Q10 of respiration in *E. japonica* was calculated to be approximately 1.4.

### Table 1. Earthworm body weights before and after EX1–EX3 experiments (35 days). The earthworms’ body weights decreased during experiments. The decreases were also observed in previous studies (e.g., Uvarov and Scheu, 2004a, b).

| Earthworms            | Body weight before experiment (g) | Body weight after experiment (g) | Decreasing rate |
|-----------------------|-----------------------------------|----------------------------------|-----------------|
| *Metaphire hilgendorfi* No1 | 4.51                             | 2.65                             | 41%             |
| *Metaphire hilgendorfi* No2 | 2.81                             | 1.65                             | 41%             |
| *Eisenia japonica* $\times$ 4 | 0.58                             | 0.45                             | 22%             |

![Fig. 1. Schematic diagram of experiment in soil and detaching from soil in EX 3.](image-url)
3.3 EX3
Changes in earthworm respiration rates under repeated conditions IS and DS are shown in Table 2. After removing the earthworms from the soil (DS1), E. japonica and M. hilgendorfi exhibited higher respiration than IS1. However, during D2–D4, earthworm respiration rates in conditions DS were lower than, or almost equal to, those with soil. Average respiration values, excluding IS1 and DS1, were 7% and 17% lower than with soil for E. japonica and M. hilgendorfi, respectively.

3.4 EX4
We analyzed three sets of data for the time series analyses (Fig. 4). Figs. 4a, 4b, and 4c show the target time series of earthworm respiration. After detrending by the smoothing technique, FFT showed maximum at 5–10 min for all the three sets of time series (Figs. 4d, 4e, and 4f).

Figs. 4g, 4h, and 4i represent the corresponding Wavelet scalograms, respectively. The Wavelet scalogram plots the absolute value of the Wavelet coefficients at each time and scale, which can capture the time series dynamics. The time dimension is represented in the horizontal axis, while the vertical axis represents the scales or frequency. The magnitude of Wavelet power is represented by color intensity where a brighter color denotes a higher value of the Wavelet power.

We observed intermittent fluctuations in the respiration time series (Figs. 4g, 4h, and 4i). For example, in Fig. 4g, the changes around time 1000 and 1500 occurred within the scale ranging from oct = 8 to oct = 10, which correspond to the fluctuations with 2–16 min, being comparable to frequencies obtained by FFT.

4. Discussion
Compared to previous studies, our study presents continuous respiration measurements in an individual earthworm for the first time using the system that employs the flow-through chamber technique (Fig. 1). Employing recent advances in the analyzers and measurement systems, the present study updated previous studies (Bolton, 1970; Phillipson and Bolton, 1976), who continuously measured earthworm respiration for a group of earthworms, as many as 20 individuals.

If respiration measurements by CO₂ emission are similar to those by O₂ consumption, the respiration rates in M. hilgendorfi and E. japonica (approximately 50 µmol CO₂ g⁻¹ h⁻¹) in this study are in the range (30–140 µmol O₂ g⁻¹ h⁻¹) of previous studies (Šustr and Pižl, 2009; Phillipson and Bolton, 1976).
However, Förster et al. (2006), who modified a photosynthetic system into a closed-chamber system for earthworms, reported a higher range (26.8–1141.8 μmol CO$_2$ g$^{-1}$ h$^{-1}$) of earthworm respiration rates in the Amazon and Germany, with our data range appearing to be in the lower limits of their data. Overall, earthworm respiration is highly variable under different species and conditions, as reported in previous studies. Sophisticated measurements, such as those from our system, are required for future studies. Our test experiments showed variations of earthworm respirations with changes in temperature for both conditions in/detaching from soil. Respiration of *E. japonica* increased with increase in temperature with Q10 = 1.4; respiration of *M. hilgendorfi* at 15°C showed larger values than at 10°C and 20°C (Fig. 3). Šustr and Pižl (2010) exhibited that respiration in *Dendrobaena mrazeki* is maximum at 25°C, which is 20°C lower than in our data for *M. hilgendorfi*. The optimal temperature for earthworm respiration is different among species and in different environments.

Earthworm respiration during four times of condition control in/detaching from soil over 30 days in/detaching from soil was not different and was within 5–17 (Fig. 1, Table 2). However, respiration in *E. japonica* and *M. hilgendorfi* No 1 were higher when the earthworms were not in soil during the first stage (D1) (Table 2). This is probably because earthworms were trying to find soil and moved a lot. However, after D2, earthworm respiration was lower under detaching from soil. Earthworms became weak during measurements, causing their respiration rates to decrease. Conversely, earthworms in soil can

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**Fig. 3.** Time series of earthworm respirations with changes in temperatures in EX 2. The basic temperature was 15°C. Solid lines show averages of two sets of individuals for *M. hilgendorfi* where one individual was put into chamber and two individuals for *E. japonica*; where were put into a chamber; error bars show the differences of the two sets of chambers.

**Table 2.** Earthworm respiration rates in and out of soil in EX3.

| Stage | Condition          | duration (day) | *Eisenia japonica* × 4 μl CO$_2$ h$^{-1}$ | *Metaphire hilgendorfi* No1 μl CO$_2$ h$^{-1}$ | *Metaphire hilgendorfi* No2 μl CO$_2$ h$^{-1}$ |
|-------|--------------------|----------------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| S1    | In soil (15°C)     | 4.27           | 22.2                                     | 145.2                                         | 233.8                                         |
| D1    | Detached from soil (15°C) | 2.81           | 24.5                                     | 150.6                                         | 184.2                                         |
| S2    | In soil (15°C)     | 3.77           | 21.5                                     | 144.7                                         | 229.3                                         |
| D2    | Detached from soil (15°C) | 2.68           | 20.9                                     | 120.8                                         | 166.6                                         |
| S3    | In soil (15°C)     | 4.10           | 22.0                                     | 141.0                                         | 189.6                                         |
| D3    | Detached from soil (15°C) | 2.77           | 22.4                                     | 110.6                                         |                                                             |
| S4    | In soil (15°C)     | 3.89           | 29.5                                     | 121.7                                         |                                                             |
| D4    | Detached from soil (15°C) | 2.64           | 23.9                                     | 86.8                                          |                                                             |
| S5    | In soil (15°C)     | 3.39           | 23.5                                     | 103.7                                         |                                                             |
| Average | In soil (15°C)     |                | 23.7                                     | 131.3                                         | 217.5                                         |
|        | Detached from soil (15°C) |                | 22.9                                     | 117.2                                         | 175.4                                         |
| Average excluding S1 and D1 | In soil (15°C) |                | 24.1                                     | 127.8                                         |                                                             |
|        | Detached from soil (15°C) |                | 22.4                                     | 106.0                                         |                                                             |
activate soil microorganisms.

The continuous measurements and time-series analyses could facilitate more studies about earthworms’ circadian rhythms by obtaining continuous earthworm respiration data. To date, there is no study on earthworms’ circadian rhythms except for diurnal variations (Chuang et al., 2004). Results of this study, using Fourier and Wavelet analyses, showed that earthworm respiration has fluctuations of 5–7 min by FFT analyses (Figs. 4d, 4e, and 4f) and 2–16 min by Wavelet analyses (Fig. 4g, 4h, and 4i). However, response time in the chamber was approximately 4–5 min and responses decreased in periods less than 5 min. The results of the Wavelet analyses demonstrated that various time-scales of respiration and, hence, movements may be observed in the earthworm respiration time series. Moreover, response time in measurements of earthworm respiration depends on both the chamber inner volume, within which earthworms may move, and the rate of flow flushing the chamber. Further improvements, such as making chamber sizes smaller or increasing flow rates through them, could facilitate more rapid responses and observations of earthworm respiration depending on research objectives.

Various studies of earthworms can be conducted using the present system. Kretzschmar (1991) reported that earthworm respiration in Aporrectodea longa is different between spring and fall. The present system can measure different temperature response in different seasons. It can also help determine whether short-term responses in earthworm respiration are applicable to seasonal differences. Further studies on the response of earthworm respiration to dry and wet conditions and irradiation conditions should be revealed by the system because of the more rapid response time in the present system (4–5 min) compared to closed-chamber systems.

In future, it may be critical to separate maintenance respiration from movement respiration in earthworms, exploring the measurement of earthworm movement and its consideration in respiration studies. Kodama et al. (2014) calculated earthworm movement using a network camera. Once a relationship between earthworm movements and respiration is established, earthworm movements can be quantitatively estimated even when they are in soil because earthworm respiration was not different (within 20%) in and out of soil.

5. Conclusions

We applied our system for the dynamic measurement of soil gas exchange to respiration measurements in earthworms: E. japonica and M. hilgendorfi. Continuous measurement of individual earthworm respiration was conducted for the first time by the system using the flow-through chamber technique. We also applied an improved system to test the performance of the system; we conducted experiments to explore how earthworm respiration changes within and out of soil conditions and with temperature changes. Temperature responses were different between species, suggesting physiological differences. Earthworm respiration was not different within 5%–10% following temporal manipulation in/detaching from soil. Moreover, continuous time-series data of earthworm respiration was analyzed by FFT and Wavelet time-series analyses because of the significance of bio-rhythm. The analyses showed the existence of 2–16 min fluctuations, indicating various time-scales of respiration phenomena. It is expected that the application of our system will facilitate earthworm studies and future material flow studies in ecosystems.

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