Current Microbiology (2020) 77:2374–2384
https://doi.org/10.1007/s00284-020-02033-z

Evaluation of the Carbon Dioxide Production by Fungi Under Different Growing Conditions

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Received: 30 December 2019 / Accepted: 14 May 2020 / Published online: 29 May 2020

Abstract

Production of carbon dioxide, as one of the ultimate products of fungal metabolism, can be used to quantify and measure their metabolic rate under different conditions, thus aiding in finding the optimal substrate and environment for cultivation of wood-destroying fungi. This study is focused on species *Pleurotus ostreatus* and *Ganoderma lucidum,*. These species are also cultivated for mycorestoration as well as their medicinal and nutritional value. To quantify their metabolical rate on various substrates (agar medium, wood chips, rye straw), multiple custom-built airtight chambers were equipped with CO_2 probes (GMP 343, Vaisala, Finland) to measure the production of carbon dioxide. The highest values were measured during the primordial production on rye straw substrate, with the average values of 1.09 g CO_2 kg^{-1} (substrate) h^{-1}. These values varied significantly between various substrates, fungal species and development stages.

Introduction

Chemical compounds contained in fungi along with their useful natural abilities are the main focus of mushroom research. They are used for nourishment, as a source of various compounds with beneficial health effects and as a tool to restore and rejuvenate the environment often damaged by human carelessness in the past and present. These are the main reasons to study and use them intensively.

Mushroom cultivation is rapidly spreading around the world for various reasons. In 2012, the production of edible and medicinal mushrooms reached approximately 31 million tons, valued at 20 billion USD [1]. In many, mostly developing countries, the main focus is to produce food, employing the disadvantaged groups of inhabitants to provide for their families [2–4]. One of the most feasible ways to cultivate mushrooms effectively is to use locally sourced lignocellulose material, which is often a waste product of agriculture and logging [2, 5–8]. Bioremediation utilising mushroom mycelium to remove and neutralise pollutants is suitable for both rich and poor countries [6, 9–12]. Using mushrooms for their medicinal properties has a strong tradition in Asian countries (China, Japan, Korea), along with Russia and America [13]. More than 130 medically beneficial properties of fungi has been confirmed, which caused great interest in the more developed countries to find the way to treat even the most severe illnesses using mushrooms [1, 13].

It is the constantly rising demand for cultivated mushrooms, which is the reason to research the cultivation process, to provide optimal growing conditions to produce fruiting bodies of the highest possible quality and quantity. Growing conditions, such as substrate composition, light schedule, temperature and chemical, physical and biological composition of air have significant impact on quality of the fruiting bodies.

Information about CO_2 production by mushrooms grown in natural or laboratory conditions are rather sparce. Nevertheless, objective quantification of CO_2 efflux during the cultivation process is crucial, considering the necessity of growing high quantities of fruiting bodies, which will indubitably have a significant impact on the environment. Scientific papers dealing with mycelia respiration of individual fungal species are mainly focused on the production of CO_2 by fungi of dead wood [14, 15]. The information about mycelial respiration is of importance not only because of the quantification of carbon emitted into the atmosphere

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due to decomposition processes of decaying wood, but also as information needed by farmers cultivating and utilisating for food and medicine demands. It is known that mycelium needs a high concentration of CO_2 at the time of substrate overgrowth. However, during the primordial growth phase, it is necessary to reduce the CO_2 concentration in the environment by intense ventilation [16, 17]. Last but not least, respiration is a reflection of the metabolic activity of mycelium during its development. Methodological approaches for determination of mycelia respiration rate have not been clearly established yet. Therefore, a research focused on environmental factors, temporal dynamic and methodological approaches for quantification of mycelial respiration is required for more precise estimation of fungal contribution to global carbon cycle as well as guidance for artificial agriculture planning.

Our study is based on measurement of the carbon dioxide production during fungal substrate overgrowth, primordial formation and growth of the fruiting bodies. We attempt to quantify the respiration rate of mycelia using commercially available instruments based on infra-red CO_2 sensors.

The aim of our work was (1) to find the most suitable procedure for the determination of fungal mycelial respiration, (2) to determine the influence of factors such as time, species of fungus, type of substrate on which the fungus grows and relation between temperature and respiration rate of mycelia, (3) to detect temporal dynamic of fungal respiration, identify presence and period of possible biorhythms in fungus development period from mycelium inoculation up to fruiting bodies stage.

Materials and Methods

Substrate Preparation

We used three types of substrate in the current study: (1) agar substrate in a Petri dish; (2) wood chips; (3) rye straw. Fungal inoculum was provided by mycological laboratory of Mykoforest Martin Rajtar Velčice, Slovak Republic.

Agar Substrate on a Petri Dish

Petri dish of 80 mm diameter was filled to 1/3 of its volume with potato dextrose agar (PDA) medium and inoculated by approximately 3 mm cube of either *Pleurotus ostreatus* or *Ganoderma lucidum* in sterile conditions of Bioair laminar flow box (Bioair Auro Vertical S.D.4, EUROCLONE S.p.A. Siziano, Italy). The cultivation vessel was then covered by Parafilm (Bemis Co., Oshkosh, WI, USA) and stored in the incubator (Heratherm IMC 18, Thermo ElectronLED GmbH, Langenselbold, Germany) at 25 °C for 3 weeks. The PDA medium was completely overgrown by *Ganoderma lucidum* and *Pleurotus ostreatus* mycelium after 3 weeks. Samples were tested by 1a set.

Wood Chips Substrate

Wood chips of oak (*Quercus petrea*) and beech (*Fagus sylvatica*) separately were mixed with nutritional additives, such as wheat bran and gypsum in 80:15:5 ratio. The chips were then mixed with clean water to achieve the moisture content of 65%. 1000 g of this substrate was then placed inside a glass bottle, which was then heated at 121 °C for 3 h in APT Line dryer (Binder GmBH, Tuttlingen, Germany). Next day, after cooling down to 25 °C, the substrate was inoculated with *Pleurotus ostreatus* (*PO*) or *Ganoderma lucidum* (*GL*). The inoculum of *Pleurotus ostreatus* (strain MFTCCA042/032017) and *Ganoderma lucidum* (strain MFTCCA010/032017), overgrown on wheat grain medium, had been prepared in specialized laboratory of Mykoforest. 100 g of the inoculum was added to the central part of the substrate in the glass bottles in sterile conditions of Bioair laminar flow box. The bottles were then covered with filters consisting of thin aluminum foil with 4 perforations of 3 mm diameter and paper wadding, covering the bottle opening fixed to the sides of the bottle with adhesive tape. CO_2 and O_2 can passively flow through the perforations in the foil, while the foil and paper wadding prevents excessive moisture loss from substrate and undesirable substrate infection from the surrounding environment. The bottles were stored in the laboratory at 22 °C with natural daylight regime. In average, total mycelial overgrowth occurred within 30 days.

Rye Straw Substrate

The main component of substrate is rye straw cut into maximum of 10 cm pieces, soaked in 1% aqueous solution of calcium hydroxide for 24 h, after which the solution was drained off and the substrate was dried to achieve moisture content of 65%; it was then inoculated with *Pleurotus ostreatus.* The substrate was then placed in the $SacO_2$ Microsac bags (The Zipper Polypropylene Microsac, Deinze, Belgium) as follows: 1000 g of prepared straw, 100 g of *PO* inoculum (hybrid HK35, strain MFTCCB179/032017 overgrown on wheat grain medium, prepared in specialized laboratory of Mykoforest); mixed thoroughly. Polypropylene bags (type PP75/BEH6/V37-53) have six filter strips, which are adapted to the application and desired gas exchange rate. Ten bags were sealed shut and placed in a custom-built automatic chamber equipped with Vaisala GMP343 CO_2 probe measuring the carbon dioxide concentration every fifteen minutes for 45 days.

Measurement of Fungal Respiration

Total respiration of soil microorganisms, live and dead plants and animals is named ecosystem respiration and plays a crucial role in global carbon cycle. Chamber method is one of the most commonly used method in ecophysiological research for estimation of respiration rate on different ecosystem compartments and scales. Chamber methods are highly precise, offering both manual and automatic functionality and are generally easy to operate. Several chamber systems are commercially available. Custom or self- built chambers offer further personalisation and functionality for specific purpose applications.

The basic principle of chamber methods is the estimation of temporal change of CO_2 concentration in defined chamber space. The CO_2 concentration is measured by infrared gasometric probes (IRGA). The method is based on absorption of infrared (IR) radiation by CO_2 molecules. The IR radiation reduction rate is a function of the CO_2 concentration. Depending on the measurement method, the chambers are devided into closed and open ones. In closed chambers the CO_2 efflux is determined from the rate of concentration increase in an isolated space. In open chamber CO_2 efflux is calculated from the difference between CO_2 concentrationn at the inlet and the outlet of the chamber [18].

Closed chambers are further subdivided into non-through flow (or static) and through flow (dynamic) types. In closed static chambers CO_2 concentration is measured iby IRGA probe in the chamber headspace. In closed dynamic chambers analyzed air is circulated from the chamber to the IRGA analyzer and back by a mixing fan. The disadvantage of chamber methods is a small time scale resolution which is currently offset by the introduction of chamber automats.

We tested four different instrument sets (Photo 1) to measure mycelial respiration:

a) Set 1—closed dynamic system, consisted of LICOR 6400 XT (Licor, Nebraska, USA) equipped with conifer chamber, chamber size was 8 × 8 × 6 cm.
b) Set 2—closed dynamic system, consisted of EGM4 (PPSystems, USA) equipped with a soil respiration chamber, chamber size was Ø 16 × 20 cm.
c) Set 3a and 3b—closed static systems, a self-built chambers of different volumes consisting of a Vaisala CarboCap CO_2 probe, model 343 and Handheld MI70 datalogger (Vaisala, Finland) and a transparent Plexiglas chamber 40 × 40 × 40 cm (3a), and 10 × 10 × 10 cm (3b).
d) Set 4a and 4b—closed static systems, a self-built automatic chambers, consisting of the Vaisala CarboCap sensor and a transparent chamber that automatically opens and closes at the required time interval. Chambers of Ø 35 cm × 100 cm (4a), and Ø 35 cm × 70 cm (4b), respectively, were used.

The following procedure was used to test appropriateness of mentioned systems for fungal respiration measurement. The opened Petri dishes with inoculated mycelium were placed inside of the tested chambers and the chambers were placed on an elastic rubber support along the chamber bottom edges to prevent gas leakage. Measurement time differed

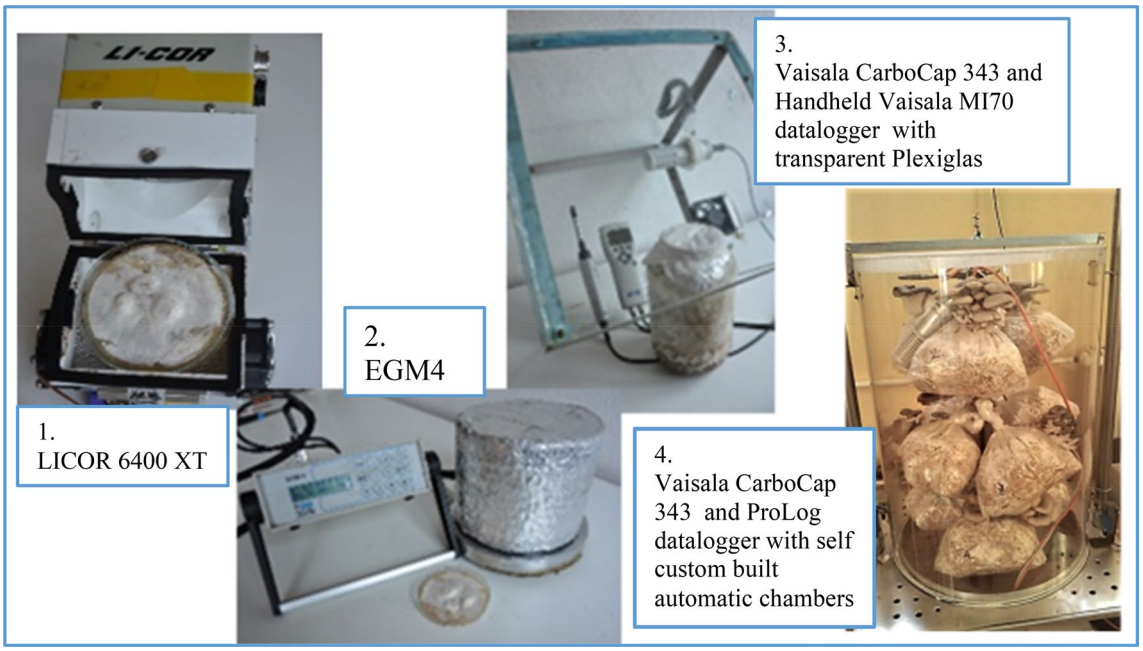

Photo 1 Instrument sets to measure mycelial respiration

according to the chamber type. In set 1 mycelial respiration was not time dependent as the system measures instantaneously. We applied 5 min adaptation time before readings. Set 2 and 3 measured accumulated CO_2 values which were time dependent. Maximum measuring time in set 2 was 5 min due to instrument limitation. Set 3 has no measuring time limits. The most sensitive system for measuring mycelium respiration rate was chosen for further analysis. To identify the relation among mycelium respiration and selected factors such as fungus species, substrate, time, temperature and their interaction we conducted three experiments.

Experiment 1: Influence of Time, Fungus Species, Substrate and Their Interactions on Mycelial Respiration

The substrate (1–10 mm fractions of beech or oak wood chips after heat and moisture treatment) was filled (1000 g) into 48 four liter glass bottles. 24 bottles with beech (FAG) wood chips substrate and 24 bottles with oak substrate (QER). Afterwards, 12 bottles of FAG wood chips were inoculated (100 g) with *GL* and 12 with *PO*. The same procedure was applied for bottles with substrate QER.

In 1-week interval 12 samples for each fungus-substrate combination was measured as well as 4 fungus-free glass bottles serving as a control (2 for each substrate). The duration of experiment lasted for 1 month until the substrate was completely overgrown with mycelium.

To measure mycelial respiration on substrate the glass bottles (covered with perforated foil and laboratory paper waddding) were placed on elastic rubber support to prevent leakage and sealed with chamber 3 b. Prior to measurement the parameters such as air pressure and air humidity were set up on recording device (Vaisala, Handheld MI70, Finland). Correction of CO_2 concentration to air temperature was done automatically. Small fan ensured sample air circulation within the chamber.

The measurement took 5 min, with the concentration being recorded every 5 s. From the CO_2 increase in chamber during the measurement we estimated the average minute increase (ΔCO_2) in the chamber (ppm min^{-1}). Under ideal conditions, the increase in CO_2 in the chamber was linear. The deviation from the linear trend, especially at the beginning of the measurement, was caused by a change in pressure gradient. For the computation of mycelium respiration we took only a linear portion of CO_2 concentration increase. Using the ideal gas law mycelial respiration was calculated with formula:

According to Drewitt et al. [19] using the ideal gas law:

$$\mathrm{Rm} = \left(\mathrm{P} \times \mathrm{V} \times \Delta \mathrm{CO}_2\right) / (\mathrm{R} \times \mathrm{T} \times \mathrm{A})$$

where: Rm—CO_2 flux (μmol CO_2 m^{-2} s^{-1}), *P*—atmospheric pressure (Pa), *V*—chamber volume (cm^3), *A*—floor plan of the chamber (cm^2), ΔCO_2—increase in CO_2 concentration (ppm min^{-1}), *T*—chamber air temperature (in °K), *R*—universal gas constant. Than, the CO_2 flux was converted to g CO_2 from 1 kg of substrate per 1 h (g CO_2 kg^{-1} h^{-1}).

The impact of factors (substrate, fungus species, time) and their interactions on mycelial respiration rate was assessed by Factorial ANOVA. The variance component analysis was used to divide the variance contribution of mycelial respiration into three components, one for each factor.

Experiment 2: The Temperature Sensitivity of Mycelial Respiration

The temperature sensitivity of mycelial respiration could be expressed as the Q_{10} index which is the increase of mycelial respiration by a 10 °C increase in temperature. For this purpose, chamber 4b was used. The influence of temperature on fungal mycelial respiration was expressed by a simple linear model:

$$\mathrm{Rm} = \alpha * e^{\beta * T}$$

where Rm is mycelial respiration, α and β are regression parameters and *T* is temperature in °C. Non-linear regression was used to fit the parameters with mycelial respiration rate. Q_{10} values was computed according to Linder and Troeng [20]:

$$Q_{10} = e^{(10 * \beta)}$$

Experiment 3: Detection of Temporal Changes, Estimation Of Biorhythms, Their Presence and Period in Time Scale from Mycelium Inoculation to Fruiting Bodies Formation

We used set 4 with automated chambers. Rye straw substrate without inoculum (control samples) and inoculated rye straw substrate with *Pleurotus ostreatus* were placed in $SacO_2$ polypropylene bags (type PP75/BEH6/V37-53 with 6 filter strips) and respiration rate in 2 automatic chambers was recorded from 290 to 331 DOY 2017.

We used wavelet analysis to identify temporal changes in mycelial respiration. Wavelet analysis is a powerful tool to analyse non-stationary signals and it permits the detection of main periodicities in a time series and the evolution of their respective amplitude, frequency, and lasting [21]. We used the Morlet wavelet which is a sinewave modulated by a classical Gaussian function, because it establishes a clear distinction between random fluctuations and periodic regions [22]. Following Grinsted et al. (2004) the dimensionless frequency was set to 6. The generated wavelet spectrum is a time-scale plot, where the *x*- and *y*-axis represent the position along time and periodicity scale, respectively, and the colour contour at each *x*/*y* point represents the magnitude of

the wavelet coefficient at that point. Statistical significance levels were estimated against a red noise model with lag-1 autocorrelations estimated from the observed time series [23].

Results

Selection of Method for Measurement of Mycelial Respiration

Respiration rate of planted mycelium measured by three systems on agar was generally at the lower limit of detectability for all systems. The reason was that the mycelium grown on the Petri dish was too small when compared to the chamber volume. This lead to very small concentration increase. The procedure based on set 3b has proven to be the most appropriate system for measuring very small CO_2 fluxes.

Unlike systems 1 and 2, the 3b system was a static chamber. In the dynamic systems 1 and 2 the air was constantly driven around the sample, which did not allow sufficient accumulation of CO_2. In the static chamber, low flow of CO_2 from mycelia could be compensated by longer measuring time. The longer the sample was stored in chamber 3b, the greater concentration of CO_2 was achieved. Using a Petri dish and a small chamber, the flows proved to be detectable in about 1–2 h. On the other hand, during such a long measurement, losses of CO_2 might occur due to imperfect tightness of the chamber. This type of measurement proved to be unsuitable for our study because of long time needed for accumulation and rise of CO_2 concentration as well as low measurement frequency (in the order of hours). However, results obtained from the static chamber 3b seem to be the most convincing method among the three tested commercially available chamber systems.

Mycelia respiration rate with wood chips and rye straw substrates were more suitable for our study aims. The amount of mycelia formed on these substrates was incomparably higher than in Petri dishes. The amount of substrate in system 3a, 4a and 4b sufficiently fills the chambers, thus the ratio between sample volume with mycelia and chamber size was much smaller than in a case of mycelia on Petri dish. Using this experimental setup, the mycelial respiration becomes detectable within few minutes.

Measurement of Mycelial Respiration in Wood Chips

Mycelial respiration rate (flux) of *GL* and *PO* on different substrate (*Fagus sylvatica* and *Quercus petraea* wood chips) during study period in g CO_2 per 1 kg of substrate and per 1 h shows variability plot in Fig. 1.

Variability plot (Fig. 1) shows that the respiration rates of mycelia were the highest on the 2nd week (2.w) of the study period and contains no significant outliers. The lowest respiration values were recorded on the 3rd week. To test for homogeneity of variance the Levene's test was applied ($P = 0.21$), so we cannot reject the null hypothesis that the variance is equal across all treatments.

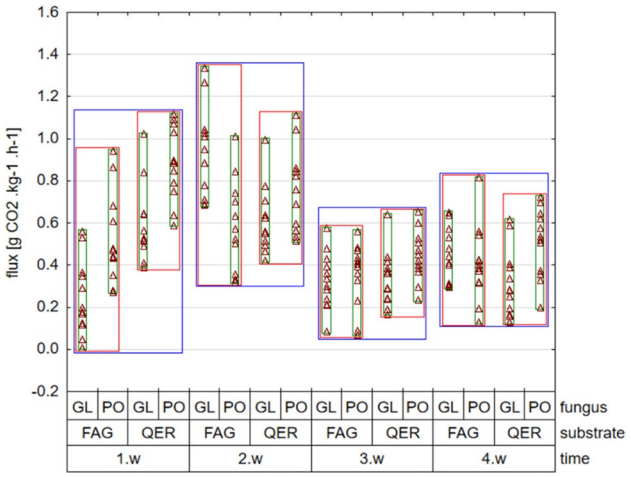

Fig. 1 One-hour mycelial respiration of *Ganoderma lucidum* (*GL*) and *Pleurotus ostreatus* (*PO*) in g CO_2 per 1 kg of beech (FAG) or oak (QER) substrate during 4 week study period

Summary table (Table 1) provides basic statistic information on mycelial respiration rates.

Multifactor ANOVA was used to assess the impact of three factors (type of fungus, substrate and incubation time) on mycelial respiration rate. Firstly, we assessed the impact of three factors without their interaction. Each of these factors have significant effect on the rate of mycelial respiration (Fig. 2), which is graphical representation of ANOVA.

The variance component analysis (Table 2) divides the variance contribution of flux into three components, one for each factor. Each factor after the first is nested in the one above. In this case, the factor contributing to the most of variance is fungus species. Its contribution represents 38.17% of the total flux variation. Factor time contribution is 23.71%. Impact of substrate on total variance is less pronounced. Error in this case 38.01 represents the unexplained variance contribution of the model.

The results of factorial ANOVA of mycelial respiration are presented in Table 3. Variability of mycelial respiration flux was decomposed according to contributions of various factors. Since Type III sums of squares have been chosen for analysis, the contribution of each factor is measured having removed the effects of all other factors. The *P*-values test the statistical significance of each of the factors. Since all seven *P*-values are less than 0.05, these factors and all the interactions have a statistically significant effect on flux (g CO_2 kg^{-1} h^{-1}) at the 95.0% confidence level.

Table 1 Mycelial respiration rate (g CO_2 kg^{-1} h^{-1}) of all treatments, where FAG and QER is abbreviation for wood chips type, *GL* and *PO* is abbreviation for fungal type, *w* is week

Treatment	Average	Standard deviation	Coeff. of variation	Minimum	Maximum	Range
FAG_GL_1.w	0.25	0.18	0.72	0.01	0.56	0.56
FAG_GL_2.w	0.95	0.21	0.22	0.69	1.34	0.65
FAG_GL_3.w	0.33	0.13	0.41	0.08	0.58	0.49
FAG_GL_4.w	0.44	0.13	0.29	0.30	0.65	0.35
FAG_PO_1.w	0.52	0.21	0.41	0.27	0.94	0.67
FAG_PO_2.w	0.57	0.22	0.39	0.32	1.01	0.69
FAG_PO_3.w	0.35	0.15	0.43	0.07	0.56	0.49
FAG_PO_4.w	0.41	0.18	0.44	0.13	0.81	0.69
QER_GL_1.w	0.59	0.18	0.30	0.39	1.02	0.63
QER_GL_2.w	0.62	0.16	0.25	0.42	0.99	0.57
QER_GL_3.w	0.34	0.13	0.39	0.17	0.64	0.47
QER_GL_4.w	0.32	0.16	0.51	0.13	0.62	0.49
QER_PO_1.w	0.88	0.17	0.20	0.59	1.12	0.53
QER_PO_2.w	0.74	0.20	0.28	0.52	1.11	0.60
QER_PO_3.w	0.44	0.12	0.27	0.24	0.65	0.42
QER_PO_4.w	0.51	0.16	0.32	0.20	0.72	0.53
Total	0.52	0.26	0.50	0.01	1.34	1.33

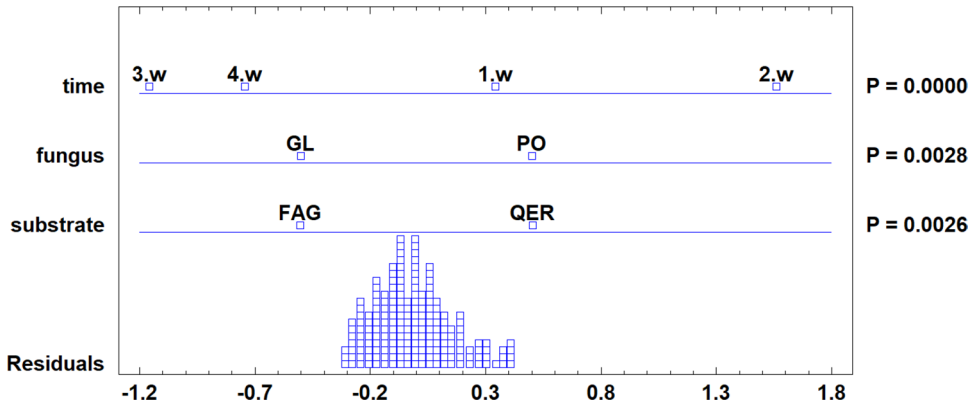

Fig. 2 Graphical representation of ANOVA for mycelial respiration (g CO_2 kg^{-1} h^{-1}) shows the deviations of each factor mean from the grand mean are scaled so that their spread may be compared to that of the residuals. Factor levels separated by more than that exhibited in the residual distribution correspond to significant differences

Table 2 Table of variance component analysis

Source	Sum of squares	Df	Mean square	Var. Comp	Percent
Total (corrected)	12.6426	191			
Time	3.5359	3	1.17863	0.0170714	23.71
Substrate	1.4356	4	0.35892	0.0000755	7.11
Fungus	2.8557	8	0.35696	0.0274788	38.17
Error	4.8153	176	0.02735	0.0273599	31.01

The interaction effect among the factors is presented in Fig. 3. The impact of interaction between fungus species and substrate on mycelial respiration is captured in Fig. 3a which displays box-plots with letters indicating difference derived by Tuckey HSD post.hoc test. Significant difference denotes different letters. From the Fig. 3a it is obvious that respiration from PO > GL on both of the substrates FAG and QER.

Interaction effect between time and fungus displays Fig. 3b. Significant difference between fungus species occurred only in the first half of the experiment. Within the observed period *PO* retains higher values of respiration except the 3rd week when the values were comparable. For *PO* the differences of mycelial respiration between first and second half of experiment were significant as indicated by different letters (Tuckey HSD post-hoc test). Respiration of

Table 3 Analysis of variance for flux (g CO_2 kg^{-1} h^{-1})—type III sums of squares

Source	Sum of squares	Df	Mean square	*F*-ratio	*P*-value
Main effects					
A:substrate	0.276219	1	0.276219	9.31	0.0026
B:fungus	0.272792	1	0.272792	9.20	0.0028
C:time	3.63509	3	1.2117	40.86	0.0000
Interactions					
AB	0.512371	1	0.512371	17.28	0.0001
AC	1.33998	3	0.446659	15.06	0.0000
BC	1.02498	3	0.341661	11.52	0.0000
ABC	0.420681	3	0.140227	4.73	0.0034
Residual	5.21976	176	0.0296577		
Total (corrected)	12.7019	191			

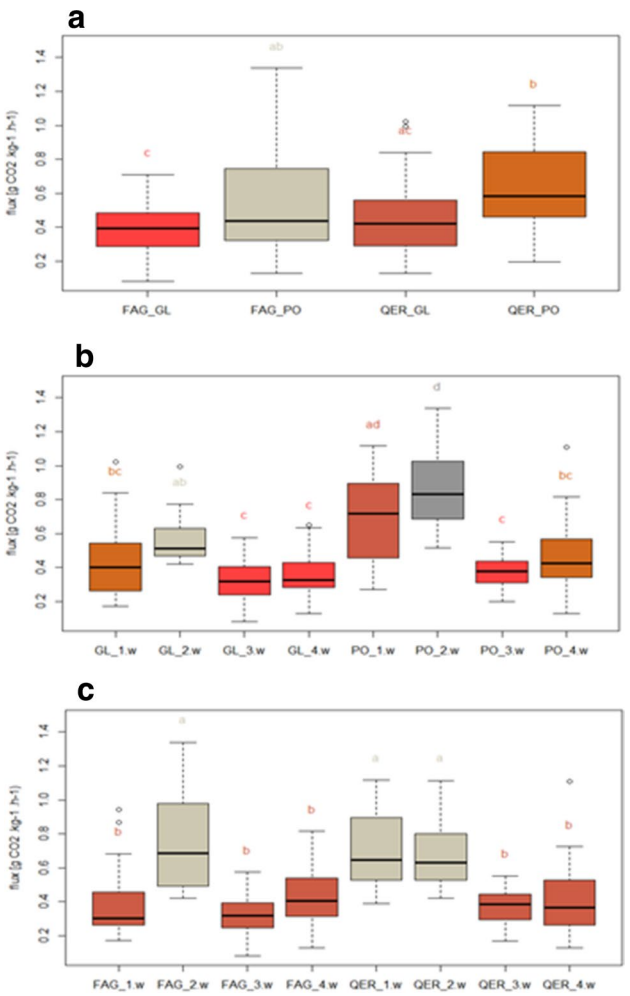

Fig. 3 Interaction effect among the factors on mycelial respiration. Interaction effect between substrate (FAG, QER) and fungus type (*PO*, *GL*) (**a**); Interaction effect between fungus (*GL* and *PO*) (**b**) and between substrate (FAG, QER) (**c**) and time of incubation in weeks

GL peaked in the 2nd week and significantly differed from the last 2 weeks.

Interaction effect between factors time and substrate on flux values is shown in Fig. 3c. According to mycelial respiration two significantly different groups were distinguished. First one with higher respiration rate contains substrate QER in first and 2nd week of experiment and substrate FAG in 2nd week. The second group with significantly lower mycelial respiration contains all remaining combination. They are forming a homogenous group with no statistical differences between mycelial respiration rate (Tuckey HSD post-hoc test).

The temperature sensitivity of mycelial respiration was estimated from 479 measurements with chamber set 4b in laboratory conditions. Temperature span ranged from 16.0 up to 23.0 °C. Figure 4 shows mycelial respiration dependence on ambient temperature. The value of derived Q_{10} was 2.19. It means that increase of ambient temperature leads to double increase of mycelial respiration rate.

Temporal Dynamic of Mycelial Respiration

Temporal dynamic of mycelial respiration was studied by automatic chambers (set 4a and 4b). Set 4a contained inoculated substrate with *PO*, while 4b served as a reference with no fungus inside. Measurement lasted from 290 to 331 DOY Fifteen minutes course of mycelial respiration is displayed in upper part of Fig. 5.

Respiration slightly declined from the beginning of observation up to 300 DOY 2017 (totally 10 days). We observed decline from 0.75 to 0.35 g CO_2 kg^{-1} h^{-1}. Mycelial respiration culminated on 306 DOY (1.6 g CO_2 kg^{-1} h^{-1}) which coincided with fungal primordia formation. On 311 DOY 2017 the formation of small fruit bodies was completed and mycelial respiration declined to 0.93 g of CO_2. Slight decline of mycelial respiration continued to the end of study period but diurnal dynamic became more pronounced. Deeper insight into respiration dynamic offered Morlet wavelet analysis which enables detection of main periodicities (or

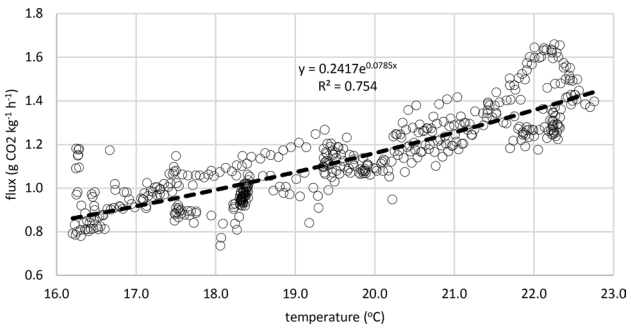

Fig. 4 Mycelial respiration rate (g CO_2 kg^{-1} h^{-1}) versus ambient temperature plot fitted with exponential curve

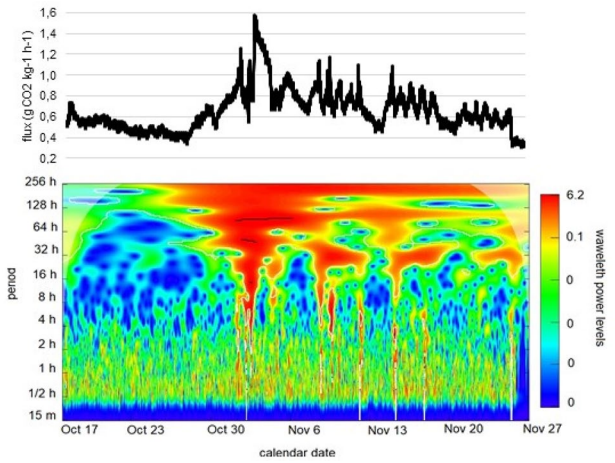

Fig. 5 Temporal course of mycelial respiration (upper part) and wavelet power spectrum. Where Oct17 = 290, Oct23 = 295, Oct30 = 303, Nov6 = 310, Nov13 = 317, Nov20 = 324, Nov27 = 331 DOY

biorhythms). A dark red color in bottom part of Fig. 5 is assigned to the highest value of the wavelet power spectrum, whereas a dark blue color is assigned to the lowest value. The wavelet analysis was performed on 15 min intervals. From beginning of experiment until 293 DOY no periodicities were observed. Periodical events with low frequencies (above 128 h) occurred since 296 DOY which coincided with the increase of mycelial respiration.

Fungal fruiting body formation was accompanied by high frequency cycles (4 h) of mycelial respiration. During this period of fruiting body formation statistically significant both high and low frequency cycles occurred. Later, when fruiting body formation was completed, in the wavelet power spectrum (region between 16 and 32 h) we observed diminishing diurnal signal (red regions disrupted with green and blue spots) on wavelet power spectrum in lower part of Fig. 5 corresponding to the dates around DOY 317 and 326. During whole experiment the periodicities of high frequencies ranging from 0.5 to 2 h were present and were discreetly distributed. The low frequency periodicities remain significant for long and continuous time periods.

Discussion

The main reason for the interest of growers, sellers and researchers in fungal respiration is to maintain high quality of fruiting bodies for as long as possible. To maintain the quality of fruiting bodies it is required to know the respiration rate to prevent undesired aging and damage [24]. The respiration rate is determined either by consumption of O_2 or CO_2 production. The ratio of produced carbon dioxide and consumed oxygen—respiratory quotient (RQ)—is a marker for the type of metabolism, as the values over 1 suggest anaerobic metabolism, while values under 1 indicate aerobic type [25].

Measuring Method

Most of the methods presented in literature for estimation of fungal respiration are rather complicated, often requiring special laboratory equipment [26, 27]. We choose those based on direct measurements by commercially available infra-red probes and closed chamber techniques which are commonly used in CO_2 flux detection in wide array of ecophysiological research, e.g. ecosystem and soil respiration [28] or deadwood respiration [29, 30]. In our study the dynamic measuring systems (Licor XT6400, EGM4 PPSystems) did not provide reliable values due to low CO_2 efflux from measured samples in Petri dishes. No doubt that technical adaptation of high-tech instruments could provide suitable tools for low rate respiration detection, but we tried to find low-cost, easy to use and reasonably reliable method. Static chambers equipped with Vaisala GMP343 probes offered longer measuring interval and easier CO_2 change detection influenced by a sample size. Fungi cultivated in 4 L bottles or bags filled with 1 kg of different substrate provide detectable fluxes. Estimation of time required from inoculation to fruiting body stage, circadian dynamic, temperature and light requirements or substrate influence on respiration rate were further advantages of bottle and bag methods. Here we have to note that automatic self-built chambers allowed continuous adaptation to growing fruiting body size and thus high temporal resolution of mycelial respiration, but on the other hand only limited sample size could be measured. Manually recorded respiration offered measurement from 48 samples with limited temporal dynamic.

Dead wood is natural substrate for saprophytic fungi. To study temporal changes of mycelial respiration in laboratory conditions would be timely demanding. To accelerate fungi growth and perform methodological verification we used wooden chips and a rye straw in our laboratory experiments.

Respiration Rate

Separation of saprophytic fungi respiration from substrate is not possible due to critical fungi dependency on decaying wood and massive substrate overgrown by mycelium hyphae. Saprofytic fungi respiration rate values are rather rare in literature. Respiration rate 2.4 g CO_2 kg^{-1} d^{-1} (equal to 566 kg C y^{-1} ha^{-1} from 1960 kg deadwood ha^{-1} reported Rinne-Garmston et al. [27]. The CO_2 emission rates for angiosperm 78.3 g C m^{-3} d^{-1} and 40.8 for conifers published Kahl et al. [29]. In Siberian taiga forest Gitarskiy et al. [30] identified 145–462 kg C ha^{-1} y^{-1} emission rate. On the 15 year old wind throw the authors of this paper measured

respiration rate of 670 kg C ha^{-1} during growing season (unpublished). More common are information about mutualistic or parasitic species. For example Heinemeyer et al. [26] published 0.2 µg C g^{-1} d^{-1} from arbuscular mycorrhizal mycelia. Average mycelial respiration rate determined in our study (0.52 g CO_2 kg^{-1} h^{-1} equals 3.3 µg C g^{-1} d^{-1}) is notably higher. Higher respiration rate in saprophytic than in mutualistic and parasitic fungal species pointed out Trocha et al. [31]. Saprophytic fungi require higher enzymatic activity than mutualistic taxa, perhaps because of the high metabolic costs of enzyme secretion to decompose litter and woody debris [32].

Mycelial Respiration Factors

We identified different rates of mycelial respiration for *PO* and *GL*. *PO* showed higher values on both of the substrate types. Large difference occurred mostly in the first 2 weeks after inoculation, later differences diminished due to earlier senescence of *PO* than *GL*. This is in accordance with findings of Sudheer et al. [33] that higher respiration rate in mushrooms with faster aging and senescence, and they explained faster maturation of fruiting bodies causing general deterioration of cells and loss of moisture from fungal tissues.

Mycelial respiration rate of saprophytic fungi is strongly influenced by substrate [29, 34]. According to Belletini et al. [35] the use of different types of substrate by fungus depends on its capacity to secret enzymes involved in lignocelluloses decomposition. We found significant difference between oak and beech substrate for both fungal species. Higher respiration rate showed oak (average 0.54 g CO_2 kg^{-1} h^{-1}) than beech (0.46 g CO_2 kg^{-1} h^{-1}). This is in contradiction to Rock et al. [36] who reported higher CO_2 rates from beech. It is rather likely that chips also used in our study might differ from intact deadwood properties, as pointed out by Kahl et al. [29]. The main drivers associated with substrate type are N and P concentrations, and C/N ratio [37].

Despite well-known relation between temperature and enzymatic processe very little research has addressed the effect on fungal respiration [38]. Muller et al. [39] reported optimal temperature span for growth and respiration rate between 22 and 28 °C. High temperatures might lead to degradation of organelle membranes, enzymes and poor function of other cellular constituents [40]. In our study we observed exponential behavior of respiration rate on temperature. This relationship is in contrast with unimodal Gausian function proposed by Lilleskov [41]. Small temperature span in our study ranged between 16 and 23 °C and led to smooth exponential respiration response to temperature ($R^2 = 0.75$). Q_{10} index derived from exponential curve showed stable values (2.19). This value is similar to Malcolm et al. [38] who reported Q_{10} from 1.7 to 2.6. Lilleskov [41] reported $Q_{10} = 3.7$ for sporocarp respiration. Q_{10} values are strongly influenced by specific field, or laboratory conditions, for this reason the results should be compared with caution.

Light is understood as controlling factor of ectomycorrhizal respiration, which is explained by coupling between host tree and mutualistic fungus. Saprophytic fungi unlike ectomycorrhizal species are not assimilate-dependant thus do not exhibit circadian course of carbon respiration. However, the impact of light on saprophytic mushrooms is evident e.g. in sporocarp shape and chemical constitution [42]. Difference between light and dark conditions respiration in our study were neglible (0.63 vs 0.68 g CO_2 kg^{-1} h^{-1}).

Mycelial respiration culminated 16 days after inoculation, which coincides with Wilkinson et al. [43] who reported peak respiration after 20 days. In our study peak respiration coincided with an early stage of the fruiting body formation when increased from 0.5 to 1.6 g CO_2 kg^{-1} h^{-1}. After fruiting body formation respiration rate decline to approx. 0.8 g CO_2 kg^{-1} h^{-1}. We can conclude that mycelium contributed approx. 0.5 and sporocarp 0.3 g CO_2 kg^{-1} h^{-1} after formation of fruiting body.

Studies on diurnal changes of fungal respiration are rare. In ectomycorrhizal species is often seen as a consequence of temperature variation and/or organic supply by host tree [26]. Stable laboratory temperature eliminated the impact of diurnal variation in our study. Also variation of daily organic supply is not a case in our study. However, wavelet analysis revealed significant daily variation which was present during fruiting body formation stage. We hypothesize these periodicities reflect endogenous circadian rhythms occurring probably only during fruiting body formation. To verify this statement further study is needed. As far as we know this is the only work exanimating endogenous cycles of saprophytic fungi respiration.

Conclusions

We compared four different systems based on commercially available CO_2 probes for estimation of mycelial respiration of two important fungal species (*Pleurotus ostreatus* and *Ganoderma lucidum*). Using Vaisala GMP343 probe in combination with simple automatic Plexiglass chamber proved to be the most appropriate method, as it is reasonably priced and resistant to fungal spores.

Besides fungal and substrate type, we identified incubation time as significant factors for mycelial respiration rate. Early stage of growth connected with mycelium formation is metabolically demanding and thus produces higher CO_2 flux. Low CO_2 flux before fructification indicates possible latent stage in mushroom metabolic activity. Presence of mushroom fruiting bodies did not stimulate higher CO_2 flux.

Acknowledgements The study was supported by grants of the Slovak Research and Development Agency No. APVV-17-0644, APVV-16-0325 and grants of the Slovak Grant Agency KEGA No. 006TU Z-4/2019 and VEGA 1/0367/16.

Author Contributions MP prepared substrates, inoculated fungi, wrote paper. PF Sr. run the experiments, interpreted the measurements. PF Jr. designed the experiment, run the statistical analysis, disccused the results. MP Jr. prepared fungal cultures and substrates, linguistic text editing. MŠ working with samples, laboratory measurements.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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