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An approach to calculate CO_2 release through Norway spruce wood decay by *Heterobasidion parviporum*

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Abstract: Fungi such as *Heterobasidion* spp., decomposing cellulose and lignin in the cell walls of tree roots and stems, cause widespread diseases in conifer forests, resulting in considerable economic losses in timber crops. In addition, they significantly contribute to increased atmospheric CO_2 levels. The aim of the present laboratory study was to evaluate the amount of CO_2 released during the decay of spruce wood samples by six isolates of *H. parviporum* over periods of 3 and 6 months. An original formula for the CO_2 release from decayed wood was developed and calculated. After 3 and 6 months, the average wood loss accounted for 13.6 and 22.1%, respectively. The estimated CO_2 release was 65.14 and 105.86 kg/m^3, respectively. The proposed conversion method represents a useful tool for the monitoring of CO_2 emissions from wood decay, esp. in the context of global climate change.

Keywords: *Picea abies*, wood decomposition, white rot fungus, CO_2 release formula

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Introduction

Wood-colonizing fungi produce a variety of enzymes, facilitating the decay of cellulose, lignin, and hemicellulose complexes in the cell walls. This contributes to the decomposition of the cell wall structure during substrate colonization (Schwarze et al., 2000; Nagy et al., 2012). The decay of cellulose bonds leads to the release of D-glucose, which is further oxygenated to water and carbon dioxide, generating substantial amounts of energy (Schwarze et al., 2000; Chapin et al., 2002; Hammel et al., 2002). Carbon dioxide release as a result of tree death and subsequent wood decay significantly contributes to increased CO_2 concentrations in the atmosphere (Kirschbaum, 2000; Watkinson et al., 2006; Hietala et al., 2015).

Generally, studies focusing on CO_2 issues provide information about the plants, the role of the oceans, or the processes responsible for the destruction of organic matter or the disturbance of soil activities

(Paul & Clark, 1996; Jobbágy & Jackson, 2000; Lal, 2008; Rykowski, 2008). Details often refer to specific biological system tests and usually provide data on the gas exchange and photosynthesis of aboveground parts, litter, fine roots, or microorganisms as the main CO_2 producers in soil, mainly under controlled conditions (Cairns et al., 1997; Oleksyn et al., 1999; Weigenand et al., 2008).

In complex ecosystems such as forests, the measurement of the carbon content in the root biomass or the amount of CO_2 released via respiration and organic matter decay in the soil is methodologically extremely difficult (Cairns et al., 1997; Rykowski, 2000; Janssens et al., 2001), irrespective of whether the values were estimated or indirectly interpolated (Newell & Fallon, 1991; Chen et al., 2000; Kueppers et al., 2004).

Pathogens of the genus *Heterobasidion* are the main perpetrators of diseases of forest trees, especially conifers, in the entire northern hemisphere. In Europe, the dieback of trees and the raw material losses (as a result of dying and decay) are estimated to reach 800 million Euros annually (Woodward et al., 1998; Asiegbu et al., 2005). The CO_2 release as a result of root decay caused by root rot pathogens (*Heterobasidion annosum* and *Armillaria* spp.) was evaluated by Sierota (2012) in 80-year-old Scots pine stands in Poland. The selected method was based on the Cartesian product calculation (the product set) $E_i = E_{i-1} * k_i$ (where E_i is the predicted value of i calculation stage and k_i is the coefficient suitable for a given calculation stage). The final algorithm contains the result of subsequent transformations and is based on the share of infected trees in a tested age class of pine stands, the weight of tree roots, the share of cellulose in the root wood, and the loss of cellulose degraded by fungi. Finally, the algorithm's result was presented using stand volume mass (G) and the calculated coefficient. Subsequently, the CO_2 release (DW) as a result of wood cellulose decay by root rot fungi of a tested stand per 1 hectare was stated as DW = 130.4*G [kg].

Sierota (2012) found that due to the impact of root pathogens in the described stand, 60.25 t/ha of CO_2 were released, amounting to a release of 16.4 t/ha of sequestered carbon. However, so far, this issue has not been investigated in spruce stands. In this context, the aim of this study was to determine the amount of CO_2 released during the decay of spruce wood by *H. parviporum* over a period of 3 and 6 months under laboratory conditions. We tested the following hypotheses: i) the different isolates of *H. parviporum* differently decay the inoculated wood samples under laboratory conditions, and ii) the pattern of the CO_2 release, as a result of wood decomposition by the pathogens, allows to calculate the share of CO_2 in the carbon balance in the atmosphere.

Material and methods

Samples of Norway spruce wood with a size of 1.0 × 2.5 × 5.0 cm were obtained from sapwood of healthy 40-year-old Norway spruce [*Picea abies* (L.) Karst.] trees 40 cm above ground level in a stand located in the mountainous Krościenko Forest District, Poland, 650 m above sea level. The selected stand has suffered serious damage by the pathogen *Heterobasidion parviporum* (Żółciak et al., 2016). The samples used in the tests had a similar wood density (Sierota et al., 2016), assessed stereometrically according to the formula: $\rho = m/V$, where m = dry mass of the sample (g) and V = volume of the sample (cm^3).

In total, 120 wood blocks were tested. To calculate initial dry wood mass, the samples were dried at 105°C, weighed, and standard autoclaved at 108°C under 0.05 MPa. The sterile samples, treated as "fresh", after saturation with sterile water to 70% (Sierota, 1997), were transferred to Kolle flasks on 2% malt-extract agar (MEA) with growing, three-week-old *Heterobasidion parviporum* mycelium and incubated at 24°C for three weeks in darkness (with 10 repetitions). Six strains of the pathogen, originated from stumps in three Norway spruce stands in the Krościenko Forest District (650–1,050 m above sea level) with previously tested different pathogenicity, were used (GenBank codes in parentheses): Hp1 (KX289697), Hp7 (KX289698), Hp3 (KU645328), Hp4 (not registered), Hp5 (KU645329), and Hp6 (KU645330) (Żółciak et al., 2016). To each flask, we added two wood samples with similar mass (10 replicates) and incubated the samples for a period of 3 (sample 1) and 6 months (sample 2). After the incubation time, the samples were removed, the mycelium was scrapped off, and the wood blocks were dried at 105°C to constant weight. The loss of dry wood represented the isolates' activity (Żółciak et al., 2016) and was used in CO_2 release calculations. The values were log-transformed and differences between means were analyzed by two-way ANOVA, post-hoc HDS Tukey´s test and Student-Newman-Keuls' test, using the software package Statgraphics Centurion 2010.

To calculate CO_2 release, the following elements of Sierota's algorithm (Sierota, 2012) were used: i) carbon (c) contribution in spruce wood as approximately 48% of the wood weight (Prosiński, 1984; Sjöström, 1993; Räisänen & Athanassiadis, 2013), ii) molecular weight of the cellulose $(C_6H_{10}O_5)_n$ as 162_U, mass of carbon dioxide as 44_U (unified atomic mass unit), and mass of carbon 12_U, which means that conversion factor from carbon mass to the carbon dioxide mass d = 3.67 (Krzysik, 1978; Prosiński, 1984).

Consequently, an algorithm for carbon dioxide release was proposed as CRI index:

$$CRI = DM/VS \times WD_x \times c \times d \ [g/cm^3] \quad [1]$$

where VS is the volume of the sample (cm^3), WD_x is the dry weight loss (%) in the period o–x, DM is the dry mass of the sample (g), c and d are defined as above.

This algorithm can further be simplified because the parameters c and d are constant value = 1.76, and DM/VS is a wood density [ρ]. Therefore,

$$CRI = 1.76 \times \rho \times WD/100 \times 1000 = 17.6 \times \rho \times WD_x \ [kg/m^3]. \quad [2]$$

In this study, the mean volume of the sample (VS) was 12.5 cm^3, while the dry weight mass (DM) prior to degradation averaged 3.4 g, with a variance coefficient < 12%; average density (ρ) was 0.272 g/cm^3.

Results

The isolates of *Heterobasidion parviporum* showed different activities in the two periods. After 3 months, dry weight loss ranged from 11.3% (Hp4) to 17.8% (Hp1), with an average WD_3 of 13.6% ($F_{3.58}$; p=0.0072) (Fig. 1). Although after 6 months, dry weight loss ranged from 19.3% (Hp4) to 24.9% (Hp3), with an average WD_6 of 22.1%, the results were not statistically different ($F_{1.41}$; p = 0.2367). Isolate Hp4 showed the lowest activity, whereas isolates Hp1 and Hp3 had the highest activities in both experiments (Fig. 1).

According to the formula proposed above, the average amounts, and SD values, of CO_2, released by cellulose degradation in the Norway spruce wood samples (CRI) by the tested isolates of the pathogen *H. parviporum* during 3 and 6 months are presented in Table 1.

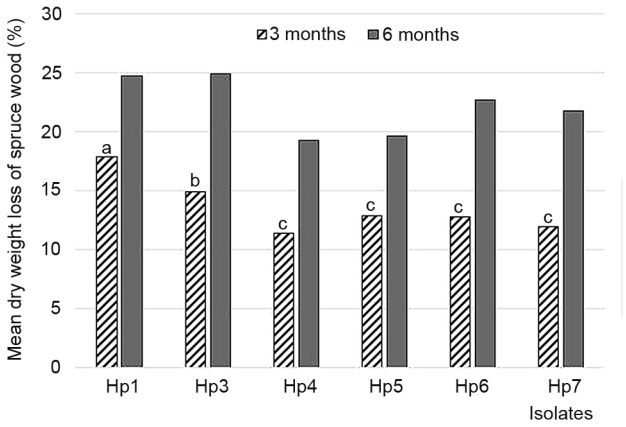

Fig. 1. Mean loss of dry mass of samples after 3 and 6 months of decay by the tested isolates. Values with the same letter are not statistically different at p ≤ .05

Converting the obtained data to units expressed in kilograms of CO_2 emitted from one cubic meter of decayed wood after 6 months of decomposition results in an average value of 105.86 kg (~106 kg).

Discussion

The amount of carbon dioxide released via wood decomposition by fungi is generally a function of both the activity of the pathogen, the wood density, and the specific environmental conditions (Rayner & Boddy, 1988; Boddy, 2001; Eriksson et al., 2012; Sierota et al., 2016; Żółciak et al., 2016). The weight of spruce wood differs depending on its moisture content; the USDA Wood Database states an average air dried weight of 405 kg/m^3, whereas Surmiński (2007) indicates a weight of 340, 470, and 680 kg/m^3, depending on the moisture content. The density

Table 1. Calculation data to assess CO_2 release by the tested *H. parviporum* isolates

Isolate	Period of decay (months)	Wood decay index [%] (WD_x)	CO_2 release (kg/m^3) (CRI)	Standard deviation (SD)
Hp1	3	17.8	85.26^{a}	0.676
	6	24.7	118.31^{A}	3.629
Hp3	3	14.9	71.37^{b}	0.705
	6	24.9	119.27^{A}	3.628
Hp4	3	11.3	54.13^{c}	0.934
	6	19.3	92.45^{C}	1.675
Hp5	3	12.8	61.31^{c}	2.368
	6	19.6	93.88^{C}	2.089
Hp6	3	12.7	60.83^{c}	2.040
	6	22.6	108.25^{AB}	2.839
Hp7	3	11.9	57.00^{c}	3.158
	6	21.8	104.42^{B}	3.132
Average	3	13.6	65.14	4.847
	6	22.1	105.86	5.035

Means with the same lowercase letter for the period of 3 months and the same capital letter for the period of 6 months do not significantly differ at p ≤ .05.

of Norway spruce wood can vary greatly, depending on the humidity at the time of measurement, tree age or site, and the climatic conditions (Chen et al., 2000; Makinen et al., 2002, 2008). Contrary, Repola (2006) found that in some sites in Finland, the density of Norway spruce wood, dried at 106°C, ranged between 384 and 386 kg/m^3. In Poland, spruce wood density ranged from 390 kg/m^3 for dried samples (Szaban et al., 2014) to 800 kg/m^3 for fresh sapwood after felling (ITD, 2006); the latter value was adopted in the current study.

Żółciak et al. (2016) have shown that during 6 months, *H. parviporum* degraded 21.6–24.7% of spruce wood mass, whereas *Phlebiopsis gigantea* could degrade up to 45% in Scots pine. In the course of one year, fungi decompose about 80% of available carbohydrates in the root wood of different tree species (Liese & Stamer, 1934; Kirk & Moore, 1972; Gilbertson & Ryvarden, 1987; Sierota, 1997); however, Nagy et al. (2012) suggest a rather slow wood decay in the reaction zone of infected Norway spruce stems.

In the present paper, the average wood decay of Norway spruce samples by different *H. parviporum* isolates was 22% after 6 months, depending on the specific enzyme activity of the pathogen. The role of enzyme activities in wood decay by *H. parviporum* was described by Żółciak and Bohacz (2016), while for *P. gigantea*, it was described by Żółciak et al. (2008, 2012). The results described by Zółciak et al. (2016), re-calculated according to the presented algorithms [1–2], show that CO_2 emission – as a result of decomposition of Norway spruce wood by *H. parviporum* – ranged between 44.6 and 51.1 t/m^3. In the present paper, such decomposition caused an average CO_2 release 65–106 kg/m^3 between 54 and 119 kg/m^3 after 6 months.

In Norway spruce stands in the Iława Forest District (Masuria, northern Poland), the amount of decayed timber as a result of *Heterobasidion* butt rot as accounted for 920.07 m^3 for pulpwood and for 162.65 m^3 for firewood, amounting to 16% (1.80 m^3/ha) of all harvested spruce timber in 2016 (personal communication). Taking into account the above data for Iława FD and the proposed algorithm, we infer that as a result of wood decay by *Heterobasidion*, about 117 kg of CO_2 per 1 hectare were released until 2016. Comparing the results with data presented by Sierota (2012) for more threaten Scots pine stands, but recalculated for 1 hectare (60.25 t/ha), we can conclude that both root rot pathogens – *H. annosum* in Sots pine and *H. parviporum* in Norway spruce stands – play an important role in the CO_2 release, both locally and globally. Cronan (2003) described that during the vegetation period, the total CO_2 flux from soil respiration was 69 g C·m^2·month^{-1}. Similarly, Borken et al. (1999) found that in 1993, the emission rate from soil was 2,981 kg C·ha^{-}1·yr^{-1}, but increased to 4,813 kg C·ha^{-}1·yr^{-1} in 1994. Lal (2008) reported that the global carbon sequestration potential of soils varies largely, from 0.4 to 1.2 Gt C/year. However, in the 1990s, the annual increment of atmospheric CO2 was 3.3 Pg C/year (Lal, 2004).

The values presented in this paper are the results of the first methodical attempt to evaluate the level of carbon dioxide release from Norway spruce wood colonized by *H. parviorum*. It is our hope that this paper stimulates further discussions about the plasticity of fungi (Wrzosek et al., 2017) in forests not only in regard to root pathogens, but also in terms of the role of saprotrophs in CO_2 release and carbon sequestration in dead wood.

Conclusion

In this study, the decomposition of spruce wood by the different isolates of the pathogen *Heterobasidion parviporum* during the course of 6 months resulted in significant, but varying carbon dioxide release (average 0.11 g/cm^3). This probably means that not all stands were equally infected with the pathogen and that the inner wood was not decayed similarly in all cases. Based on our results, we inter that the decay of 1 m^3 of spruce wood would result in the emission of ~106 kg of CO_2 into the atmosphere. Although the proposed algorithm is based on the results of laboratory tests, it seems to be a useful, albeit simplified method to calculate CO_2 release from wood decay by wood-colonizing fungi. Given the specific nature of *H. parviporum* decay in roots, stumps, and in the heartwood of standing spruce trees (Garbelotto & Gonthier, 2013), as well as the volume of decayed wood (timber), we can calculate the CO_2 release according to the scale of the threat, stand age, and pathogen activity.

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