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Correct calculation of CO_2 efflux using a closed-chamber linked to a non-dispersive infrared gas analyzer

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Summary

1. Improved understanding of the carbon (C) cycle is essential to model future climates and how this may feedback to affect greenhouse gas fluxes.

2. We summarize previous work quantifying respiration rates of organic substrates and briefly discuss how advances in technology, specifically the use of chambers linked to a non-dispersive infrared gas analyzer (NDIR), can be applied to assess carbon dynamics from short-term field measurements. This technology hastens measurement and is relatively inexpensive, enabling researchers to increase replication and investigate temporal and spatial variation.

3. We describe the theory behind calculations of CO_2 efflux released through organic substrates, when using a closed-chamber linked to a NDIR. These methods can in principle be extended to any chamber-based measurement of gas fluxes, including partially closed chambers as used for soil surface CO_2, nitrous oxide or methane effluxes and stem CO_2 respiration, although additional assumptions may apply.

4. We show that incorrect application of formulae in some earlier studies resulted in either under- or over-estimation of CO_2 effluxes. Of the studies, we reviewed measuring the respiration of woody debris, leaf litter or woody stems using closed chambers linked to a NDIR, only 22% (11 of 51) provided the equations used to calculate CO_2 efflux, and 72% (8 of 11) of those provided contained basic errors. Using our data on the decomposition of woody debris as an example, we found that such mistakes resulted in anywhere from 8% underestimation to 22% overestimation of CO_2 efflux. The errors varied among studies and hence may limit understanding of the factors affecting emissions of CO_2 and our ability to incorporate this knowledge into global carbon models.

5. We provide formulae for the correct calculation of respiration rates in future studies using closed chambers and thus provide a basis for comparative studies of factors affecting CO_2 efflux from woody debris, leaf litter and other substrates. Ultimately, this will contribute to improved parameterization of forest respiration.

Key-words: carbon cycle, climate change, decomposition, ecosystem process, ideal gas law, infrared gas analyzer (IRGA), leaf litter, prediction, stem, woody debris

Introduction

Improved understanding of the carbon (C) cycle is essential to model future climates (Luo, Keenan & Smith 2015). The ecosystem C cycle consists of assimilation of C through photosynthesis and C release through respiration (Cornwell & Weedon 2014). The first has been widely studied and can be modelled at various scales with reasonable confidence. However, respiration remains relatively poorly understood (Subke, Inglima & Cotrufo 2006; Trumbore 2006). Respiration includes components from living above-ground biota, decaying material in soil, including roots and leaf litter, and the decomposition of woody debris (Tang *et al.* 2008). Relatively few studies have investigated woody debris and leaf litter respiration (Wu *et al.* 2010; Yoon *et al.* 2014). The residence time of carbon, defined as the difference between the time when CO_2 is assimilated into a given system and the time at which CO_2 returns into the atmosphere (Baldocchi 2014), varies substantially among different carbon pools (Luyssaert *et al.* 2007). Woody debris has longer residence times than leaf litter and fine roots. Hence, the paucity of empirical studies on the contribution of some carbon pools, such as woody debris, to the C cycle results in a poor understanding of C dynamics.

Recent advances in technology to monitor trace gases, including carbon dioxide (CO_2), with accurate, less time-

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consuming and cost–effective devices (Huitchinson & Livingston 2001; Yasuda, Yonemura & Tani 2012; Harmon *et al.* 2015) offer opportunities to extrapolate results from short-term measurements to long-term process predictions. Infrared gas analyzers, of which non-dispersive infrared gas sensors (NDIR) are by far the most common in operation today, can be connected to chambers to measure gas efflux. Over the past decade, researchers have applied such methods to measure respiration of woody debris and other substrates, such as leaf litter. Chamber-based methods allow researchers not only to conduct experiments under near-natural conditions, but also to obtain more data and greater replication over relatively short times (Davidson *et al.* 2002; Ngao *et al.* 2006). These advantages have enabled researchers to study temporal and spatial variation in respiration (Vanderhoof *et al.* 2012). Improved accuracy and a greater range in field conditions examined will ultimately reduce uncertainties in predictive models.

However, improved accuracy in measurements of CO_2 concentrations cannot help whether inappropriate formulae or incorrect parameters are applied when calculating CO_2 effluxes. The issues discussed here are relevant to any gas measurement from organic substrates, including soil, stem, leaf litter and woody debris respiration using chambers linked to a NDIR. However, we focus on methods employing a closed chamber because of the simpler set of assumptions, as compared to partially open systems. Many specific methodological issues with the use of partially open chambers are far from settled. For example, for the measurement of soil surface fluxes, there is still considerable debate concerning, (i) whether an assumption of gaseous equilibrium between soil and chamber is reasonable, (ii) the effects of variation in gas diffusion gradients within the soil, (iii) the origin of emitted gas and (iv) the effects of collar insertion into the soil (Heinemeyer *et al.* 2011). For measurements of stem respiration, debate still surrounds the issue of how to estimate the escape of dissolved CO_2 and its translocation from emission source *via* the xylem stream.

From our review of the literature, we found 36 publications on woody debris respiration (Table S1, Supporting information), six on leaf litter respiration and 48 on the respiration of living stems, of which 25, 6 and 20, respectively, used closed chambers linked to a NDIR. Researchers using the earlier methods based on trapping CO_2 through alkali or soda lime, devoted considerable time to explaining the methods being employed (Yoneda 1975; Boddy 1983). However, none of the papers we reviewed presented the theory and formula used to convert measured CO_2 concentrations into actual CO_2 effluxes from samples, when using closed chambers linked to a NDIR. Moreover, the methods and calculations varied substantially among the studies we reviewed. Indeed, only six (24%) of the studies on respiration of woody debris, two (33%) on leaf litter respiration and three (15%) on stem respiration supplied the formula used. Of those, 67%, 100% and 67%, respectively, contained errors. Hence, there is a crucial need for a common and standardized framework for studies using closed chambers linked to a NDIR (Mackensen & Bauhus 2003). Reviewing the methods applied to date, we find that although some researchers cited earlier publications or asserted they were following prior methods, they often failed to correctly apply formulae. Moreover, formulae have sometimes deviated from the ideal gas law. This is evident from the lack of fundamental variables, such as the pressure (P), in the calculations. Finally, even where researchers applied correct formulae, some appeared to have employed inappropriate measurements for some important variables. For example, where the appropriate value for temperature should be that of the gas under measurement (to some extent the chamber's temperature or even the ambient air temperature may be acceptable), in some cases it was replaced by the internal temperature of the NDIR (e.g. Jomura *et al.* 2007, 2008). Our specific objectives are as follows: (i) to present the theory behind the CO_2 flux calculation when using a NDIR linked to a closed chamber and (ii) to illustrate the shortcomings in previous studies and their effects on CO_2 efflux calculations. We provide a methodological framework that will facilitate repeatable cross-study comparisons. This will in turn enable researchers to improve predictions of climate change impacts on ecosystems and ecosystem functioning.

Review of methods for measuring respiration

Methods for measuring respiration have received impetus from technological advances. Improved spatial and temporal scales of measurement in studying trace gas fluxes from organic substrates have been achieved, and researchers have tried to tackle shortcomings related to older approaches (Baldocchi 2014). Laboratory studies initially employed either chemical absorption with soda lime or an alkali solution to trap the CO_2 produced by a sample of organic substrate. These methods are based on a simple chemical principle, where a known mass of soda lime, or known CO_2 concentration of an alkali solution, is incubated with the organic substrate. After a pre-determined exposure time (usually 4–24 h), the soda lime mass or the CO_2 concentration of the alkali solution, is remeasured. The difference between the final and initial measurements equals the amount of CO_2 released from the sample during the period of exposure. Alkali solution methods usually employed NaOH or KOH and determined the concentration of CO_2 by titration (e.g. Yoneda 1975; Yoneda, Yoda & Kira 1977; Carpenter *et al.* 1988; Marra & Edmonds 1994; Progar *et al.* 2000; Hicks & Harmon 2002; Mackensen & Bauhus 2003; Barker 2008; Herrmann & Bauhus 2008). In the laboratory, some researchers employed gas chromatography to quantify CO_2 production (e.g. Boddy 1983; Wu *et al.* 2010). However, as some studies on soil respiration have revealed (Bekku *et al.* 1997; Grogan 1998), these methods can systematically bias estimates of CO_2 effluxes. In addition, it is difficult to perform such measurements outside the laboratory and they are time consuming which limits sample replication. Some measurements of respiration in the field also

used alkali or soda lime traps (Forrester *et al.* 2012). However, these have been progressively replaced with chambers connected to a NDIR, as these are more time efficient, portable and accurate.

Details of the chambers used and how these relate to the calculation of CO_2 efflux have been described elsewhere (e.g. Bekku *et al.* 1997; Rochette *et al.* 1997; Bain *et al.* 2005; Ngao *et al.* 2006), including some inter-study comparisons that investigated artefacts and biases (e.g. Lund *et al.* 1999; Longdoz, Yernaux & Aubinet 2000; Huitchinson & Livingston 2001; Davidson *et al.* 2002; Livingston, Hutchinson & Spartalian 2006; Riveros-Iregui *et al.* 2008). These were studies of soil respiration, but they are applicable to woody debris, leaf litter and stem CO_2 efflux measurements with some specific adaptations (Herrmann & Bauhus 2008). Chambers can be categorized as running in either steady-state or non-steady-state modes; although some researchers prefer to classify chambers according to a combination of steady state and whether the chamber is subject to flow through (Pumpanen *et al.* 2004; Bain *et al.* 2005). Hence, we might have three types of chamber: 'static chamber' also called 'non-flow-through steady-state chamber', 'open dynamic chamber' or 'flow-through steady-state chamber', and 'closed dynamic chamber' or 'flow-through non-steady-state chamber' (Pumpanen *et al.* 2004; Bain *et al.* 2005). This last category applies to fully enclosed chambers, where the substrate emitting the gas under measurement is completely contained within the chamber. Such chambers are typically employed for measurement of CO_2 efflux from smaller pieces of woody debris and sometimes leaf litter (e.g. Ataka *et al.* 2014a) and are our focus here. However, the above categorization can be expanded to include partially enclosed chambers used for surface CO_2 flux measurements, where only some part of the surface of the object emitting the gas is covered by the chamber. Such chambers are commonly used for measurements of soil respiration and can be adapted to measure respiration from living stems (e.g. Stahl *et al.* 2011) and from larger pieces of woody debris (e.g. Forrester *et al.* 2012). The equation developed below is appropriate for all above types of chambers, although for partially enclosed chambers estimation of the volume or mass of the emitter under measurement involves further assumptions. These methods are also not affected by the type of gas analyzer employed, although NDIRs are by far the most common gas analyzers employed today. It should also be noted that we are concerned here with the methods for calculating the CO_2 efflux given accurately measured CO_2 concentrations. Most NDIRs have built-in corrections to adjust for differences in the internal pressure, temperature and concentration of water (H_2O) vapour which can affect measurement of CO_2 concentration.

With closed dynamic chambers, the whole system is closed (Fig. S1) and a NDIR is linked to the chamber to determine concentrations of CO_2 in the air circulating within the system. The amount of CO_2 produced by the substrate is determined by assessing the rate of increase in the CO_2 concentration within the system. To measure another trace gas flux, one needs to replace the CO_2 components and related values (such as the molar mass) with appropriate values for the different target trace gas in the equations below.

Theory and formulae for calculation of CO_2 efflux for closed chambers

THE SYSTEM AND ITS FUNCTION

Prior to considering theory behind the calculations, we clarify the basics of the system and how it works. First, the system is closed (Fig. S1) and the ideal gas law governs closed systems.

$$PV = nRT, \qquad \text{eqn 1}$$

where P = pressure in Pascals (Pa), V = volume in cubic metres (m^3), n = number of moles of molecules of all kinds in the gas, R = universal gas constant (8·314 J K^{-1} mol^{-1}) and T = temperature of the gas under study expressed in Kelvin (K).

The temperature in eqn 1 is that of the circulating gas, the air in the chamber (typically the same as the ambient temperature or the sample surface temperature) not the temperature in the optical path of the NDIR. The optical path is heated to a constant temperature (≈51 °C) to make its ends strictly isothermal, which ensures that the measured absorption of light is constant for a given CO_2 concentration. Because the volume of the optical path is small compared to that of the chamber, the temperature of the gas in the system as a whole does not change measurably.

FORMULA DEVELOPMENT

This formula development holds true for any gas efflux estimation using any closed-chamber-based method linked with a NDIR. The following section assumes the NDIR is functioning properly. Biases related to the functioning of NDIR such as interference of gases (e.g. water vapour, see Kondo *et al.* 2014), internal temperature and pressure, NDIR calibration, response time, etc. are beyond the scope of this paper. However, it is worth emphasizing that for some of these NDIR-related issues (e.g. temperature and pressure instability) manufacturers have built in some automatic correction functions. Such automatic corrections do not pertain to the calculation of gas efflux, which is dependent on the ideal gas law and the temperature and pressure of the gas under measurement (i.e. the gas in the chamber).

Eqn 1 implies that:

$$n = \frac{PV}{RT} \qquad \text{eqn 2}$$

In a closed system, containing a CO_2 source, the measured CO_2 concentration increases linearly over typical measurement intervals, *c.* 5–10 min. Partially closed or open systems may show an asymptotic increase (if so, an exponential function is needed). With a linear increase, ordinary least square (OLS) regression of measured CO_2 concentrations [CO_2] against time reveals CO_2 efflux rates.

Thus:

$$[CO_2] = \Delta CO_2 * t + \beta, \quad \text{eqn 3}$$

where ΔCO_2 = change in CO_2 concentration per unit time, t = time and β is an intercept not important for these calculations. As such, the unit of ΔCO_2 depends on the units of both $[CO_2]$ and time. For example, if the unit of $[CO_2]$ is ppm, and the unit of t is seconds, then the unit of ΔCO_2 is ppm s^{-1}. Usually ppm given by a NDIR is equivalent to micro-mol mol^{-1}.

Let b be the rate of increase in CO_2 in micromoles per unit time (i.e. the CO_2 moles produced per unit time). This can be calculated as follows:

$$b = \Delta CO_2 * n, \quad \text{eqn 4}$$

where b is in micromole per unit time, ΔCO_2 micromole per mole per unit time and n is in moles. When we replace n from eqn 2 in eqn 4 we have

$$b = \Delta CO_2 \frac{PV}{RT} \quad \text{eqn 5}$$

The gas volume $V = V_c - V_s$, where V is the volume of gas under analysis with the assumption that the volume of air within the gas emitter sample (in pores, etc.) is negligible compared with $V_c - V_s$, V_c is the volume of the chamber including any tubing part of the effective chamber volume, and V_s is the sample volume. Also, $T = T_i + T_c$, where T is temperature of the air in K, T_i converts degrees Celsius to K, and T_c is the temperature of the gas under study in degrees Celsius. T_c can be averaged over the measurement period, though it is an assumption that variation during measurement has a negligible effect. Then b becomes

$$b = \Delta CO_2 \frac{P(V_c - V_s)}{R(T_i - T_c)} \quad \text{eqn 6}$$

Respiration rate of the organic substrate sample (R_S) can be expressed as moles of CO_2 produced per unit of dry mass (W_s) of the sample per unit time. W_s can be either assessed at the end of the experiment or, for prolonged experiments, by taking a wood core or disk in the case of woody debris and stem, or taking a known amount of leaf litter to calculate it for a set of measurements. Thus:

$$R_S = \Delta CO_2 * \frac{P(V_c - V_s)}{R(T_i + T_c)} * \frac{1}{W_s} \quad \text{eqn 7}$$

The units of R_S depend on the units of ΔCO_2 and of W_s.

Since, $\frac{P(V_c - V_s)}{R(T_i + T_c)}$ is in moles, if $\Delta CO_2 = \frac{dCO_2}{dt}$ is in units of ppm t^{-1} where t is in seconds, this unit is also equivalent to micromole $mole^{-1}$ t^{-1} and W_s is in units of grams then the respiration rate is expressed in micromoles of CO_2 per gram (g) of dry mass of the sample per unit of time (μmol CO_2 g^{-1} s^{-1}).To obtain respiration rate in microgram (μg) of CO_2 per gram of dry weight of sample per unit of time (μg CO_2 g^{-1} s^{-1}), we need to multiply by the molar mass of CO_2 (M_{CO2} = 44·01 g).

Equivalently, the universal gas constant, R can be substituted with

$$R = \frac{P_i V_i}{T_i}, \quad \text{eqn 8}$$

where standard pressure P_i = 101·325 kPa (1 atm), standard temperature T_i = 273·15 K, and V_i = 22·41 L (volume of a mole of air at 0 °C).

Making this substitution in eqn 7 results in the formula:

$$R_S = \Delta CO_2 \frac{P}{P_i} * \frac{(V_c - V_s)}{V_i} * \frac{T_i}{(T_i + T_c)} * \frac{1}{W_s} \quad \text{eqn 9}$$

The respiration rate can be obtained using either eqn 7 or eqn 9. In addition, the respiration rate may be expressed on a dry mass (W_s), volume (V_s) or surface area (A_s) basis of the sample. In eqns 7 and 9 above, one simply replaces the variable W_s as appropriate. For comparability among studies, we recommend reporting CO_2 on a dry mass basis. At a minimum, authors should provide sufficient information for recalculation of values on a dry mass basis, even if they express it otherwise.

The remaining defining parameters in calculating CO_2 efflux in eqns 7 and 9 are air pressure, the chamber air temperature and volume of circulating gas. The presence of P accounts for any difference in ambient pressure from standard pressure. Air pressure is usually recorded by the NDIR, but otherwise it needs to be measured.

Air temperature can be measured with different devices either placed in the chamber (ideally) or near the chamber. In most cases, the researcher will need to record the chamber's air temperature during measurement.

Finally, the volume of circulating gas is reduced by the presence of a sample inside the chamber. If the sample volume is overestimated, then the volume of circulating gas will be underestimated and the CO_2 efflux will be underestimated, and *vice versa*. The magnitude of any error depends on the relative difference in the sample and chamber volume. If the sample is a 'close fit' inside the chamber, the magnitude of the error increases. Chambers that are much larger than the sample have the disadvantage that more time is required to obtain accurate CO_2 concentration increases, but results will be proportionately less affected by sample-volume measurement errors. We suggest using a chamber that is two to three times the volume of the sample. For partially closed chambers, because the chamber sits on the surface of the substrate, the volume of air circulating within the system is simply the volume of the chamber. The volume of woody debris is most often determined by dimensional measurements applying one of several geometric formulae (Table S2, Appendix S3). Fraver, Ringvall & Jonsson (2007) discussed woody debris volume estimation error related to different formulae and recommended Newton's formula. An alternative more accurate but time-consuming method is the water-displacement method (Williamson & Wiemann 2010), in which the wood is submerged in water and the displaced volume is measured. For leaf litter, its volume may be estimated from the fresh weight, leaf area or leaf density (Huxley 1971) or a sufficiently large chamber should be used

enabling the volume of the leaf litter to be treated as being negligible, although this necessarily means more time may be required for the measurement.

If one wishes to express the respiration in terms of the carbon content of the dry sample, one should be aware that the commonly applied conversion ratio of 0·5 between carbon concentration and biomass can be misleading, as demonstrated by Harmon *et al.* (2013). If measuring another trace gases, such as nitrous oxide or methane, one needs to use the appropriate molar mass (M).

PARAMETER ESTIMATION IN ABSENCE OF DIRECT MEASUREMENTS

In the absence of direct measurements of air pressure and temperature, they must be estimated assuming that data obtained from the nearest weather station are adequate to avoid substantial errors. Air pressure may fluctuate temporally and spatially with regard to prevailing local climatic conditions and temperature may vary with topography and habitat derived microclimatic effects, so local measurement of these parameters is always preferable. Nevertheless, air pressure and temperature can be adjusted for elevation, if the difference in altitude between the field site and the nearest weather station is known.

Air pressure changes with altitude and can be approximated with eqn 10.

$$P_z = 100 * \left(\frac{44331 \cdot 514 - z}{11880 \cdot 516}\right)^{1/0{\cdot}1902632} \qquad \text{eqn 10}$$

P_z is the pressure in Pascals (Pa) and z is altitude in metres (m) (Anonymous 2004) and the standard lapse rate for temperature is $-6{\cdot}5$ °C km^{-1}.

Application: Case study and estimation of errors in the previous studies

CASE STUDY

The example used here is from an ongoing wood decomposition project in Mengsong, Xishuangbanna, SW China. In this study, freshly cut logs were placed on the forest floor and monitored over a period of 3 years to assess the effects of forest structure on decomposition rates. Hence, a non-invasive method for repeated measurement of woody debris respiration was required, so we employed a closed-chamber linked to a NDIR (LI-820, Lincoln, NE, USA). The Fig. S2 shows the measured CO_2 concentration for an example log (*Castanopsis mekongensis* A. Camus, Fagaceae) after 6 months of incubation. The noisy data at the start of the graph represent distortions in measured CO_2 due to closing the chamber and the CO_2 remaining in system tubing from the previous measurement. For this reason, we discard the first 1 min of recorded data (Jomura, Kominami & Ataka 2012; Yoon *et al.* 2014). Some authors suggest only considering the middle section of the graph and discarding first and last minutes of the recorded data, when using an automated closed chamber (Dannoura *et al.* 2006), because of possible nonlinear increases in CO_2 concentration.

In the Supporting information, we provide an R script for calculating CO_2 efflux (Appendix S4). The first function handles output files from a LI-820 to generate a dataframe of the sample details [file name, date-time, slope (k), mean temperature (of the optical path) and mean pressure]. Note again that the optical path temperature is not appropriate for calculating the CO_2 efflux. The temperature of the circulating gas must be measured separately. The second and third functions calculate CO_2 efflux according to eqns 7 and 9, respectively.

FORMULA APPLICATION

The characteristics of the log considered here were as follows:

L = 50·0 cm, d_b = 10·5 cm, d_m = 10·0 cm, d_t = 9·6 cm, V_s = 3·94 L (calculated using Newton's formula (Table S2)), V_c = 11·84 L (including tubing volume), W_s = 2559·84 g, T_i = 273·15, T_c ambient = 25·87 °C (measured with HOBO SMART SENSOR Humidity & Temperature Tester AR827), and P = 83·02 kPa (from the NDIR).

From the OLS regression method, ΔCO_2 = 1·38 ppm s^{-1}. With all of these, we calculate the respiration rate (R_S) using eqns 7 and 9 developed above (here, we multiplied by M_{CO2} to express the result in mass per unit time), R_S = 0·0063 μg CO_2 g^{-1} (dry weight) s^{-1} or R_S = 22·68 mg CO_2 kg^{-1} (dry weight) h^{-1}.

ERRORS IN THE CALCULATION OF CO_2 EFFLUX IN EARLIER STUDIES AND THEIR CONSEQUENCES

As mentioned in the introduction, some studies have incorrectly calculated CO_2 effluxes. The errors found in those studies can be divided into (i) major errors, including failure to account for ambient pressure and use of the wrong temperature and (ii) minor errors such as slightly different values for the absolute zero temperature, incorrect descriptions of variables, or non-matching units. It is worth noting that, although the error may be minor, non-matching units could result in a substantial miscalculation (see Fig. 1 on the calculation of the effects of the mistakes and Appendix S1 on previous studies and formulae). Here, we describe and discuss each of these mistakes and then discuss how each in isolation would affect the calculated CO_2 efflux in the example given above. The associated error was calculated as

$$\text{Associated error} = \frac{(R_{\text{rep.}} - R_{\text{cor.}})}{R_{\text{cor.}}}, \qquad \text{eqn 11}$$

where $R_{cor.}$ = the correct respiration and $R_{rep.}$ = the reported respiration rate. Hence, positive values of the associated errors indicate overestimation and negative values underestimation of respiration rates. The most common major error was failure to account for deviations of ambient air pressure at the time of measurement from standard pressure, as reflected by failure to include these variables in the formula (Jomura *et al.* 2007, 2008; Stahl *et al.* 2011; Rowland *et al.* 2013; Ataka *et al.* 2014a,b; Katayama *et al.* 2014; Yoon *et al.*

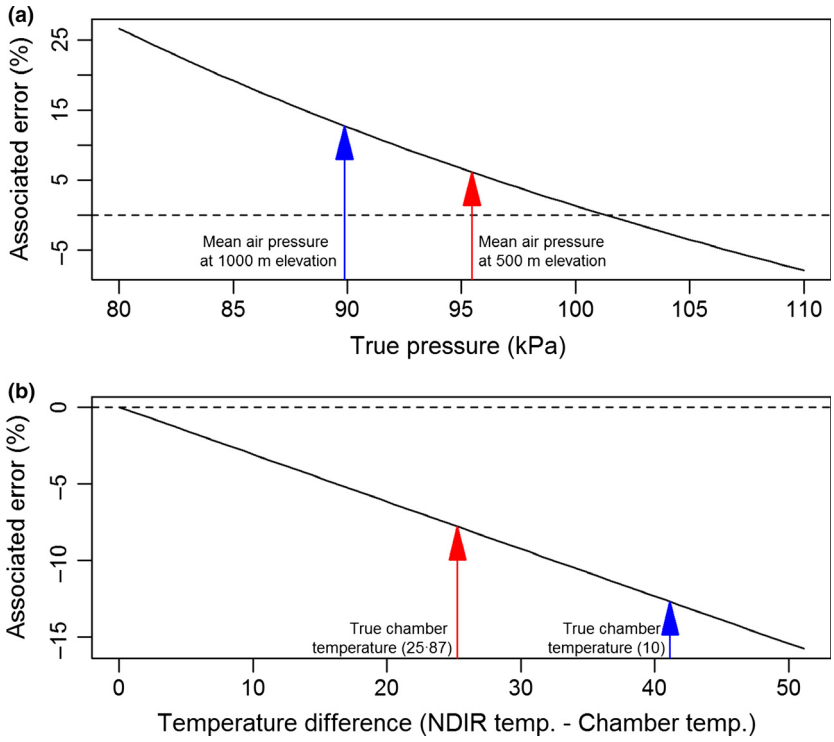

Fig. 1. Estimated errors in the CO_2 efflux calculation due to errors encountered in the literature. The associated error was calculated as $(R_{rep.} - R_{cor.})/R_{cor.}$, where $R_{cor.}$ is the correct respiration rate and $R_{rep.}$ is the reported respiration rate. (a) Associated error when the local air pressure during measurement is omitted in the formula used to calculate the gas efflux. The dashed line indicates the associated error when the difference between pressure of the gas under measurement and the pressure at standard conditions is zero. The red and blue arrows show the magnitude of error if air pressure is omitted for measurement conducted at 500 and 1000 m elevations, respectively, under otherwise standard conditions. (b) Associated error when the internal temperature of the non-dispersive infrared gas analyzer (NDIR) is used instead of the temperature of the gas under measurement. The dashed line indicates the associated error when the difference between internal temperature of NDIR and that of the chamber is zero. Red and blue arrows show the magnitude of errors if the differences between internal temperature of NDIR (e.g., 51·15 °C) and true chamber temperature (e.g., 25·87 and 10 °C) are 25·28 and 41·15, respectively.

2014). Figure 1a shows the associated error if the air pressure is not included in the calculation of CO_2 efflux rate. For a given pressure less than P_i = 101·325 kPa, CO_2 efflux is overestimated; for a given pressure higher than Pi, efflux is underestimated. In the case study above (where actual pressure was 83·02 kPa), this would overestimate CO_2 efflux by 22%. The studies reviewed here were conducted at sites with altitudes from 5 to 701 m. After applying eqn 10, the ambient pressure under otherwise standard conditions among those sites ranges from 101·265 to 93·183 kPa, which on its own could result in an overestimation of CO_2 efflux of up to 9% in the cases where pressure was not measured (weather-related differences in ambient pressure from the elevation-specific mean could of course lead to higher or lower errors).

The other major error was in the use of the wrong temperature. The temperature in the formula should be the temperature of the air under analysis, which can be approximated to the chamber temperature or the ambient air temperature at the time of measurement. Instead, we found that some studies (e.g. Jomura *et al.* 2007, 2008) used the NDIRs internal temperature. Using the NDIR temperature instead of chamber's temperature produces an under-estimation of the CO_2 efflux (Fig. 1b). In the case study above, this mistake would underestimate the CO_2 efflux by 8%.

With regard to the minor errors, sometimes the unit of CO_2 efflux based on a given formula does not match the unit of CO_2 efflux reported in the results section. For example, Rowland *et al.* 2013 employed a formula based on surface area, but they reported results on a unit mass basis. This may lead to confusion especially if there is no attempt to explain the discrepancy. In addition, we noticed some inconsistencies in attributing the recommended value of T_i (273·15 K, as recognized by the International Union of Pure and Applied Chemistry (IUPAC; Cohen & Taylor 1987) to convert temperature in degrees Celsius to Kelvin. For instance, studies used 273 K (Stahl *et al.* 2011; Liu *et al.* 2013; Rowland *et al.* 2013), 273·2 K (Jomura *et al.* 2007, 2008; Ataka *et al.* 2014a, b; Katayama *et al.* 2014), and 273·5 K (Olajuyigbe, Tobin & Nieuwenhuis 2012). While the first two values represent the same number rounded to a different number of decimal places, the last is clearly incorrect. The level of precision of this constant should be at least as high as that of the measurement. In addition, some authors (e.g. Rowland *et al.* 2013) used a variable described as the molar volume but in fact, they were referring to its inverse. Although these errors might be considered insignificant, they are easily avoided.

Considering the above errors, some studies incorporated two of these mistakes, but their influences were in opposite

directions. For example, in our case study, the combination of these two errors would result in overestimation of CO_2 efflux by 13%. For instance, Jomura *et al.* (2007, 2008) failed to account for the ambient pressure and used the temperature of the optical path. Because such errors can cancel one another out, this may have limited the researchers' ability to detect their mistakes.

Conclusion

Climate change is being driven by greenhouse gas increases from anthropogenic activities and among the greenhouse gases, CO_2 is the most important. A deeper understanding of the global carbon cycle is therefore crucial to accurately model and predict climate-change impacts. The use of a closed chamber linked to a NDIR offers tremendous advantages over earlier methods to measure the respiration of organic substrates, such as woody debris and leaf litter. Besides offering increased accuracy, it is faster, which enables researchers to increase replication and investigate more thoroughly environmental factors affecting respiration rates in the field. However, we encountered several errors in the literature concerning the calculation of CO_2 efflux when a closed-chamber linked to a NDIR was employed. Moreover, a large proportion of the reviewed studies did not supply a formula or a citation for the calculations used. The application of incorrect formulae in the calculation of CO_2 sheds doubt on some previous results and hinders development of this field. We draw attention to those shortcomings, and describe appropriate methods for calculating CO_2 effluxes.

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Data accessibility

The data for this paper are presented in the main article and its supplementary information files. The R-script to process output files from a non-dispersive gas analyzer (NDIR, e.g. LICOR 820) is given in the supplementary information and is also available from the following website https://github.com/dossag/Infra-red-gas-analyzer-code. The Licor data used in the case study are deposited in the Dryad repository: https://datadryad.org/resource/doi:10.5061/dryad.8cj64 (Dossa *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Examined studies and constituent formulae **.**

Appendix S2. Closed dynamic chamber system and example of recorded CO_2 concentration data.

Table S1. Summary of woody debris, living stems and leaf litter respiration as recorded in previous studies

Table S2. Summary of formulae to calculate the volume of woody debris found in previous studies.

Appendix S3. Geometrical shapes and formulae employed to obtain an estimated volume of woody debris.

Appendix S4. R script for calculating CO_2 efflux using a closed-chamber linked to an NDIR.

Fig. S1. A closed-chamber system for measuring WD respiration with a non–dispersive infrared sensor (NDIR).

Fig. S2. CO_2 concentration of a closed-chamber system measuring respiration of a piece of woody debris (WD) (Fig. S1) using a Licor 820.