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Carbon-isotopic composition of soil-respired carbon dioxide in static closed chambers at equilibrium

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The carbon-isotopic composition ($\delta^{13}C$) of soil-respired CO_2 has been employed to evaluate soil carbon-cycling processes and the contribution of soil CO_2 emissions to canopy and tropospheric air. These evaluations can be successful only when accurate isotope values of soil-respired CO_2 are available. Here, we tested the robustness of $\delta^{13}C$ values of soil-respired CO_2 obtained after long incubations in static closed chambers that were initially flushed with soil air. The rationale of this approach is that the equilibrium carbon-isotope values of chamber-headspace CO_2 are theoretically equal to those of CO_2 produced within the soil. Static closed chambers were installed in replicated grass monocultures, and measurements of headspace CO_2 concentrations and $\delta^{13}C$ values were performed at regular time intervals for 24 h in July 2005. The results revealed no significant effects of grass species on headspace CO_2 concentrations or $\delta^{13}C$ values (repeated measures analysis of variance (ANOVA), $P > 0.1$). As predicted by theory, isotope values asymptotically approached equilibrium conditions, which in our experimental setting occurred after 10 h. This good match between model predictions and our results suggests that an accurate determination of $\delta^{13}C$ values of CO_2 produced within soils is obtained through the isotopic measurement of chamber-headspace CO_2 once equilibrium conditions have been reached with the underlying soils. An additional advantage of this approach is that only one sample per chamber is required, which, combined with the low uncertainties of these measurements, facilitates the investigation of the spatial (landscape) variability of soil-respired CO_2. Copyright © 2007 John Wiley & Sons, Ltd.

The carbon-isotopic composition of soil-respired CO_2 (the flux out of a soil) is an important parameter that has been used to evaluate soil carbon dynamics[1–4] and the role of soils in ecosystem carbon budgets in local,[5,6] regional,[7] and global investigations.[8] Despite the importance of accurately determining $\delta^{13}C$ values of soil-respired CO_2, no consensus exists about the best methodology to measure this value. One of the most commonly employed methods includes the use of static closed chambers. Specifically, a chamber is inserted into the soil profile or is installed on top of the soil and, when closed, creates a headspace where soil-respired CO_2 is allowed to accumulate. In the transient approach of this method, headspace air samples are taken at regular short-term intervals, and CO_2 concentrations and carbon-isotope ratios are both determined on the collected samples.[4,6] A two end-member mixing model is then employed to estimate the $\delta^{13}C$ value of the soil-respired CO_2, under the assumption that atmospheric and soil-respired CO_2 contribute CO_2 to the chamber headspace. This estimation follows the approach developed by Keeling,[9,10] in which the isotopic composition of the non-atmospheric end-member (soil-respired CO_2, in the current case) corresponds to the intercept of the least-squares linear relationship established by regressing the isotopic composition of sampled CO_2 against the inverse concentrations of CO_2. Although this approach requires a fairly significant number of samples to establish a linear relationship with a high r^2 value, the uncertainty associated with the intercept is large.[11,12] Ultimately, this uncertainty in the determination of the $\delta^{13}C$ value of soil-respired CO_2 generates a large uncertainty in estimations of soil contributions to atmospheric CO_2.[13]

An alternative approach is to allow headspace CO_2 to reach isotopic equilibrium with soil CO_2, which is advantageous because the isotopic composition of headspace CO_2 at equilibrium equals that of the CO_2 produced within the soils.[14] Despite its advantage, no study so far has established the robustness of the isotope results obtained through this equilibrium approach. The purpose of the present study is to

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evaluate the uncertainty associated with measurements of the carbon-isotope composition of soil-respired CO_2 in closed static chambers under equilibrium conditions.

THEORETICAL BACKGROUND

The CO_2 in soil pore spaces (or soil CO_2 as defined by Dörr and Münnich[15] and Cerling *et al*.[16]) derives mainly from atmospheric sources and the respiration of heterotrophic and autotrophic organisms. In general, the isotopic composition of soil CO_2 reflects that of the plants growing on those soils,[17] but it typically deviates from plant $\delta^{13}C$ values as a result of a number of processes. For instance, the microbial degradation of plant material produces CO_2 with an isotopic composition that is different from that of the plant material.[18] In addition, $\delta^{13}C$ values of soil organic matter (SOM) typically vary with soil depth[19] due to preferential degradation,[20,21] past changes in $\delta^{13}C$ value of atmospheric CO_2,[22] or, in some cases, past changes in the type (C3 vs. C4) of vegetation cover.[23] As a result, the $\delta^{13}C$ values of SOM may differ from those of standing vegetation, and the $\delta^{13}C$ values of CO_2 produced by the decay of SOM may differ both from those of the CO_2 produced by the respiration of roots and rhizosphere organisms, and from SOM itself. Moreover, the movement of CO_2 through the soil column produces a kinetic isotope effect associated with the faster diffusivity of $^{12}CO_2$ than that of $^{13}CO_3$.[24] As a result, soil CO_2 is preferentially enriched in ^{13}C by a theoretical value of 4.4‰ relative to the CO_2 originally produced by biological activity. Thus, the carbon-isotopic composition of soil-produced CO_2 must be determined directly, *in situ*, if the information derived is to be useful for understanding soil carbon-cycling process.

Cerling[24] modeled the isotopic composition of soil CO_2 considering the biological production of (respired) CO_2 and the kinetic isotope effect associated with diffusion in the following terms:

$$\partial C/\partial t = D\,\partial^2 C/\partial z^2 + \Phi \quad (1)$$

where C is CO_2 concentration, t is time, D is the effective diffusion coefficient of CO_2, z is soil depth, and Φ is biological CO_2 production. As indicated by Amundson *et al*.,[14] this model serves to explain a number of observations, including the increase of soil CO_2 concentrations with depth, the variability of soil CO_2 isotope values with depth, particularly near the soil/atmosphere interface, and the ^{13}C-enrichment of soil CO_2 relative to that produced during the metabolic activity of roots and soil microorganisms. This isotope enrichment results from the kinetic isotope effect produced by the diffusion of CO_2 though the soil. Because the same isotope effect occurs during the passage of CO_2 from soil pores to the atmosphere, soil-respired CO_2 is enriched in ^{12}C relative to soil CO_2.[15,16] An important aspect highlighted by Amundson *et al*.[14] on the basis of finite difference solutions of Eqn. (1) is that the carbon-isotopic composition of soil-respired CO_2 at equilibrium must be equal to that of CO_2 produced by soil microorganisms and roots. This property then permits the determination of the isotopic composition of biologically produced CO_2 in soils from measurements of equilibrium $\delta^{13}C$ values of soil-emitted (respired) CO_2. In this study, we employ this equilibrium approach to determine the isotopic composition of soil-respired CO_2 in static chambers that are flushed with soil air. On the basis of theoretical considerations, we will determine whether the results from this approach reflect the isotope value of the CO_2 produced within the soil.

EXPERIMENTAL

The study was conducted in C3 grass monocultures located in Marshall County, Iowa, USA (42.00°N, 93.25°W). The climate is strongly continental with a mean annual temperature of 8.7°C and a mean annual precipitation is 880 mm, most of which occurs during the growing season. We established a randomized block field experiment on Nodaway soils where tilled corn crops were formerly planted. The experimental setup consisted of four 45 m × 15 m blocks containing 15 m × 15 m plots that were planted with one of the following grass species in August 2001: *Bromus inermis*, *Dactylis glomerata* or *Phalaris arundinacea*. The chambers employed in this experiment to collect soil-respired CO_2 consisted of PVC (polyvinyl chloride) tubes of 10.16 cm diameter and 25 cm in height. The tubes contained numerous holes of 6 mm in diameter that were drilled in the lower 12.5 cm of each tube to allow the growth of roots. The PVC tubes were pounded into the ground with a hammer, encompassing existing grasses, in each plot in June 2002, leaving about 5 cm above the soil (headspace). The bottom of the PVC tubes was left open throughout the experiment.

Samples of soil-respired CO_2 were collected on July 3 and 4, 2005, with the aid of 10-mL septum-capped vials (Labco Ltd., High Wycombe, UK), which have proven to be reliable in isotope studies of soil and canopy CO_2.[25,26] Between 24 and 48 h before collection, a small amount (~2 g) of anhydrous magnesium perchlorate was introduced into the vials to keep the system dry and to prevent any isotopic exchange of the collected CO_2 with moisture. Prior to capping the vials, they were manually flushed with helium at a flow rate of 20 mL/min for 1.5 min to remove atmospheric CO_2. Isotopic analyses of these flushed vials (n = 5) yielded no signal, thus indicating the absence of CO_2 in the vials. Helium-flushed vials were then transported to the field. Prior to collecting soil gas samples, the field chambers were capped with a specially designed PVC cap, containing a sampling port that was created by drilling an opening into the cap and attaching a Swagelok fitting with epoxy resin adhesive (907 Miller-Stephenson Chemical Co., Danbury, CT, USA). The caps were then sealed with high-vacuum grease to prevent the entrance of atmospheric air and the leakage of soil-emitted gases. Once the chamber had been tightly capped, air in the headspace was flushed with soil air with the aid of a manual pump. When the flushing was complete, a Swagelok screw with septum was screwed on to the sampling port. The helium-filled vials were then attached to the Swagelok fitting with the aid of a sharp 1.1 mm diameter hollow needle, which allows the free exchange of gas between the vial and the chamber headspace. In lab tests, the helium-flushed vials were attached with the same hollow needle to 2-L glass flasks containing both 5% and 0.3% CO_2, of known $\delta^{13}C$ values, in helium.

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After 2 h, the measured $\delta^{13}C$ values and CO_2 concentrations in the vials were not significantly different (n = 4, $P = 0.195$, paired *t*-test) from those in the flasks, thus indicating that no isotope fractionation and no concentration bias occurred through the use of the hollow needles.

The vials were allowed to collect soil-respired CO_2 for 2, 4, 8, 12 and 24 h. At each time the chamber headspace was flushed with soil air, i.e. the initial conditions were reset to time = 0 h before each subsequent sample collection. In addition, two air samples were collected in the field 2.5 m above the ground by opening the flushed vials in line with the prevailing wind direction for 1 min. The carbon-isotopic composition of the collected CO_2 and its concentration were measured within 48 h of collection, using a Finnigan GasBench II fitted to a Finnigan Delta Plus XL isotope-ratio mass spectrometer (Finnigan, Bremen, Germany). For carbon-isotope normalization, the samples were analyzed against two calibrated lab standards. CO_2 concentrations were determined on the basis of the *m*/*z* 44 signal in the isotope-ratio mass spectrometer in a similar fashion to that described by Steinmann *et al*.[27] Calibration was accomplished with vials filled with three different CO_2 concentrations (0.3, 5 and 10%). The overall analytical uncertainty was better than 6% for CO_2 concentrations and 0.12‰ for $\delta^{13}C$ values (against the internationally accepted Vienna Pee Dee Belemnite (VPDB) standard, which has a defined value of 0‰).

RESULTS AND DISCUSSION

No significant effects of grass species on headspace CO_2 concentrations or $\delta^{13}C$ values were found at a given sampling time (repeated measures analysis of variance (ANOVA), $P > 0.1$, Fig. 1). Consequently, the results for the species were combined for analysis (Table 1). Values of $\delta^{13}C$ for soil-respired CO_2 asymptotically decreased through time from −18.24‰ (95% confidence interval (CI) = 0.15) to −24.42‰ (95% CI = 0.24) in the studied plots (Fig. 1). Similarly, CO_2 concentrations increased through time from 0.22% (95% CI = 0.03) to 1.62% (95% CI = 0.06) (Fig. 1). The $\delta^{13}C$ for near-surface atmospheric CO_2 was −7.63‰ and its abundance was 386 ppmv.

The observed gradual decrease of isotope values for soil-respired CO_2 with time, coupled with a gradual increase in CO_2 concentrations, is consistent with theoretical[24] and modeling[14] evaluations of the factors that control the isotopic composition of soil-respired CO_2. Increasing CO_2 concentrations result from a greater contribution of respired CO_2 over atmospheric inputs, which also explain the decreasing trend in $\delta^{13}C$ values through time. Other field experiments with shorter (<2 h) collection times of soil-respired CO_2[6,12] have also yielded increasing headspace concentrations and decreasing isotope values through time. However, our results show that the $\delta^{13}C$ values of respired CO_2 asymptotically reach an equilibrium value over time (Fig. 1), changing by less than 0.05‰ per hour after 10 h of collection (Fig. 2). This rate of change was calculated from the first derivative of the asymptotic curve presented in Fig. 1. As indicated by theoretical considerations,[4,14,16] the importance of this equilibrium $\delta^{13}C$ value is that it equals that of the CO_2 produced

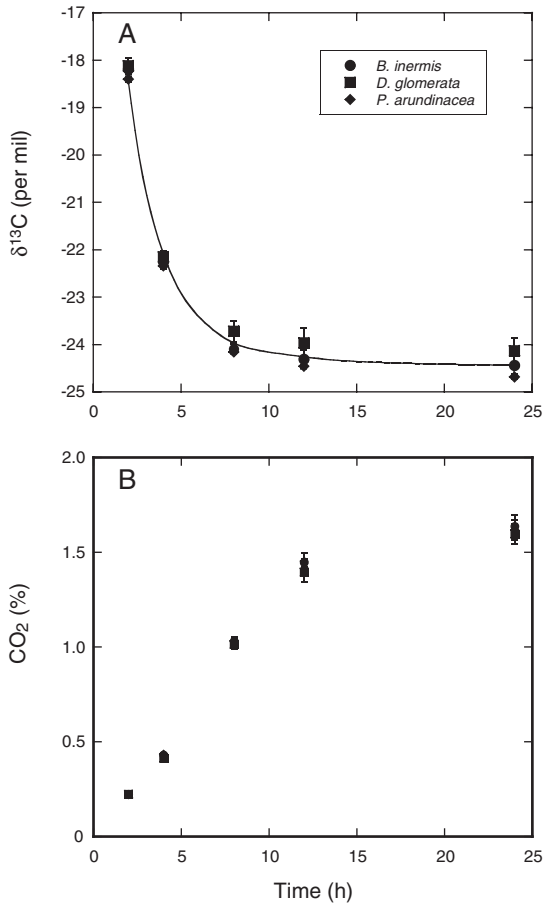

Figure 1. Variation in (A) carbon isotopic composition of CO_2 and (B) CO_2 concentrations over time in static closed chambers installed in three grass monocultures. At each time period n = 12, with n = 4 per species. Vertical bars on each symbol represent ± 1 standard error. The curve in (A) represents a spline fit of the data ($r^2 = 0.978$). Note that the $\delta^{13}C$ values of chamber CO_2 reach an equilibrium value of −24.42‰ after 10 h. This value falls between those of soil organic matter (−21.39‰, n = 6) and of grass leaves (−27.37‰, n = 12).

in the soil by roots and microorganisms. In our experimental setting, SOM exhibits an average $\delta^{13}C$ value of −21.39‰ (95% CI = 0.32, n = 6), while leaves of the planted grasses show an average $\delta^{13}C$ value of −27.37‰ (95% CI = 0.19, n = 12). The obtained equilibrium $\delta^{13}C$ value of −24.42‰ (95% CI = 0.24) falls between the two sources of biologically produced CO_2, thus providing support to the possibility of using this approach to evaluate belowground carbon dynamics.

Although the study of the isotopic composition of soil-respired CO_2 often involves its collection in pre-flushed chambers,[6,28,29] Ohlsson *et al*.[12] demonstrated that the level of flushing has an important effect on transient $\delta^{13}C$ values of soil-respired CO_2, and proposed that flushing creates an initial disturbance to the diffusion process, which affects the transient $\delta^{13}C$ values that form the raw data of Keeling plot analyses. Because of this potential effect, Ohlsson *et al*.[12] suggested that non-flushed chambers should be employed to determine the isotopic composition of soil-respired CO_2. We

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Table 1. Average CO_2 concentrations (in %) and their average carbon-isotopic compositions (in ‰) through time (in hours) in static closed chambers that were installed in plots planted with three different grass species. Values in parentheses represent standard errors (n = 4 for each species and n = 12 for all three species)

	Bromus inermis		*Dactylis glomerata*		*Phalaris arundinacea*		*All three species*	
Hour	$\delta^{13}C$ (‰)	CO_2 (%)	$\delta^{13}C$ (‰)	CO_2 (%)	$\delta^{13}C$ (‰)	CO_2 (%)	$\delta^{13}C$ (‰)	CO_2 (%)
2	−18.19 (0.12)	0.22 (0.007)	−18.12 (0.18)	0.22 (0.004)	−18.40 (0.05)	0.22 (0.002)	−18.24 (0.08)	0.22 (0.001)
4	−22.25 (0.16)	0.43 (0.008)	−22.15 (0.12)	0.41 (0.006)	−22.34 (0.03)	0.44 (0.004)	−22.25 (0.07)	0.43 (0.004)
8	−24.09 (0.11)	1.03 (0.010)	−23.72 (0.22)	1.01 (0.028)	−24.16 (0.02)	1.03 (0.024)	−23.99 (0.10)	1.03 (0.012)
12	−24.31 (0.20)	1.44 (0.022)	−23.96 (0.31)	1.39 (0.051)	−24.46 (0.04)	1.45 (0.045)	−24.24 (0.13)	1.43 (0.023)
24	−24.44 (0.22)	1.63 (0.060)	−24.13 (0.27)	1.59 (0.054)	−24.68 (0.05)	1.62 (0.053)	−24.42 (0.13)	1.62 (0.030)

argue, however, that flushed chambers can also be used for this purpose but only when measurements are performed at or near equilibrium conditions. In retrospect, pre-flushing the chamber headspaces is not a requirement since the initial conditions should have no influence on the isotopic equilibrium that is obtained after long incubations, but we did not specifically test that deduction. As predicted by theoretical considerations,[24] numerical models,[14] and empirical data (this study), this approach then permits the determination of the carbon-isotopic composition of biologically produced CO_2, without the need for a correction factor. The disadvantages are that achievement of isotope equilibrium conditions requires longer collection times, particularly for soils with low respiration rates, thus precluding the study of belowground processes operating on short time scales (e.g., hourly), and that samples with very high CO_2 concentrations (>1.6% in our case) are generated. Moreover, preliminary measurements may be needed to determine the minimum length of the incubation period that is needed in other locations, but both modeling[14] and our empirical data suggest that 10–12 h should be sufficient. The advantages, however, are that only one sample per chamber is needed, thus reducing costs; that the obtained isotope values represent those of the CO_2 produced by belowground metabolic processes, and possibly those of soil-respired CO_2; and that the overall uncertainty of these isotope values originates from an analytical error, which is typically <0.1‰. In contrast, the standard error associated with a Keeling-type approach is in most cases no better than 0.3‰.[12] As pointed out by Phillips and Gregg,[13] improved precision in the determination of the isotopic value of soil-respired CO_2 permits the estimation of soil contributions to atmospheric CO_2 with high confidence, even if only a small difference in isotope values exists between the soil and plant sources. For example, this equilibrium approach facilitates evaluation of the spatial variability of soil-respired CO_2 $\delta^{13}C$ values, which would then allow a better assessment of soil carbon dynamics and the role of soils in carbon budgets.

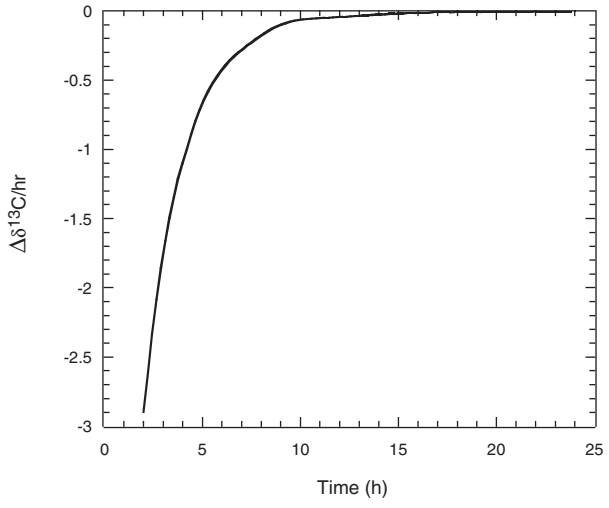

Figure 2. Rate of change in the carbon-isotopic composition of chamber headspace CO_2 through time, which was obtained from the first-derivative of the line in Fig. 1. Note that after 10 h, the isotope values varied within the analytical uncertainty of our measurements.

CONCLUSIONS

Our study of the accumulation of CO_2 in static closed chambers installed in grass monocultures revealed that the variability of chamber-headspace CO_2 $\delta^{13}C$ values over time is consistent with the predictions of a numerical model developed by Amundson *et al.*,[14] indicating that $\delta^{13}C$ values for soil-respired CO_2 gradually decrease with time as the system reaches equilibrium conditions. This theoretical model also indicates that this isotope value equals that of the biologically produced CO_2 in soils. Our results suggest that measurements of $\delta^{13}C$ values of headspace CO_2 in static closed chambers performed at or near equilibrium accurately reflect those of the CO_2 produced by belowground metabolic processes.

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REFERENCES

1. Rochette P, Flanagan LB, Gregorich EG. *Soil Sci. Soc. Am. J.* 1999; **63**: 1207.
2. Hanson PJ, Edwards NT, Garten CT, Andrews JA. *Biogeochemistry* 2000; **48**: 115.
3. Ehleringer JR, Buchmann N, Flanagan LB. *Ecol. Appl.* 2000; **10**: 412.
4. Soe ARB, Giesemann A, Anderson TH, Weigel HJ, Buchmann N. *Plant and Soil* 2004; **262**: 85.

DOI: 10.1002/rcm

5. Broadmeadow MSJ, Griffiths H, Ehleringer JR, Hall AE, Farquhar GD. *Stable Isotopes and Plant Carbon-Water Relations*. Academic Press: San Diego, 1993.
6. Flanagan LB, Brooks JR, Varney GT, Berry SC, Ehleringer JR. *Global Biogeochem. Cycles* 1996; **10**: 629.
7. Nakazawa T, Sugawara S. *J. Geophys. Res.* 1997; **102**: 3843.
8. Ciais P, Tans PP, Trolier M, White JWC, Francey RJ. *Science* 1995; **269**: 1098.
9. Keeling CD. *Geochim. Cosmochim. Acta* 1958; **13**: 322.
10. Keeling CD. *Geochim. Cosmochim. Acta* 1961; **24**: 277.
11. Pataki DE, Ehleringer JR, Flanagan LB, Yakir D, Bowling DR, Still CJ, Buchmann N, Kaplan JO, Berry JA. *Global Biogeochem. Cycles* 2003; **17**: 1022. DOI: 10.1029/2001GB001850
12. Ohlsson KEA, Bhupinderpal S, Holm S, Nordgren A, Lövdahl L, Högberg P. *Soil Biol. Biochem.* 2005; **37**: 2273.
13. Phillips DL, Gregg JW. *Oecologia* 2001; **127**: 171.
14. Amundson R, Stern L, Baisden T, Wang Y. *Geoderma* 1998; **82**: 83.
15. Dörr H, Münnich KO. *Radiocarbon* 1980; **22**: 909.
16. Cerling TE, Solomon DK, Quade J, Bowman JR. *Geochim. Cosmochim. Acta* 1991; **55**: 3403.
17. Fritz P, Mozeto AA, Reardon FJ. *Chem. Geol.* 1985; **58**: 89.
18. Šantrůčková H, Bird MI, Lloyd J. *Func. Ecol.* 2000; **14**: 108.
19. Nadelhoffer KJ, Fry B. *Soil Sci. Soc. Am. J.* 1988; **52**: 1633.
20. Benner R, Fogel ML, Sprague EK, Hodson RE. *Nature* 1987; **320**: 708.
21. Bird M, Kracht O, Derrien D, Zhou Y. *Aust. J. Soil Sci.* 2003; **41**: 77.
22. Fung I, Field CB, Berry JA, Thompson MV, Randerson JT, Malmstrom CM, Vitousek PM, Collatz GJ, Sellers PJ, Randall DA, Denning AS, Badeck F, John J. *Global Biogeochem. Cycles* 1997; **11**: 507.
23. Mora G, Pratt LM. *Quat. Sci. Rev.* 2002; **21**: 985.
24. Cerling TE. *Earth Planet. Sci. Lett.* 1984; **71**: 229.
25. Tu KP, Brooks PD, Dawson TE. *Rapid Commun. Mass Spectrom.* 2001; **15**: 952.
26. Midwood AJ, Gebbing T, Wendler R, Sommerkorn M, Hunt JE, Millard P. *Rapid Commun. Mass Spectrom.* 2006; **20**: 3379.
27. Steinmann KTW, Siegwolf R, Saurer M, Korner C. *Oecologia* 2004; **141**: 489.
28. Buchmann N, Ehleringer JR. *Agricult. Forest Meteor.* 1998; **89**: 45.
29. Andrews JA, Harrison KG, Matamala R, Schlesinger WH. *Soil Sci. Soc. Am. J.* 1999; **63**: 1429.

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