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Soil moisture effects on the carbon isotope composition of soil respiration

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The carbon isotopic composition ($\delta^{13}C$) of recently assimilated plant carbon is known to depend on water-stress, caused either by low soil moisture or by low atmospheric humidity. Air humidity has also been shown to correlate with the $\delta^{13}C$ of soil respiration, which suggests indirectly that recently fixed photosynthates comprise a substantial component of substrates consumed by soil respiration. However, there are other reasons why the $\delta^{13}CO_2$ of soil efflux may change with moisture conditions, which have not received as much attention. Using a combination of greenhouse experiments and modeling, we examined whether moisture can cause changes in fractionation associated with (1) non-steady-state soil CO_2 transport, and (2) heterotrophic soil-respired $\delta^{13}CO_2$. In a first experiment, we examined the effects of soil moisture on total respired $\delta^{13}CO_2$ by growing Douglas fir seedlings under high and low soil moisture conditions. The measured $\delta^{13}C$ of soil respiration was 4.7‰ more enriched in the low-moisture treatment; however, subsequent investigation with an isotopologue-based gas diffusion model suggested that this result was probably influenced by gas transport effects. A second experiment examined the heterotrophic component of soil respiration by incubating plant-free soils, and showed no change in microbial-respired $\delta^{13}CO_2$ across a large moisture range. Our results do not rule out the potential influence of recent photosynthates on soil-respired $\delta^{13}CO_2$, but they indicate that the expected impacts of photosynthetic discrimination may be similar in direction and magnitude to those from gas transport-related fractionation. Gas transport-related fractionation may operate as an alternative or an additional factor to photosynthetic discrimination to explain moisture-related variation in soil-respired $\delta^{13}CO_2$. Copyright © 2010 John Wiley & Sons, Ltd.

There is great interest in employing carbon isotopes to identify the sources and drivers of soil respiration,[1,2] which is often the largest flux of CO_2 from terrestrial ecosystems.[3–5] A number of isotope studies have suggested that the supply of recently assimilated photosynthates has a substantial influence on the $\delta^{13}CO_2$ of soil respiration.[6–9] In these studies, soil-respired $\delta^{13}CO_2$ was found to correlate with recent atmospheric humidity, in a similar way to how photosynthetic discrimination is known to respond to humidity.[10] These correlations have been interpreted as indirect evidence that plant photosynthates are rapidly transported below ground and consumed, so the isotopic composition of soil respiration reflects the recent moisture status of plants. However, other work has shown that $\delta^{13}CO_2$ respired directly from plants[1,11,12] and from soil[13,14] can exhibit complex temporal dynamics that are independent of substrate $\delta^{13}C$. These studies call into question whether there is a direct causal relationship between plant moisture status and soil $\delta^{13}CO_2$ flux, and justify a closer look at moisture impacts on soil-respired $\delta^{13}CO_2$.

When C_3 plants experience moisture limitations, either due to low soil moisture, or to high transpiration demands, they discriminate less against the heavy $^{13}CO_2$ isotopologue during photosynthesis, and assimilate carbon that is enriched in ^{13}C compared with non-water-stressed plants.[10,15,16] The sensitivity of photosynthetic discrimination to plant moisture status is explained by the theoretical model first proposed by Farquhar *et al.*,[17] which describes photosynthetic discrimination for C_3 plants as a function of stomatal openness, expressed as the ratio of CO_2 inside and outside the leaf. A study by Pate and Arthur[15] indicated that the Farquhar model could potentially also explain $\delta^{13}C$ variation in carbon pools beyond leaves. Their results

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showed variation of up to 8‰ in the $\delta^{13}C$ of phloem sugars, corresponding with seasonal patterns of plant water stress.

A number of more recent studies, however, have shown that on timescales of hours to days, plant-respired $\delta^{13}CO_2$ is independent of putative respiratory substrates, which raises some questions about the predictive ability of the Farquhar model on short timescales for describing plant-respired $\delta^{13}CO_2$ (see review by Bowling *et al.*[1]). Both Werner *et al.*[11] and Hymus *et al.*[18] have shown that large diurnal changes exceeding 7‰ occur in leaf-respired $\delta^{13}CO_2$, while the $\delta^{13}C$ of carbohydrate substrates remains relatively constant. Similarly, Gessler *et al.*[19] found that day-to-day variations in root-respired $\delta^{13}CO_2$ were not significantly correlated with $\delta^{13}C$ of water-soluble root compounds.

Furthermore, soil respiration is composed of CO_2 fluxes from soil microbes in addition to roots. A potential complication in determining the cause of soil-respired $\delta^{13}CO_2$ moisture dynamics is a lack of information on how moisture affects soil microbial-respired $\delta^{13}CO_2$. Moisture-related changes in microbial communities[20] or in their respiratory substrates[21] could potentially alter respired $\delta^{13}CO_2$. We are unaware, however, of any previous studies that have explicitly examined the day-to-day or seasonal isotope dynamics of heterotrophic soil fluxes.

In addition to biological influences, the physical effects of gas transport have large influences on soil-respired $\delta^{13}CO_2$ that are rarely addressed in ecological studies. The diffusion of CO_2 has a large theoretical fractionation (4.4‰), which in soil systems causes heavier ^{13}C to reside longer within soil pore space than ^{12}C. Although this fractionation effect is always present, Cerling *et al.*[22] demonstrated that if soil respiration is at a diffusive steady-state, the $\delta^{13}C$ of soil surface flux should theoretically match the $\delta^{13}C$ produced within the soil. More recent work examining diffusion under non-steady-state conditions,[13] however, has shown that changes in soil CO_2 concentration gradients, or in soil gas diffusivity, cause transient changes in relative diffusion rates of ^{12}CO and ^{13}CO isotopologues, due to the more rapid response and equilibration of the lighter ^{12}C isotope. Such transient changes can cause the $\delta^{13}C$ of surface flux to deviate from the $\delta^{13}C$ of CO_2 produced within the soil. Furthermore, in systems that experience pulses of advection,[23,24] mixing between isotopically heavy atmospheric CO_2 and more depleted soil-produced CO_2 can similarly cause transient changes in the $\delta^{13}C$ of soil fluxes. This can greatly complicate interpretations of soil-respired $\delta^{13}CO_2$, because changes in surface flux may be due either to changes in the $\delta^{13}C$ of soil-produced CO_2 or to transport-related effects. Non-steady-state diffusion or pulses of advection that are brought about naturally by environmental changes,[13] atmospheric turbulence,[23] or caused by sampling itself,[25] may influence the $\delta^{13}C$ of the surface flux. We call the apparent fractionation that is a by-product of non-steady-state gas transport 'dynamic fractionation'. Soil moisture may impact the magnitude of dynamic fractionation, because moisture affects both the rate of biological CO_2 production and soil gas diffusivity.

The influences of sampling techniques on dynamic fractionation have been examined in two recent modeling studies,[14,26] and these findings raise a potential challenge to previous interpretations of soil-respired $\delta^{13}CO_2$. Many studies that identified moisture-related soil respiration dynamics employed various types of soil surface chambers to sample respired $\delta^{13}CO_2$, and models of soil gas diffusion have since revealed that surface chambers alter CO_2 concentration gradients between the soil and atmosphere, creating non-steady-state conditions that change the $\delta^{13}CO_2$ of surface flux during measurements. Furthermore, the magnitude of the disturbance created by chambers depends on the soil CO_2 production rate, and the time required for re-equilibrium depends on the soil gas diffusivity, both which vary with soil moisture conditions. For many surface chamber measurement techniques, $\delta^{13}CO_2$ measurements from dry soils are more biased towards enriched values than $\delta^{13}CO_2$ measurements from high-moisture soils.[25] This finding opens the possibility that previous correlations between moisture and soil-respired $\delta^{13}CO_2$ may have resulted from fractionation related to soil gas transport, rather than to biogenic causes.

The purpose of our study was to examine two mechanisms other than respiration of recently fixed photosynthates that may explain moisture-related changes in soil-respired $\delta^{13}CO_2$. These alternatives do not exclude the important influence of recent photosynthates on soil respiration, and evidence from studies using several other techniques has also indicated close links between canopy carbon supply and soil respiration, such as in phloem girdling studies,[27,28] studies across natural gradients of root abundance,[29] and studies involving isotopic labeling of photosynthates.[30,31] However, because most previous natural abundance $\delta^{13}C$ studies have focused on the isotopic composition of recently assimilated photosynthates as a single explanation, it is not clear whether other mechanisms may have operated at the same time. Natural abundance $\delta^{13}C$ measurements hold important potential for studying canopy-soil carbon linkages *in situ*, but their full utility can only be realized if all the processes that fractionate carbon along plant-soil pathways are known. In contrast to photosynthetic discrimination, which has been thoroughly described and is mechanistically well understood, far less is known about the fractionation that occurs during post-photosynthetic processing within plants and soil, and whether this fractionation is moisture-dependent.

In this study we performed two greenhouse experiments in combination with model simulations to examine two potential post-photosynthetic mechanisms for moisture-related variations in soil-respired $\delta^{13}CO_2$: (1) that variations in soil moisture have an impact on the magnitude of dynamic fractionation resulting from non-steady-state soil CO_2 transport, and (2) that variations in soil moisture affect the $\delta^{13}CO_2$ of soil microbial respiration. In the first experiment we grew Douglas fir seedlings in soil columns under well-watered and drought-stressed conditions, and compared measurements of soil-respired $\delta^{13}CO_2$ taken with surface chambers. We then used the experimental data in conjunction with an isotopologue-based gas diffusion model[14] to test the hypothesis that moisture-related differences in the measured $\delta^{13}CO_2$ were caused by transport-related, dynamic fractionation. To compare the magnitude of impacts caused

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by dynamic fractionation to the potential impacts of photosynthetic discrimination, we also used foliar gas-exchange measurements in conjunction with the equation of Farquhar *et al*.[17] to calculate the theoretical photosynthetic discrimination of the seedlings, and the potential influence of recent photosynthates on soil-respired $\delta^{13}CO_2$. In the second experiment we assessed the role played by soil microbes, and tested the hypothesis that $\delta^{13}CO_2$ of heterotrophic soil respiration does not vary with moisture. We incubated root-free soils in sealed chambers to minimize the possibility for dynamic fractionation during sampling, and also validated this sampling approach with the isotopologue diffusion model.

EXPERIMENTAL

Soil description

For both greenhouse experiments, soil columns (15 cm i.d. × 38 cm height, non-transparent PVC pipe) were filled with a 1:1 mixture of perlite and cobbley silt-loam soil collected from the HJ Andrews Experimental Forest in the western Oregon Cascade Range, USA. The soil is derived from alluvium of volcanic origin, part of the Quentin series, and the dominant vegetation is mature Douglas fir forest.[32] Soil was removed from the O, A, and B1 horizons to 15 cm depth, homogenized, and large roots and rocks were removed by sieving to 2 cm.

The soil was mixed with horticultural perlite to reduce compaction during prolonged watering. Perlite is an inert, neutral, amorphous volcanic glass that is heated until it expands, forming granules with a closed cell interior structure. In a pre-test, we verified that perlite does not release detectable amounts of CO_2. We filled three replicate 12 mL Exetainer™ vials (Labco, High Wycombe, UK) with moistened perlite, flushed the air space overnight with CO_2-free air, and then allowed the perlite to incubate for ~8 h before measuring the CO_2 concentration in the headspace.

The soil columns were supported by 1 mm fiberglass mesh on the bottom to facilitate drainage, and had 3 cm headspace at the top. The dry bulk density was approximately 0.5 g cm^{-3}, and the effective porosity was 59%.

Experiment 1: Impact of surface chamber sampling on total soil-respired $\delta^{13}CO_2$

To measure total soil respiration in a controlled setting, ten Douglas fir seedlings (each 2 years old) from Weber Forest Nursery in Olympia, WA, USA, were planted in soil columns in April 2006, and watered for 3 months in a sheltered outdoor area at Oregon State University, Corvallis, OR, USA, to become established. In July the columns were moved to a greenhouse and randomly selected for either a high- or a low-moisture treatment. Columns in the high-moisture treatment were watered to field capacity every evening with tap water, and columns in the low-moisture treatment were allowed to dry over 5 weeks.

The soil moisture was also measured continuously with CS-610 TDR moisture probes (Campbell Scientific, Logan, UT, USA), using calibration coefficients that we determined by comparing the sensor voltage with the volumetric water content of the packed soil columns (determined as gravimetric water content × bulk density). The air temperature in the greenhouse ranged approximately from 21 to 30°C.

We measured the soil respiration weekly throughout the equilibration period and just prior to isotope sampling with a LI-6400 portable gas exchange system (Licor Environmental, Lincoln, NE, USA). A miniature soil respiration chamber was constructed to fit inside the planted soil columns and connect to the LI-6400. This miniature soil chamber was constructed of a non-transparent PVC end cap (5.15 cm i.d.), and had the same features as the commercially available LI-6400-19 soil chamber, including an E-type thermocouple, pressure release vent, and a rim with a closed-cell foam gasket to interface with soil collars. Soil collars were inserted into the soil to a depth of 2 cm. The standard LI-6400 soil chamber automatic program was used to measure respiration, with the chamber mixing fan on the low speed setting. The accuracy of the custom soil chamber plus collar was validated by a series of comparisons with results obtained from a LI-6400-19 in unplanted soil columns (regression slope = 1.00 ± 0.03, $R^2 = 0.99$, n = 9).

Isotope samples were collected using a smaller version of the chamber system described by Ekblad and Högberg[6] to fit within the soil columns. The soil collars were capped with a PVC end cap (total volume 178 cm^3) fitted with a stainless steel union holding an acetyl-butyl septum. A series of four gas samples was taken from the chamber headspace, at 3 min intervals for high-moisture soils and 5 min intervals for low-moisture soils, with the goal of capturing CO_2 levels spanning at least 200 ppm. A two-end-member mixing model, or Keeling plot, was used to calculate the $\delta^{13}C$ of the soil respiration. The $\delta^{13}C$ values of air samples from the chamber headspace were plotted against their corresponding $1/[CO_2]$ values, and ordinary least-squares regression was used to extrapolate the Keeling intercept.[33]

To assess potential photosynthetic discrimination, we also monitored plant water stress by measuring stomatal conductance and photosynthesis mid-morning once per week. These foliar gas exchange measurements were performed with a LI-6400 using a conifer leaf chamber at ambient light conditions. Leaf areas (one-sided) were measured following harvest by scanning the needles in a flat bed digital scanner and calculating the needle areas with ImageJ software.[34]

We calculated the theoretical photosynthetic discrimination from these gas exchange data and the model described by Farquhar *et al*.:[35]

$$\Delta = a + [b - a][C_i/C_a] \quad (1)$$

where Δ is the total discrimination due to photosynthesis, a is the fractionation due to diffusion in air (4.4‰), b is the net fractionation caused by carboxylation (27‰), and C_i and C_a are leaf internal CO_2 and ambient CO_2 concentration, respectively. We calculated Δ for each plant using mid-morning C_i and C_a values measured during week 5 of the moisture treatments. The $\delta^{13}C$ of photosynthates was assumed to equal the daytime CO_2 in the greenhouse (estimated as −10‰) minus the photosynthetic discrimination, Δ.

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We estimated the maximum potential impact of photosynthetic discrimination on the $\delta^{13}C$ of soil respiration by calculating theoretical values for soil-respired $\delta^{13}CO_2$ based on several simplifying assumptions. First, we assumed that autotrophic soil respiration derived exclusively from recently assimilated photosynthates, so that the $\delta^{13}C$ of autotrophic soil respiration was the same as that of the photosynthates calculated with the Farquhar model. Second, we assumed that the $\delta^{13}CO_2$ of heterotrophic respiration remained static at −28.4‰ and did not vary with soil moisture (based on findings from Experiment 2, described below). We estimated heterotrophic respiration rates from the root-free soils used in Experiment 2, and estimated autotrophic respiration rate by difference for planted and root-free soils with the most similar moisture contents (rates were also normalized to 25°C for comparison following Campbell and Law[36]). We used these autotrophic and heterotrophic respiration rates and isotope values in a two-member mixing Equation to calculate the expected impacts of photosynthetic discrimination on the $\delta^{13}C$ of total soil respiration for each soil column.

Experiment 2: Impact of soil moisture on heterotrophic soil-respired $\delta^{13}CO_2$

Nine soil columns were packed with perlite-amended soil to the conditions described above. To achieve a range of soil moistures, all the columns were initially watered and were kept moist by saturating the bottom 2 cm in a shallow pan of water to permit capillary draw. Each week for a total of 5 weeks, 1–2 columns were set aside to commence drying. Soil-respired CO_2 was sampled for C isotope analysis from all the soil columns at the end of this period, with water content ranging from 10 to 35% (v/v). The water contents were determined gravimetrically. Soil respiration was measured immediately prior to isotope sampling using a LI-6400 gas-exchange system with a LI-6400-19 soil chamber.

To sample soil-respired $\delta^{13}C$, we sealed the soil columns by capping the top and bottom with PVC end caps and sealing the edges with silicone vacuum grease, creating a 700 mL headspace. The columns were incubated overnight for a total of 22 h to allow soil-derived CO_2 to build up to high concentrations, and for gas in the soil pores and chamber headspace to come to isotopic equilibrium. The headspace was vented to an airlock during the incubation period to allow pressure venting, and was switched with a three-way valve to a syringe needle during sampling.

Because these soils did not become as dry as the soil columns in Experiment 1 that contained plants, at a later date we also incubated small quantities of air-dry soils directly in 12 mL Exetainer vials. Three replicate 5 g samples of air-dry soil were sealed in Exetainer vials, and flushed with CO_2-free air overnight to purge the airspace (approximately 6 mL). Because of low production rates, these soils were incubated for 72 h to allow CO_2 concentrations to reach similar concentrations as in the soil column incubations. We simulated the large soil columns with a gas diffusion model (details below) to quantify any potential residual isotopic disequilibrium following the incubation period, and to ensure comparable results between the two incubations techniques.

Sampling and analysis of $\delta^{13}CO_2$

In both experiments, gas samples were collected from the headspace of the soil chambers into 12 mL Exetainer vials pre-flushed with N_2. A handpump connected to a three-way valve was used to evacuate the Exetainers and immediately take a sample. Because evacuation was incomplete, we sampled standard gases with the handpump and calculated concentration dilution factors to account for residual N_2. The dilution averaged 9.38% (std dev = 0.83%) over the course of all the experiments. After sampling, vials were capped with silicone adhesive and analyzed within 24–72 h using a DeltaPlus XL isotope ratio mass spectrometer interfaced to a GasBench II automated headspace sampler (Finnigan MAT, Bremen, Germany). For the air-dried soils incubated in Exetainers, the headspace $\delta^{13}C$ and CO_2 were measured directly on the Gas Bench II, similarly to the procedure described by Crow *et al*.[37] The ratio of heavy to light isotopes of each sample (R) was related to the Vienna PeeDee Belemnite (PDB) standard (R_{std}) in order to express the carbon isotope ratios as $\delta^{13}C$ (‰).

$$\delta = \left(\frac{R}{R_{std}} - 1\right) \times 1000 \quad (2)$$

In the first experiment, samples were analyzed at the College of Oceanic and Atmospheric Science (COAS), Oregon State University. In the second experiment, samples were analyzed at the Institute for Stable Isotope Research Facility (ISIRF) at the Environmental Protection Agency Western Ecology Division, Corvallis, OR, USA. The same instrument model was used at both locations. The combined standard uncertainties of the measurements, which include sampling and instrument uncertainties, were determined based on replicate analyses to be 0.4‰ for the PDB standard and 2.51% of CO_2 concentration for the ISIRF instrument, and 0.13‰ for the PDB and 3.75% of CO_2 concentration for the COAS instrument.[38]

Modeling simulations: Assessing impacts of non-steady-state diffusive fractionation

To assess how gas transport under sampling conditions may have affected measured $\delta^{13}CO_2$ values, we simulated the diffusive processes in each experiment with an isotopologue diffusion model. We simulated the surface chamber used in the first experiment with a three-dimensional (3-D) version of the model to account for feedbacks between the chamber and the surrounding soil surface. Details of the 3-D model and experimental validations are described by Nickerson and Risk.[14] We used a one-dimensional (1-D) version of the model[13] for the sealed soil columns in the second experiment. The performance of the 1-D model was validated by comparing modeled and observed values of chamber headspace $\delta^{13}C$ and CO_2 following incubation.

To briefly describe the model, both versions of the model calculated fluxes of $^{12}CO_2$ and $^{13}CO_2$ using Fick's First law with isotopologue-specific gas diffusivities. The modeled environment assumed a well-mixed atmospheric boundary layer and a soil profile divided into uniform units (100 horizontal layers for the 1-D model, and 10 horizontal × 5 radial layers for the 3-D model). The model allows gas

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exchange between neighboring units following concentration gradients according to Fick's Law.

A constant atmospheric upper boundary layer was set at 380 ppm and −10‰ (in addition, a lower atmospheric boundary layer was simulated in Experiment 1, representing the screened bottom of the soil column). The modeled soil profile consisted of solids, and water- and air-filled pore space. Water- and air-filled porosity were determined from volumetric water content and bulk density measurements, and were assumed to be uniform through the profile. The model was also parameterized using chamber surface area and volume, and the following soil properties: soil depth, effective gas diffusivity, and total CO_2 production rate. Air-filled pore space was used to calculate the effective gas diffusivity with the Millington-Quirk relationship.[39] We also independently calculated the effective diffusivity of the perlite-amended soil across a range of moisture levels and found close agreement with the Millington-Quirk relationship (regression slope = 0.96, $R^2 = 0.76$, n = 9).

The total CO_2 production rate for each soil column was estimated from the surface CO_2 flux rate measured just prior to isotope sampling. For the first experiment, this rate was multiplied by two to account for equal fluxes from the top and the screened bottom of the soil column. The production of CO_2 was assumed to be equal throughout the soil profile. Model runs were initialized with open tops and bottoms until the soil CO_2 and $\delta^{13}C$ profiles stabilized, and the boundary conditions were then modified to model the presence of sampling chambers.

RESULTS

Experiment 1: Impact of surface chamber sampling on total soil-respired $\delta^{13}CO_2$

At the end of 5 weeks of soil drying, soils in the low-moisture treatment averaged 5.6% (v/v), compared with 39.4% (v/v) in the regularly watered high-moisture treatment. The soil-respired $\delta^{13}CO_2$ values determined from Keeling intercepts (Fig. 1) were 4.7‰ higher in the low-moisture treatment than from the high-moisture treatment ($p = 0.0004$, Welch's t-test). The average Keeling intercepts for the low- and high-moisture treatments were -20.0 ± 2.4‰ and -24.7 ± 1.3‰, respectively. Because gas samples were collected with surface chambers, however, these raw Keeling intercepts are likely to have some artifacts caused by non-steady-state gas transport that must be corrected for to obtain the true isotopic signature of soil respiration.[14] To evaluate potential errors in the Keeling intercepts caused by non-steady-state diffusion during the sampling process we simulated the sampling conditions with the 3-D gas diffusion model, parameterizing the model as described above, with an initial estimate of −28‰ for soil-respired $\delta^{13}CO_2$. We assessed the ability of the 3-D diffusion model to replicate our sampling conditions by comparing measured and modeled values of CO_2 and $\delta^{13}C$ from the sampling chamber over time, and found that the measured values leveled off more rapidly than predicted by the gas diffusion model (Fig. 2(a)). Keeling plots constructed from small chamber measurements also exhibited greater curvature and had more enriched intercepts than predicted by the diffusive model (Fig. 2(b)). Because the gas diffusion model did not fit our data well, we did not apply it further to estimate Keeling intercept biases caused by non-steady-state diffusion. We instead assessed potential causes of the disparity between the measured and modeled values.

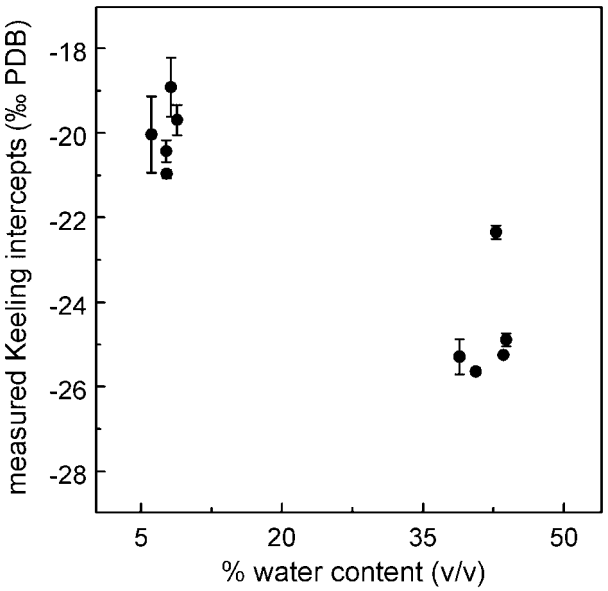

Figure 1. Keeling intercepts at high- and low-moisture levels for total soil respiration with Douglas fir seedlings. Error bars are SE for the intercepts of ordinary least-squares linear regressions.

We examined whether the data-model mismatch could be due to uncertainty in CO_2 production, measured just prior to isotopic sampling, by varying production rate in the model while holding other parameters constant. We found that increasing production in the model did predict more rapid chamber equilibration, but also consistently predicted chamber $\delta^{13}C$ values that were more depleted than those observed. We also examined whether the data-model mismatch could be due to uncertainty in CO_2 diffusivity, by varying diffusivity in the model and assessing the best fit between measured and modeled CO_2 and $\delta^{13}C$ with Pearson's correlation coefficient. The fit consistently improved as we increased the diffusivity in the model, and the 'true' diffusivity appeared to exceed the range of values that would be reasonable for soil. Furthermore, the highest diffusivity level fit the data from low-moisture and high-moisture soils almost equally well (R^2 between measured and modeled CO_2 concentration averaged 0.987 vs. 0.996 for low- and high-moisture chambers, respectively), suggesting that CO_2 transport in our soil columns was far less sensitive to soil moisture than would be expected under diffusion-dominated conditions. A logical explanation of the high apparent diffusivity and the lack of sensitivity to moisture is that advection occurred during the sampling process. Extraction of the four 12 mL air samples used to construct the Keeling plots would have displaced approximately 27% of the chamber headspace volume, thereby drawing CO_2 into the chamber from the soil profile. Although we are unable to confirm that advection occurred without differential pressure data, there are isotopic signals

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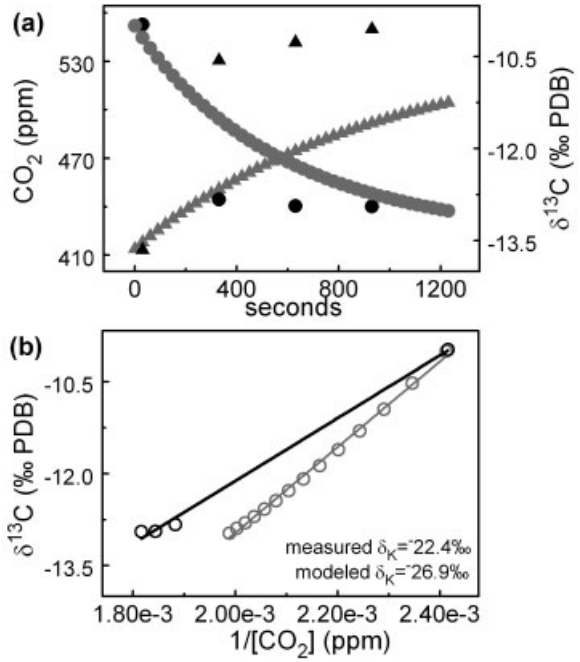

Figure 2. Example of measured and modeled $\delta^{13}C$ and CO_2 concentrations for a surface chamber from Experiment 1, with 6% soil water content (v/v) and 1.2 μmol CO_2 m^{-2} s^{-1} surface flux rate. (a) Time series of measured (black) and modeled (grey) $\delta^{13}C$ and CO_2 (circles and triangles, respectively). (b) Resulting Keeling plots fit with ordinary least-squares regression for measured (black) and modeled (grey) data. Keeling intercept values (δ_K) are also shown. Under theoretical diffusive conditions (modeled results) Keeling plot curvature occurs because surface chambers reduce soil-atmosphere concentration gradients and slows diffusion of ^{12}C faster than ^{13}C. Curvature in measured Keeling plots was greater than theoretical diffusive conditions, potentially due to advective removal of soil gas during sampling.

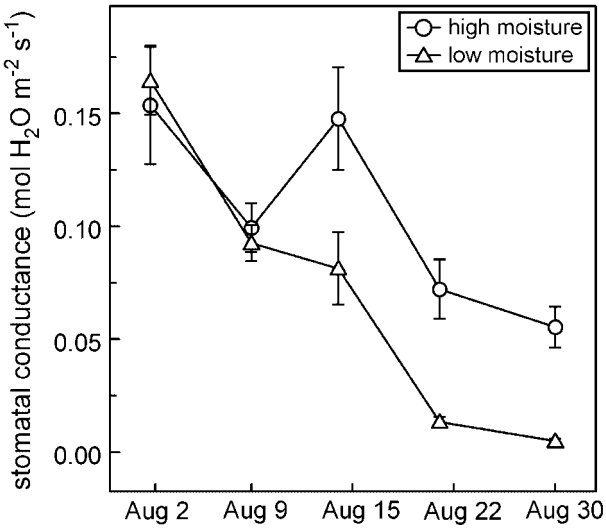

Figure 3. Mean mid-morning stomatal conductance under natural light over the course of moisture treatments. Error bars are 95% confidence intervals.

of advection present in our data set. In the discussion, we examine the potential impacts of advective sampling on the measured difference in $\delta^{13}C$ between moisture treatments.

The moisture treatments also had an impact on plant moisture stress, and produced large differences in photosynthetic discrimination estimated with the model of Farquhar *et al*. (Eqn. (1)). Mid-morning stomatal conductance indicated that low-moisture seedlings experienced physiological stress by the fourth week of soil drying (Fig. 3). In the high-moisture treatment, the stomatal conductance showed expected increases and decreases over time in response to natural light conditions. In the low-moisture treatment the stomatal conductance decreased monotonically over time, from an initial value of 0.12 mmol H_2O m^{-2} s^{-1}, similar to the high-moisture treatment, to 0.02 mmol H_2O m^{-2} s^{-1} by week 4. Using Eqn. (1), we calculated that recently assimilated photosynthates were theoretically 8.1‰ more enriched in the low-moisture than in the high-moisture treatment (95% confidence interval (CI) = −10.6 to −5.5‰). To estimate the maximum potential difference that this could cause in soil-respired $\delta^{13}CO_2$, we examined a hypothetical case in which this difference also represents the variation in the $\delta^{13}C$ of autotrophic soil respiration, and the $\delta^{13}C$ of heterotrophic soil respiration does not vary with soil moisture. Under these theoretical conditions we estimated that the total soil-respired $\delta^{13}CO_2$ would be 3.7‰ more enriched in the low-moisture treatment than in the high-moisture treatment (Fig. 4). Therefore, the measured 4.7‰ difference in Keeling intercepts may not be explained entirely by variation in photosynthetic discrimination.

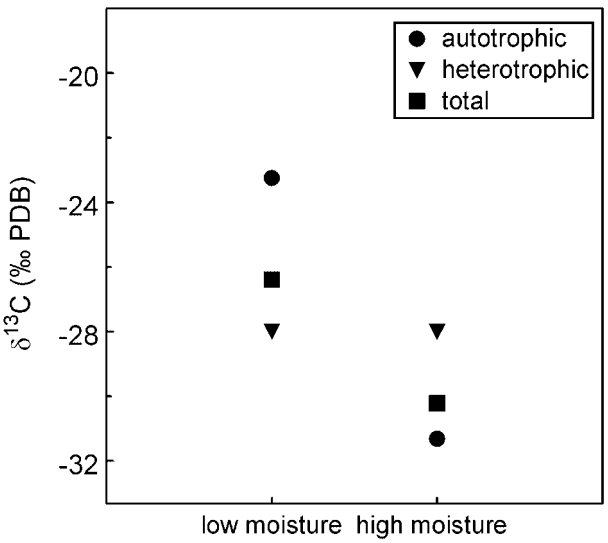

Figure 4. Theoretical $\delta^{13}C$ of soil respiration calculated with the model of Farquhar *et al* for photosynthetic discrimination (Eqn. (1)). The $\delta^{13}C$ of heterotrophic respiration was held constant (−28.4‰), $\delta^{13}C$ of autotrophic respiration was calculated from Eqn. (1) using parameters from foliar gas exchange measurements, and $\delta^{13}C$ of total soil respiration was calculated using a two-member mixing equation. Relative contributions of autotrophic and heterotrophic sources were determined from respiration rates measured from intact and root-free soils.

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Experiment 2: Impact of soil moisture on heterotrophic soil-respired $\delta^{13}CO_2$

To test the impact of microbial respiration on soil $\delta^{13}CO_2$ moisture dynamics, we incubated soils without plants across soil moisture contents ranging from 10 to 35% (v/v) in sealed soil columns. After 22 h of incubation, the headspace concentration ranged from 1.1 to 2.0% CO_2 and the $\delta^{13}C$ averaged -27.3 ± 0.2‰ (Fig. 5, open circles). We also incubated smaller amounts of air-dried soils in Exetainer vials, which had a moisture content of 4 to 7% (v/v), and reached an average of 1.2 ± 0.2% CO_2 and -29.0 ± 0.4‰ $\delta^{13}C$ after 72 h of incubation (Fig. 5, triangles). These raw measurements indicated a relatively constant $\delta^{13}C$ across a large moisture range in the large soil columns, but a more depleted $\delta^{13}C$ in the drier soils incubated in Exetainer vials. We hypothesized that the difference between these two types of incubation chambers was a result of incomplete diffusive equilibration in the large volumes of the soil columns.

To test this, we parameterized the 1-D isotopologue model for each soil column using measured water contents and flux rates. The true values for soil $\delta^{13}C$ were unknown and were initially estimated as -28‰ across all moisture levels, and then reiterated at -28.4‰ to better match measured chamber values. In contrast to the simulations in Experiment 1, the diffusive model was able to predict chamber $\delta^{13}C$ and CO_2 fairly closely in Experiment 2 (Fig. 6). For CO_2 concentration, the slope of modeled vs. measured CO_2 was not significantly different from 1 for all the chambers, across a large range of post-incubation concentrations (slope 95% CI = 0.748–1.66, $R^2 = 0.82$). The measured $\delta^{13}C$ values following the incubation period were similar for all chambers, varying by less than the measurement uncertainty of ±0.4‰. Modeling a single soil $\delta^{13}C$ value of -28.4‰ across all moisture levels predicted the experimental chamber values to within ±0.5‰.

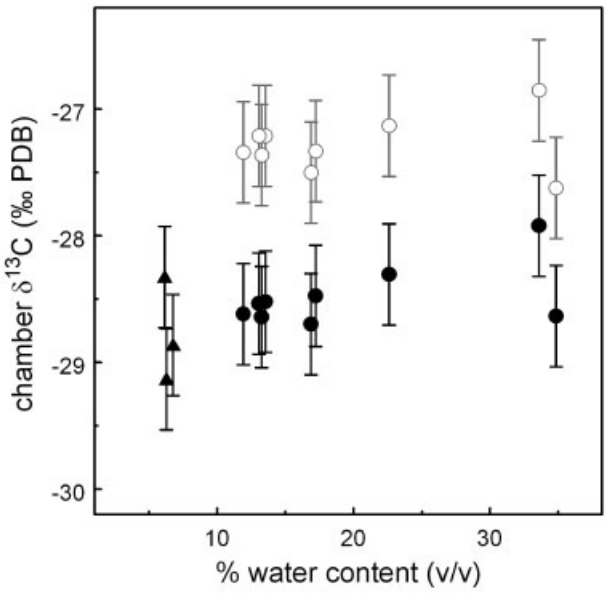

Figure 5. Heterotrophic soil respiration $\delta^{13}C$ across a range of soil moistures. Unadjusted $\delta^{13}C$ measurements from large chambers (○), large chamber measurements adjusted by modeled estimates of residual isotopic disequilibrium (●), and unadjusted $\delta^{13}C$ measured from small Exetainer vials (▲).Error bars represent measurement uncertainty (0.4‰).

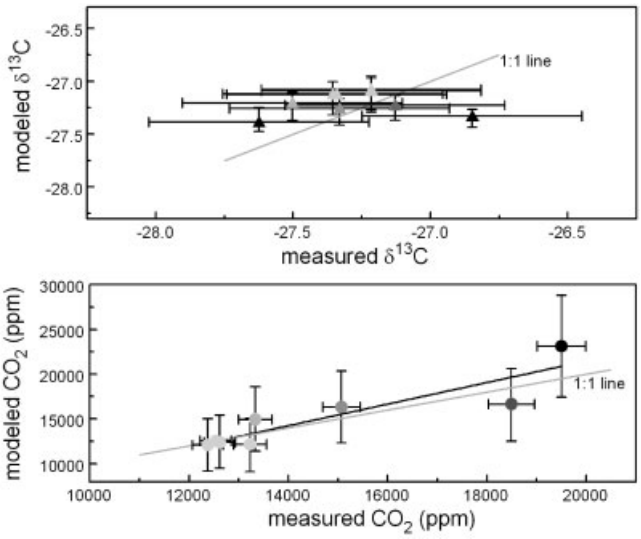

Figure 6. 1-D model performance for estimating chamber headspace $\delta^{13}C$ (top) and CO_2 concentration (bottom), following a 22 h incubation period. Horizontal bars represent combined measurement uncertainty from hand-pump sampling and mass spectrometer ($\delta^{13}C$ ±0.4‰, CO_2 concentration ±2.5%) Vertical error bars represent model output for ± 25% of the CO_2 production rate measured prior to sealing the pots, to account for uncertain fluctuations in temperature during the incubation period. Colors of points from darkest to lightest indicate highest to lowest soil moisture.

Model simulations indicated that the headspace $\delta^{13}C$ was still more enriched than the soil-produced $\delta^{13}C$ after the incubation period due to incomplete diffusive equilibration, by 1.0–1.3‰. Simulations showed that after 48 h the headspace $\delta^{13}CO_2$ would have still been 0.58–0.61‰ higher than the production value, suggesting that it would take several days for the sealed soil columns to approach isotopic equilibrium. When we subtracted the residual enrichment of each soil column from the measured chamber $\delta^{13}C$ values, we found no significant difference between the $\delta^{13}C$ values from the soil columns and Exetainer vials (Fig. 5, closed symbols). A slight linear trend towards lighter $\delta^{13}C$ at low moisture was found, but the slope was not significantly different from zero (95% CI = −0.002-0.148).

DISCUSSION

The purpose of this experiment was to determine whether variation in (1) dynamic, transport-related fractionation or (2) the $\delta^{13}C$ of soil microbial respiration can help to explain moisture-related changes in soil-respired $\delta^{13}CO_2$. The first experiment demonstrated a 4.7‰ difference in measurements of soil-respired $\delta^{13}CO_2$ from moisture-sufficient and moisture-limited seedlings (Fig. 1). Although the plants in this experiment experienced extremes of high and low moisture, and theoretical photosynthetic discrimination estimated with the model of Farquhar *et al*. differed substantially between the treatments, we estimated that

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even if autotrophic soil respiration was produced entirely from recently assimilated carbon, photosynthetic discrimination could not account entirely for the difference in measured Keeling intercepts (Fig. 4). Subsequent modeling of gas transport also indicated that the system was not at isotopic equilibrium and therefore these results may have been influenced by transport-related fractionation, although not due to non-steady-state diffusion as we originally expected. The mismatch between our measurements and a diffusion model (Fig. 2) provided evidence that our sampling approach of repeatedly filling Exetainers from a surface chamber caused advection. As discussed below, the presence of advection may partially explain the measured difference in $\delta^{13}CO_2$ from high- and low-moisture soils.

Experiment 2 ruled out the possibility that moisture-related differences in soil $\delta^{13}CO_2$ were related to changes in heterotrophic respiration (Fig. 5). Although initial isotope measurements indicated that very dry soils incubated in Exetainer vials produced more depleted $\delta^{13}C$ than the more moist soils incubated in larger soil columns, subsequent modeling showed this difference was due to incomplete diffusive equilibration in the larger containers. The measured headspace CO_2 and $\delta^{13}C$ values agreed closely with predicted values from the gas diffusion model (Fig. 6), which allowed us to estimate the actual $\delta^{13}C$ of soil-produced CO_2, in contrast to knowing only the $\delta^{13}C$ measured from the chamber headspace. Using these estimates, we found no significant differences in the $\delta^{13}CO_2$ of heterotrophic respiration across a large range of soil moisture conditions.

Impacts of advection on measured $\delta^{13}C$

Although few studies have explicitly examined the impacts of advection on soil-respired $\delta^{13}CO_2$, recent work on non-steady-state transport kinetics provides a conceptual framework for understanding the potential impacts of advection during sampling. Numerical simulations of soil gas diffusion during sampling with surface chambers have shown that chambers disrupt concentration gradients between the soil and the atmosphere, and cause the $\delta^{13}C$ of surface flux to change as the system approaches a new equilibrium.[14,26] Both theoretical[14] and experimental evidence[40] have further demonstrated that the CO_2 concentrations and $\delta^{13}C$ signatures of surface chambers equilibrate to soil values at the depth to which chambers or collars are inserted. Pulses of advective transport, which draw soil gas into the surface chamber, are likely to increase the speed with which chambers approach and equilibrate with the soil profile $\delta^{13}C$ and CO_2. This should produce Keeling plots with more curvature than expected for purely diffusive gas transport, as we observed in Experiment 1 (e.g. Fig. 2(b)). In addition to advancing the equilibration between the sampling chamber and soil, advection would also alter the measured $\delta^{13}CO_2$ by drawing gas from soil pores that is more enriched than biological respiration, due to diffusive fractionation and atmospheric invasion. Drawing isotopically heavy CO_2 into surface chambers should further exacerbate the non-linearity of Keeling plots, and bias Keeling intercepts towards values that are more enriched than the actual $\delta^{13}CO_2$ produced in the soil.

Advective sampling conditions help to explain why the high- and low-moisture treatments had relatively enriched Keeling intercepts compared with both the incubations in Experiment 2, and the expected $\delta^{13}C$ values for C_3 plant-derived substrates.[41] Under moist conditions, we expected the $\delta^{13}C$ of total soil respiration to be similar to the average value obtained in Experiment 2 (−28.4‰). Instead, the measured Keeling intercepts in Experiment 1 were considerably more enriched, averaging −24.7 and −20.0‰ for high- and low-moisture treatments, respectively. In a study of the impacts of advection on isotopic measurements, Kayler *et al.*[24] applied a brief and strong vacuum at the soil surface and found that it altered $\delta^{13}CO_2$ measurements by only 1‰. While this bias resulting from advection was small, their results are difficult to compare with those from our study because they used a different type of sampling chamber (mini-tower), drew only a single sample, and performed their test with only moist soil conditions.

The significant difference in the $\delta^{13}C$ that we measured between high- and low-moisture treatments may have resulted in part from moisture-related differences in the soil $\delta^{13}C$ profiles from which CO_2 was transported during sampling. As explained by Cerling *et al.*,[22] soils with low moisture (high diffusivity) experience greater atmospheric invasion than comparable soils with high moisture, and therefore have lower CO_2 concentrations and more enriched $\delta^{13}C$ values throughout the soil profile. In dry soils, low rates of biological CO_2 production further exacerbate atmospheric incursion into soil profiles. Advectively removing a gas sample would therefore draw more enriched air into the surface chamber from a dry than from a moist soil, resulting in greater non-linearity in Keeling plots, and intercepts more biased towards enriched values. It follows from this reasoning that the difference in measured Keeling intercepts between moisture treatments may have resulted at least in part from the advection introduced by sampling, and not entirely from actual variation in soil-produced $\delta^{13}CO_2$.

Distinguishing photosynthetic discrimination and dynamic fractionation

Our results indicate that any potential isotopic variation in autotrophic soil respiration resulting from photosynthetic discrimination would be dampened by heterotrophic fluxes that remain isotopically static across moisture levels. Although we cannot account for microbial behavior in the presence of roots, our soil incubations suggested that $\delta^{13}CO_2$ from microbes consuming soil organic matter is not sensitive to soil moisture. Even under the extreme soil moisture levels in Experiment 1, which produced large differences in theoretical photosynthetic discrimination, the estimated impacts of photosynthetic discrimination on total soil respiration were smaller than the fractionation associated with some sampling techniques.[25] In this study, we were unable to quantify the bias in Keeling intercepts that may have been caused by advective sampling; however, in a comparative analysis of several types of surface chambers, Nickerson and Risk[25] found that non-steady-state diffusion created biases with some sampling designs exceeding 4‰. They also found that the biases of most designs were higher

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in dry than in moist soils, because disturbance effects propagated further and more quickly through soils with high gas diffusivity. Dynamic, transport-related fractionation can therefore act in the same direction as expected for photosynthetic discrimination and, with some sampling techniques, the impacts of dynamic fractionation can also be similar in magnitude. Because dynamic fractionation and photosynthetic discrimination can have similar effects, they are unlikely to be distinguished without careful analysis of soil gas transport kinetics.

In field studies, Ekblad and Hogberg[6] found a seasonal range in soil-respired $\delta^{13}CO_2$ of 4.9‰ in a boreal mixed coniferous forest, and Fessenden and Ehleringer[8] found a range of 6.2‰ in a temperate coniferous forest. These studies utilized static chambers with fairly small estimated biases related to non-steady-state transport.[25] Therefore, these ranges are unlikely to be due to abiotic causes alone. Nevertheless, they demonstrate that the range of measured variation in soil-respired $\delta^{13}CO_2$ is fairly small in natural environments, and dynamic fractionation may therefore have important impacts on interpretations of ecological studies.

In the present study, model simulations demonstrated substantial influences from dynamic fractionation in both experiments, which altered the interpretation of our results. The simulations demonstrated two fundamental difficulties created by gas transport that studies must resolve before $\delta^{13}CO_2$ dynamics can be attributed to biological causes. The first difficulty is to sample soil-respired $\delta^{13}CO_2$ in a way that does not alter gas transport during the sampling process. The second difficulty is to determine the $\delta^{13}C$ of soil production, which can differ from measured $\delta^{13}C$ as a result of sampling, environmental changes, or prior soil disturbances. By carefully validating and executing isotope sampling techniques, sampling-related biases may be minimized, but non-steady-state conditions are likely to be common in natural soils, particularly near the soil surface, and are unlikely to be completely avoided. Fortunately, transport-related fractionation follows physical principles that are amenable to simulation, and a growing number of studies[24,25,40,42] demonstrate the utility of coupling experiments with modeling tests to help to address these difficulties.

CONCLUSIONS

In this study we examined whether moisture-related changes in the $\delta^{13}C$ of soil respiration could be explained in part by microbial respiration or gas transport-related effects. Measurements of total soil respiration with surface chambers showed a significant difference in $\delta^{13}C$ at high and low soil moistures, but the difference exceeded expected impacts from photosynthetic discrimination, and subsequent simulations of gas transport also indicated that advection probably occurred during the sampling process and may have accounted in part for the measured difference. We ruled out variations in heterotrophic respiration as a potential influence, because we found soil-microbial $\delta^{13}CO_2$ was constant across a large range of moisture conditions. It is important, however, for ecological studies of soil-respired $\delta^{13}CO_2$ dynamics not to overlook potential influences from transport-related, dynamic fractionation, which may have impacts similar in direction and magnitude to those expected from photosynthetic discrimination.

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