A considerable fraction of soil-respired CO₂ is not emitted directly to the atmosphere

Enrique P. Sánchez-Cañete1,2,5, Greg A. Barron-Gafford1,3 & Jon Chorover4

Soil CO₂ efflux \( (F_{\text{soil}}) \) is commonly considered equal to soil CO₂ production \( (R_{\text{soil}}) \), and both terms are used interchangeably. However, a non-negligible fraction of \( R_{\text{soil}} \) can be consumed in the subsurface due to a host of disparate, yet simultaneous processes. The ratio between CO₂ efflux/O₂ influx, known as the apparent respiratory quotient (ARQ), enables new insights into CO₂ losses from \( R_{\text{soil}} \) not previously captured by \( F_{\text{soil}} \). We present the first study using continuous ARQ estimates to evaluate annual CO₂ losses of carbon produced from \( R_{\text{soil}} \). We found that up to 1/3 of \( R_{\text{soil}} \) was emitted directly to the atmosphere, whereas 2/3 of \( R_{\text{soil}} \) was removed by subsurface processes. These subsurface losses are attributable to dissolution in water, biological activities and chemical reactions. Having better estimates of \( R_{\text{soil}} \) is key to understanding the true influence of ecosystem production on \( R_{\text{soil}} \) as well as the role of soil CO₂ production in other connected processes within the critical zone.

Soil carbon dioxide (CO₂) efflux \( (F_{\text{soil}}) \) is the second largest contributor to terrestrial CO₂ exchanges, similar in scale to uptake by terrestrial photosynthesis. Soil CO₂ efflux \( (F_{\text{soil}}) \) is defined as the rate of CO₂ exchange between soil and atmosphere, and it is the result of soil CO₂ production \( (R_{\text{soil}}) \) and its transport to the atmosphere. Rates of \( R_{\text{soil}} \) are the result of heterotrophic respiration during the decomposition of organic matter by microbes and autotrophic respiration by roots. Both \( F_{\text{soil}} \) and \( R_{\text{soil}} \) act together in response to the interactions between biotic and abiotic factors. Generally, \( F_{\text{soil}} \) increases with the productivity of an ecosystem, driven by increases in temperature and precipitation. With ample water, temperature is the dominant driver of \( F_{\text{soil}} \); however, in arid and semiarid ecosystems, patterns of \( F_{\text{soil}} \) are often driven by precipitation pulses and variation in soil moisture.

\( F_{\text{soil}} \) can be measured using manual or automatic chambers that capture CO₂ emitted from the soil surface to the atmosphere or estimated by the gradient method through measures of the soil CO₂ molar fraction at multiple depths. Commonly, \( F_{\text{soil}} \) is considered equal to \( R_{\text{soil}} \) and the two terms are used interchangeably within the literature and in land surface models. However, a considerable fraction of the \( R_{\text{soil}} \) can fail to actually emerge from the soil surface \( (F_{\text{soil}}) \) due to a host of different processes, such as aqueous phase partitioning, calcite dissolution reactions, gravitational percolation due to a higher density CO₂, or CO₂ dissolution in xylem water. Therefore, simple estimations of \( F_{\text{soil}} \) are likely lower than actual rates of \( R_{\text{soil}} \). Misrepresenting \( F_{\text{soil}} \) as \( R_{\text{soil}} \) can have significant consequences for interpretation of both biotic and abiotic processes because it not only underestimates the contributions of aboveground function to belowground processes, but it also yields a misguided understanding of the rates and drivers of subsurface biogeochemistry and the potential for carbon exports from the system through hydrological transport.

The importance of these alternative CO₂ loss pathways is illustrated when considering that soil can store an order of magnitude greater CO₂ as dissolved inorganic carbon (DIC) inclusive of dissolved CO₂, carbonate acid, bicarbonate, and carbonate) in the aqueous-filled relative to gas-filled pore space. As a result, large CO₂ losses can be produced by DIC leaching in all ecosystems around the world, with increased CO₂ losses in ecosystems with higher precipitation and higher soil solution pH. In semiarid regions, this DIC leaching may explain a portion of the missing terrestrial carbon sink. For this reason, distributed measures of O₂, which has an aqueous solubility 29.7 times lower than CO₂ at 15 °C and does not form additional chemical species by dissolution in water, provides a useful constraint on determining soil CO₂ production that might otherwise be missing from \( R_{\text{soil}} \).

1B2 Earthsciences, Biosphere 2, University of Arizona, Tucson, 85721, USA. 2Departamento de Física Aplicada, Universidad de Granada, Granada, 18071, Spain. 3School of Geography and Development, University of Arizona, Tucson, 85721, USA. 4Department of Soil, Water and Environmental Science, University of Arizona, Tucson, 85721, USA. 5IISTA-CEAMA, Instituto Interuniversitario de Investigación del Sistema Tierra en Andalucía, Granada, 18006, Spain. Correspondence and requests for materials should be addressed to E.P.-C. (email: enripsec@ugr.es)
Traditional measures of soil respiration

Using ARQ to account for subsurface CO$_2$ transport and consumption

\[ R_{\text{loss}} = R_{\text{soil_ARQ}} - F_{\text{soil}} \]

Dissolution in water
Transport to depth
Vertical and lateral transport
Mineral weathering along flowpaths
Potential degassing down gradient

Figure 1. Measurements of apparent respiratory quotient (ARQ), i.e., the ratio of soil CO$_2$ efflux/O$_2$ influx, have the potential to provide improved quantification of soil respiration, and partitioning of soil respiratory CO$_2$ into vertical (upward) gaseous and lateral or downward dissolved fluxes. Here, $R_{\text{soil}}$ is the soil CO$_2$ production measured, either using the traditional efflux method ($R_{\text{soil,trad}}$) or on the basis of ARQ ($R_{\text{soil,ARQ}}$). $F_{\text{sub}}$, which is the soil CO$_2$ (upward) efflux estimated by the CO$_2$ gradient method, is typically equated to soil respiration ($R_{\text{soil,trad}}$). However, direct continuous measurements of ARQ as conducted in the current work, reveal that a significant fraction of CO$_2$ produced by soil respiration is transported or consumed in the subsurface, and not locally emitted to the atmosphere. Hence, a substantial amount of respired CO$_2$ – unaccounted for by quantifying $F_{\text{sub}}$ alone, and denoted here as $R_{\text{loss}}$ – can be estimated on the basis of concurrent measures of O$_2$ influx to soil. The ARQ method reveals a significant component of soil respiration ($R_{\text{soil}}$) that is not emitted locally to the atmosphere, $R_{\text{loss}}$ is the CO$_2$ produced, but unaccounted for, in traditional measures of CO$_2$ surface efflux. This $R_{\text{loss}}$ is consumed by subsurface processes attributable to dissolution in water, vertical and lateral transport along hydrologic flow paths, chemical reactions (including, e.g., silicate and carbonate mineral weathering), and potential degassing upon groundwater discharge (e.g., to streams). Non-negligible values of $R_{\text{loss}}$ indicate that (i) flux based measurements alone significantly underestimate local soil respiration and (ii) an important fraction of soil respiratory CO$_2$ production is consumed in subsurface reactions. $R_{\text{soil,ARQ}}$ is the total soil CO$_2$ production, and the sum of $R_{\text{soil}}$ and $R_{\text{loss}}$.

The ratio of soil CO$_2$ efflux to O$_2$ influx, known as the apparent respiratory quotient (ARQ), allows one to estimate the CO$_2$ losses from $R_{\text{soil}}$\textsuperscript{2}. A diagram, with the main variables involved in exchange and loss of CO$_2$, is shown in Fig. 1. Here we present the first study using continuous ARQ estimates to evaluate annual CO$_2$ losses of carbon from $R_{\text{soil}}$ ($R_{\text{soil,ARQ}}$, where $R_{\text{soil,ARQ}} = R_{\text{soil}} + R_{\text{loss}}$). Our goals were (i) to quantify the values, patterns, and seasonality of ARQ at different soil depths within a semi-arid coniferous forest and then (ii) to estimate the amount of soil CO$_2$ removed through biological and non-biological processes ($R_{\text{soil,ARQ}}$) (iii) in order to illustrate the disparity between $F_{\text{sub}}$ using traditional assumptions that $R_{\text{soil}} = F_{\text{sub}}$ and an estimate of $F_{\text{sub}}$ that takes into account CO$_2$ losses ($R_{\text{loss}}$) and actual rates of $R_{\text{soil}}$, as determined using the ARQ. Having better estimates of $R_{\text{soil}}$ is key to understanding the true influence of aboveground production on $R_{\text{soil}}$ CO$_2$-induced mineral weathering, and other biologically-driven processes within the critical zone.

Results

The annual time series of climatic and edaphic variables are shown in Fig. 2. During 2015, mean air temperature was 9.4 °C, ranging from −10 to 22 °C with synoptic scale fluctuations driven by atmospheric pressure variations associated with passing of frontal systems (Fig. 2a). Mean soil temperature across all depths was ca. 9.3 °C, with variability decreasing in amplitude with depth (Fig. 2b). Volumetric soil water content (VWC) averaged 20% across all depths with variation over time driven by rainfall events, falling mainly during the monsoon period. Precipitation intensity was greater than 3 mm in 30 min, the delay between precipitation and a VWC response was less than 30 min.

Dynamics of the variables considered to control soil gas concentrations and their exchange with the atmosphere are shown in Fig. 2d–g. Mean CO$_2$ volumetric fraction increased with depth, with average values of 0.25, 0.57 and 0.64% at 10, 30 and 60 cm, respectively. We found a clear annual pattern analogous to the temperature and seasonality of ARQ at different soil depths within a semi-arid coniferous forest and then (i) to estimate the CO$_2$ losses from $R_{\text{soil}}$\textsuperscript{2}. A diagram, with the main variables involved in exchange and loss of CO$_2$, is shown in Fig. 1. Here we present the first study using continuous ARQ estimates to evaluate annual CO$_2$ losses of carbon from $R_{\text{soil}}$ ($R_{\text{soil,ARQ}}$, where $R_{\text{soil,ARQ}} = R_{\text{soil}} + R_{\text{loss}}$). Our goals were (i) to quantify the values, patterns, and seasonality of ARQ at different soil depths within a semi-arid coniferous forest and then (ii) to estimate the amount of soil CO$_2$ removed through biological and non-biological processes ($R_{\text{soil,ARQ}}$) (iii) in order to illustrate the disparity between $F_{\text{sub}}$ using traditional assumptions that $R_{\text{soil}} = F_{\text{sub}}$ and an estimate of $F_{\text{sub}}$ that takes into account CO$_2$ losses ($R_{\text{loss}}$) and actual rates of $R_{\text{soil}}$, as determined using the ARQ. Having better estimates of $R_{\text{soil}}$ is key to understanding the true influence of aboveground production on $R_{\text{soil}}$ CO$_2$-induced mineral weathering, and other biologically-driven processes within the critical zone.

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Dynamics of the variables considered to control soil gas concentrations and their exchange with the atmosphere are shown in Fig. 2d–g. Mean CO$_2$ volumetric fraction increased with depth, with average values of 0.25, 0.57 and 0.64% at 10, 30 and 60 cm, respectively. We found a clear annual pattern analogous to the temperature pattern, with maxima in summer and minima in winter. Superimposed on this seasonal trend is pulsed increases in the volumetric fraction of CO$_2$ driven by precipitation events, with larger amplitude responses during warmer months. Mean O$_2$ volumetric fraction decreased with increasing depth from 20.27%, to 19.27% and 18.04% at 10, 30 and 60 cm, respectively. The mean O$_2$ volumetric fraction was significantly different at the three depths, and this difference was sustained through the entire year ($F_{\text{sub}} = 213.9; P < 0.05$). Minimum O$_2$ values occurred in the deepest depths during the snowmelt period, and O$_2$ variations were anti-correlated with CO$_2$ at 10 cm ($R^2$ 0.94, p < 0.05) and 30 cm ($R^2$ 0.89, p > 0.05) throughout the year. However, at 60 cm a poor correlation ($R^2$ 0.11, p > 0.05) was found due to the decoupling during the snowmelt. When the snowmelt period (from January 8
to February 20) was excluded from regression analysis, the correlation between O₂ and CO₂ increased notably in deeper layers, with R² values of 0.95, 0.92 and 0.46 at 10, 30 and 60 cm, respectively. Large O₂ fluctuations at 60 cm during the snowmelt period could be due to the snowmelt during daytime producing a wetting front that percolates to lower permeability soil horizons (higher clay content) at depth, stimulation of soil respiration and hence O₂ consumption, but with near saturation conditions limiting diffusion of O₂ into the soil from above. ARQ showed similar mean values at all depths (ca. 0.3), reaching minimum values at 60 cm during snowmelt (January-February) and maximum values at 10 cm in April. Fsoil was at its maximum during summer and minimum during winter, with an annual mean of 1.64 µmol m⁻² s⁻¹. Means, standard deviations, minima, maxima, and correlation coefficients for variables shown in Fig. 2 are included in Supplementary Information (Tables 1S and 2S). Monthly descriptive statistics for edaphic variables and ARQ are also included there (Fig. 1S).

We also examined, in one soil pedon at 30 min averages, the dynamic behaviour of CO₂ and O₂ through several rain pulse events to capture their combined effects on ARQ (Fig. 3). ARQ slightly increased at 10 cm and 30 cm in response to rain pulses, but remained stable at 60 cm. Interestingly, the rapid increases in CO₂ induced by rain events were counteracted by rapid decreases in O₂, causing only small variations in the ARQ range (ca. 0.2–0.3). The time to return to values similar to those prior to the precipitation event for CO₂, O₂, ARQ and VWC was not delayed with depth. At 10 cm depth, diurnal ARQ fluctuations showed a higher amplitude than at deeper depths, driven by higher amplitude in the O₂ fluctuations at 10 cm.

The annual cumulative Fsoil, including consideration of the CO₂ loss (Rsoil,ARQ, 2012 ± 223 gC m⁻²) was 3.2 times higher than traditional estimates of Fsoil derived using the gradient method (622 ± 86 gC m⁻², using eq. 1). This suggests that ca. 1400 gC m⁻² were removed from Rsoil (Fig. 4) prior to efflux from the soil surface. These ca. 1400 gC m⁻² represent the soil CO₂ efflux not emitted to the atmosphere (Rloss) in the vicinity of production. If Rsoil was fully emitted to the atmosphere locally, by upward gaseous diffusion processes, with zero Rloss, then Fsoil would accurately reflect Fsoil. However, this was not the case. The smallest differences between Fsoil using the traditional assumption of equalling Rsoil versus using Rsoil,ARQ were in March, April, September and October, but even then, our recalculated Fsoil was still 2.7–3.0 times higher (Fig. 4). Maximum differences were produced in January.

Figure 2. Time series of daily-averaged values for the three pedons of air temperature (Air T), atmospheric pressure (P), soil temperature (Soil T), volumetric water content (VWC), precipitation, CO₂ volumetric fraction, oxygen volumetric fraction, apparent respiratory quotient (ARQ) and soil CO₂ efflux (Fsoil) at 10, 30, and 60 cm depth during 2015. The standard error for each variable is shown with shading. The period studied in Fig. 2 is highlighted with shading.
Figure 3. Half-hour averaged values of CO$_2$ volumetric fraction, O$_2$ volumetric fraction, apparent respiratory quotient (ARQ) and volumetric water content (VWC) at 10, 30 and 60 depth in a single instrumented pedon (north-facing) during the summer monsoon of 2015.

Figure 4. Monthly cumulative soil CO$_2$ efflux ($F_{\text{soil}}$) (with uncertainty represented as the standard error) and monthly cumulative soil CO$_2$ efflux, accounting for the CO$_2$ removed from the soil respiration ($R_{\text{soil ARQ}}$, calculated as $R_{\text{soil}}$ multiplied by 0.9 ± 0.1/ARQ). The values above of each bar indicate the ratio $R_{\text{soil ARQ}}/F_{\text{soil}}$. The inset figure shows the annual cumulative of $F_{\text{soil}}$ and $R_{\text{soil ARQ}}$ and its uncertainty.
and December, when our recalculated $F_{\text{soil}}$ was 5.3–5.6 times higher. Our two estimates of $F_{\text{soil}}$ (with and without accounting for $R_{\text{soil}}(\text{ARQ})$) followed similar monthly patterns despite the differences found in ARQ. The degree of agreement between $F_{\text{soil}}$ estimated using the gradient method (Fig. 2g), and periodic chamber measurements of $F_{\text{soil}}$ can be found as Supplementary Information (Fig. 2S).

**Discussion**

Given the significant role of soil carbon dynamics in determining other bio-hydro-geochemical processes in the critical zone, there is a need to better understand the dynamic nature of $\text{CO}_2$ production and loss from an ecosystem. The low ARQ values we found here (ARQ $\approx$ 0.3, Fig. 2 and Table 1S) in comparison to oxidative ratios expected for natural organic matter (i.e., moles of $\text{O}_2$ consumed per mole of $\text{CO}_2$ produced during respiration of organic matter, which average ca. 1.1 (equivalent to ARQ $= 0.9$)), highlight the important role of subsurface biological and non-biological processes in removing $\text{CO}_2$ from $R_{\text{soil}}$. These processes are discussed further below.

If all $R_{\text{soil}}$ were emitted directly to the atmosphere by gaseous diffusion processes (that is, if $F_{\text{soil}} = R_{\text{soil}}$), as is commonly assumed, $F_{\text{soil}}$ would be on average approximately three times higher (due to ratio between ARQ theoretical/ARQ measured, 0.9/0.3). Therefore, assuming that all $\text{O}_2$ consumption is associated with respiration, in this semiarid forest only 1/3 of $R_{\text{soil}}$ is emitted directly to the atmosphere and 2/3 are removed by subsurface processes. These results are actually quite similar to those found in the only other paper that has calculated in situ ARQ for estimates of $F_{\text{soil}}$ (23), which reported a mean ARQ of 0.26 and, therefore, an $R_{\text{soil}}$ that is 3.8 times higher than $F_{\text{soil}}$ estimated in their experimental site (Yatir Forest). In that study, researchers collected $\text{CO}_2$ and $\text{O}_2$ samples in a pine forest overlying chalk and limestone bedrock with a mean annual precipitation of 280 mm. Despite their site receiving only 1/3 of the precipitation of our site, and therefore less potential for $\text{CO}_2$ reaction with soil water, a similar ARQ was obtained. This could be attributed to a different composition (and hence oxidative ratio) of the soil organic matter undergoing decomposition, and the effect of $\text{CO}_2$-consuming calcium carbonate dissolution reactions in their soils. Here, we used ARQ $= 0.9$ as a representative respiratory quotient (RQ) value since it is the mean value corresponding to biomolecular components of natural organic matter (24), but if we had used for example the 0.74 value measured for a grassland soil (25), the calculated annual $F_{\text{soil}}$ would be 1023 g C m$^{-2}$, which would be only 1.6 times higher $F_{\text{soil}}$ (assuming that all $R_{\text{soil}}$ is emitted by diffusion processes). This highlights the fact that the contribution of $R_{\text{soil}}(\text{ARQ})$ to $F_{\text{soil}}$ will depend on the oxidative ratio of the organic matter undergoing degradation, which could potentially change seasonally or with location. Nonetheless, our results are in accordance with Angert et al. (22) and underscore the important contribution of subsurface processes in removing $\text{CO}_2$ (or $\text{O}_2$) from the soil gas phase prior to its efflux from the soil surface, and the need for a better understanding of the mechanisms involved in those losses.

Prior measurements of RQ have been mostly limited to laboratory experiments using air samples from natural soils or incubated soils, and we do not know of any other studies with in-situ and continuous estimates of RQ as a function of soil depth. In our case, assuming that only $R_{\text{soil}}$ and diffusion of $\text{O}_2$ and $\text{CO}_2$ give rise to ARQ, ARQ will be equal to RQ and the oxidative ratio (OR) of organic matter undergoing degradation. In this study, the annual mean RQ (calculated as ARQ/0.76) across all depths was 0.38, which was lower than RQ values for some soils ranging from 0.82 to 1 (26–28), but similar to or exceeding those of other soils ranging from 0.21 to 0.40 (29–33). Incubation studies have found a decrease in RQ values with time, often attributed to a depletion of labile organic matter (organic acids and carbohydrates). In such conditions, the microbiota shift to metabolizing less energetically favourable compounds with lower RQ values, such as lipids, lignin and protein (34). Therefore, the low RQ values found here might suggest that the carbon in the organic matter undergoing degradation was of relatively low oxidation state. However, RQ values were far lower than the common values of 0.88 for lignin and 0.73 for lipids (35), suggesting that low RQ substrates cannot alone explain our results; there must also be $\text{CO}_2$ or $\text{O}_2$ consuming processes contributing to these very low values.

Significant soil $\text{CO}_2$ losses can also be driven by DIC drainage and chemical reactions in the soil. The solubility of $\text{CO}_2$ in water is described by Henry's law, which states that the number of moles of dissolved $\text{CO}_2$ plus carbonic acid per liter of water (collectively referred to as $[\text{H}_2\text{CO}_3]$) is directly proportional to the $\text{CO}_2$ partial pressure and inversely proportional to temperature. In this study, based on aqueous geochemical calculations (36), the potential $\text{CO}_2$ removed as DIC during the whole year would be 15.35 g C m$^{-2}$. This would represent roughly 2.5% of the cumulative $F_{\text{soil}}$ (622 g C m$^{-2}$) and a 1.1% in the C removed from the cumulative $R_{\text{soil}}$ (1390 g C m$^{-2}$). These low values of downward DIC transport to groundwater are consistent with the low values of flux estimated globally (37). Since they only had individual measurements taken at specific time points, Angert et al. (22) posited that measurements and considerations of ARQ might become less important on annual and longer timescales when respect to chemical reactions, only those that consume $\text{CO}_2$ or $\text{O}_2$ lead to a decrease in RQ. Potential $\text{CO}_2$ consuming reactions include those wherein $\text{CO}_2$ is a reactant in mineral dissolution, such as the dissolution of primary and secondary silicates (41), (oxyhydr)oxides or calcite.

Given that plagioclase is a kinetically labile primary silicate mineral present in the soil profiles of our study site, it is reasonable to expect that some portion of the resired $\text{CO}_2$ is consumed in its weathering to form kaolinite, also observed in our profiles (Table 1). The $\text{CO}_2$-driven weathering of plagioclase to kaolinite consumes two moles of $\text{CO}_2$ per mole of plagioclase. Numerous prior laboratory and field studies have measured rates of
plagioclase dissolution at pH values similar to those of the pore waters at our site (ca. pH 5.4). Laboratory-derived weathering rates of plagioclase are typically two to three orders of magnitude higher than those derived from field data (White & Buss, 2014). Hence, whereas steady state laboratory rates are approximately $1.5 \times 10^{-12}$ moles m$^{-2}$ s$^{-1}$, field-measured rates are closer $1 \times 10^{-11}$ moles m$^{-2}$ s$^{-1}$ or lower (normalization in this case is to plagioclase surface area). Given the mass fraction of plagioclase in the study soils, a soil bulk density of 1.5 g cm$^{-3}$, and assuming a specific surface area for the plagioclase as 5.6 m$^{2}$ g$^{-1}$ (estimated as 3/(particle density × particle radius))$^{35}$, we calculate that the steady state rates of plagioclase dissolution could account for consumption of ca. 3.0 to 230 g C m$^{-2}$ year$^{-1}$. Importantly, plagioclase is only one of the primary silicates present in our soils; other labile silicates, such as K-feldspar and mica, will consume comparable quantities of CO$_2$ during dissolution and both are present at higher mass concentrations. Nonetheless, it seems clear that silicate dissolution alone is unlikely to explain all of the CO$_2$ removed in our study.

O$_2$ consuming reactions include the oxidation of Fe(III), NH$_4^+$, NO$_3^-$, mineral sulfides, H$_2$S and SO$_4^{2-}$. The rates of pyrite (FeS$_2$) oxidation in regolith are controlled by the delivery of O$_2$ to the weathering zone, which consumes 3.75 moles of O$_2$ per mole of pyrite oxidized, and hence this can be a significant sink for O$_2$ in soil systems$^{45}$. In our site, this potential contribution may be limited (though not negligible) because of low pyrite content in the schist-derived mineral assemblage. However, biotite (mica) content in our micaceous schist derived soil is significant, representing up to 14% of the bulk soil mineral mass (Table 1), and it can contain up to three moles of Fe(II) per mole of formula, with 0.25 moles of O$_2$ being consumed per mole of Fe(II) oxidized to Fe(III) during biotite weathering. Although nitrification processes were already considered in the RQ values previously shown, the deposition of calcareous atmospheric dust along with high inputs of Ca$^{2+}$, Mg$^{2+}$, K$^+$, Na$^+$, as found in the region$^{33}$, could have contributed to lowering RQ values due to chemical reactions. Calcite dissolution plays an important role in producing and consuming CO$_2$ in carbonate-containing soils$^{19}$, with one mole of CO$_2$ consumed per mole of calcite dissolved. The relative contribution of this reaction to subsurface CO$_2$ consumption is unclear because CaCO$_3$ does not accumulate to levels quantifiable by X-ray diffraction and soil pH (5.4) is moderately acidic. Nonetheless, the mineralogical and geochemical composition of the soil (Table 1) indicate that all of the previously mentioned reactions could consume CO$_2$ and O$_2$ to varying degrees, contributing to the low ARQ value we measured here.

Microbial composition likely also impacts the ARQ observed in a given soil. The moles of CO$_2$ produced per mole of O$_2$ consumed depends, in part, on the microbial carbon-use efficiency (i.e., the ratio of growth to carbon assimilation) of the heterotrophic community$^{47}$. Hence, microbial community composition and environmental conditions (e.g., temperature, tends to decline carbon-use efficiency with increasing temperature) will likewise influence the moles of CO$_2$ produced per mole of O$_2$ consumed for a given substrate. The minimum ARQ was obtained at 60 cm during the snowmelt period (Fig. 2f) induced by the minimum O$_2$ values. However, the maximum ARQ occurred in April. We speculate that this may be the result of the accumulation, over winter, of labile and energetically favourable organic compounds (organic acids and carbohydrates) that are oxidized by a heterotrophic microbial community activated by increasing spring temperatures. Oxidation of such compounds, containing carbon in a higher oxidation state, results in a higher ratio of moles of CO$_2$ produced per mole of O$_2$ consumed. Furthermore, chemolithoautotrophic and photoautotrophic organisms can assimilate CO$_2$ without O$_2$ production using different metabolic pathways. Photoautotrophic and chemoautotrophic organisms that fix CO$_2$ and transform it into microbial biomass have been found to be highly abundant in forests$^{48}$, with a global rate for microbial synthesis of organic C of 4.9 to 37.5 g C m$^{-2}$ year$^{-1}$ in different soils$^{49}$. Methanogenic bacteria that metabolize CO$_2$ to decompose organic matter to CH$_4$ under anaerobic conditions$^{50}$, have been observed even in well aerated soils such as those found in deserts$^{51}$. Therefore, the low ARQ and RQ values found in our soils could indicate one or several processes whereby (i) CO$_2$ is being removed laterally as dissolved H$_2$CO$_3^-$, (ii) CO$_2$ and O$_2$ are consumed in geochemical reactions, or (iii) a biological O$_2$ consumption occurs without emission of CO$_2$ and vice versa.

Subsurface CO$_2$ consumption has been studied both in soil-atmosphere CO$_2$ exchanges and in CO$_2$ exchanges at the ecosystem level. Roland et al.$^{52}$ used a chemical carbonate weathering model to explain non-biological fluxes detected at ecosystem scale in a karst, finding that the CO$_2$ coming from deeper layers at night could be stimulating carbonate dissolution and, thus, consuming CO$_2$. Hamerlynck, et al.$^{53}$ found a negative F$_{net}$ at night in a Chihuahuan desert shrubland, both using an automatic soil chamber and using the gradient method with CO$_2$ sensors buried in the shallower layer, similarly attributing the CO$_2$ consumption to carbonate dissolution. Additionally, temperature influences on the solubility of CO$_2$ (Henry’s Law) were suggested in explaining negative F$_{net}$ in Antarctic dry valley ecosystems$^{54,55}$, and soil electrical conductivity and pH were correlated with CO$_2$ uptake in alkaline desert soils$^{56}$. All of these studies found negative F$_{net}$, highlighting that CO$_2$ consumptive processes in the soil were higher than CO$_2$ production processes. This is not unexpected in such ecosystems.

### Table 1. Soil physicochemical characteristics and mineralogical composition.

| Depth (cm) | Quartz (%) | Plag-Feldspar (%) | K-Feldspar (%) | Iron Oxides (%) | Mica (%) | 2:1 Clay (%) | 1:1 Clay (%) | Others (%) |
|-----------|------------|--------------------|---------------|----------------|---------|-------------|-------------|-----------|
| 0–20      | 44.0       | 7.1                | 8.2           | 1.0            | 13.1    | 17.1        | 4.7         | 3.0       |
| 20–40     | 46.2       | 5.8                | 6.9           | 0.5            | 14.2    | 18.5        | 5.9         | 2.3       |
| 40–80     | 40.1       | 4.8                | 6.1           | 0.7            | 13.3    | 16.3        | 5.9         | 4.6       |

| Depth (cm) | Clay (%) | Silt (%) | Sand (%) | pH | Ec (µS/cm) | LOI (%) |
|------------|----------|----------|----------|----|------------|---------|
| 0–20       | 19.8     | 48.8     | 31.4     | 5.7 | 196.1      | 21.2    |
| 20–40      | 27.5     | 44.0     | 28.5     | 5.3 | 199.1      | 5.8     |
| 40–80      | 31.8     | 31.4     | 36.8     | 5.1 | 122.1      | 5.0     |
where \( R_{soil} \) is very low due to low biological activity and therefore even small changes in \( R_{soil} \) can change the sign of the soil-atmosphere CO\(_2\) gradient. In our ecosystem, \( F_{soil} \) was always positive, but the complementary O\(_2\) measurements provided a novel insight, confirming that even in ecosystems with high biological production, non-biological processes are masked by high \( R_{soil} \) and therefore, are difficult to detect from \( F_{soil} \) measurements alone.

In conclusion, this study highlights the important and dynamic, but often overlooked, roles played by subsurface transport and weathering processes that differentiate \( R_{soil} \) from surface measures or estimates of \( F_{soil} \). As Angert et al. noted, variations in the ARQ in acidic and neutral soils (as we have here) are likely tied to substrates and processes not well understood at present, and such processes warrant further research. Therefore, we must change our point of view regarding \( R_{soil} \) studies from an inappropriately conceived system in which all CO\(_2\) is produced by biology, to a dynamic system where the soil CO\(_2\) is produced and removed by the interaction of combinatorial biological processes, hydrologic transport, and associated geochemical reactions. Because the fraction of \( R_{soil} \) contributing to \( F_{soil} \) depends on the ARQ chosen, we recommend that future \( F_{soil} \) studies use a combination of soil CO\(_2\) and O\(_2\) sensors to determine ARQ values. Such an approach can yield important information to quantify the CO\(_2\) removed by biological and non-biological processes. ARQ and RQ values are key in estimating CO\(_2\) sinks deduced from changes in atmospheric O\(_2\) concentration and are highly influential in evaluating ecosystem productivity. Currently, ecosystem productivity is estimated using values of net ecosystem exchange, as the sum of gross primary production (GPP) and ecosystem respiration (\( R_{soil} \)). This may be problematic because that \( R_{soil} \) consists of an aboveground component attributed to plant respiration and a belowground component, \( F_{soil} \) that we now know may incompletely quantify soil respiration. In our ecosystem, if soil CO\(_2\) losses were calculated from \( F_{soil} \) alone, GPP estimates would be erroneously low, and if this is consistent across other ecosystems, it could have enormous implications on carbon exchange studies from ecosystem to global scale.

Material and Methods

Site description. The field site is a mixed conifer forest located at 2573 m a.s.l. on Mt. Bigelow north of Tucson, Arizona, in the Santa Catalina Mountains-Jemez River Basin Critical Zone Observatory. The climate is semi-arid, with a mean annual temperature of 9.4 °C and mean annual precipitation of 750 mm, falling mostly during the summer monsoon. Snow falls during winter, usually persisting from December to March. Ponderosa pine (Pinus ponderosa) and Douglas fir (Pseudotsuga menziesii) dominate the site with a mean canopy height of 10 m. The soil has a sandy loam texture of 32.3% sand, 41.4% silt and 26.4% clay with a pH of 5.4 and a depth to bedrock of ca. 1 m. Additional information about mineral composition and other soil properties can be found in Table 1.

Experimental design. Field measurements were conducted during the complete calendar year of 2015. Three instrumented pedons were equipped to measure each of the following, using co-located sensors: temperature and humidity (5TM, Decagon, USA), O\(_2\) molar fraction (SO-110, Apogee, USA; Manufacturer reports a sensitivity of 26µV per 0.01% and repeatability < 0.1% of reading), and CO\(_2\) molar fraction at 10, 30 and 60 cm depth. A drift correction was applied to the O\(_2\) sensors assuming a constant linear signal decrease as the manufacturer reported (1 mV per year). The measurement range of the CO\(_2\) sensors was up to 10,000 ppm at 10 cm and 20,000 ppm at 30 and 60 cm (GMM222 and GMM221, Vaisala, Finland; accuracy 1.5%, repeatability 2% of reading). Both CO\(_2\) and O\(_2\) values were corrected for variations in temperature, humidity, and pressure per instructions from the manufacturer. Atmospheric pressure, air temperature, and precipitation were obtained from a meteorological tower. Data-loggers (CR1000, Campbell scientific, USA) collected measurements every 30 s and stored 30 min averages. The instrumented pedons are separated from each other by distances of ca. 10 meters, and they are located, respectively, on a south facing slope, a north facing slope, and in a convergent valley position within a bedrock of ca. 1 m. Additional information about mineral composition and other soil properties can be found in Table 1.

Procedure to estimate \( F_{soil} \). Estimates of \( F_{soil} \) were obtained using the gradient method through the equation:

\[
F_{soil} = -pk_\theta \frac{\partial c}{\partial x}
\]  

(1)

where \( F_{soil} \) (µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)), \( \rho \) is the air density (mol air m\(^{-3}\)), \( \partial c \) is the CO\(_2\) molar fraction gradient (µmol CO\(_2\) mol air \(^{-1}\)) calculated using the difference between atmospheric CO\(_2\) molar fraction (400 ppm) and the CO\(_2\) value at 10 cm depth, \( \partial x \) is the vertical gradient (m) and \( k_\theta \) is the in situ CO\(_2\) transfer coefficient (m\(^2\) s\(^{-1}\)) obtained by rearranging Eq. 1:

\[
k_\theta = \frac{-F_{chamber} \partial x}{\rho \partial c}
\]  

(2)

where \( F_{chamber} \) was measured by a portable soil CO\(_2\) efflux chamber (Li-8100, Li-Cor, USA) from 18 collars around the instrumented pedons, follow a transect from the south face to the north face going through the valley, every two weeks during the months without snow cover (n=20). Later, \( k_\theta \) was modelled using a power function (\( k_\theta / D_s = a \theta_s^b \)) of the soil air porosity (\( \theta_s = \) soil porosity-soil water content), where \( D_s \) is the diffusion coefficient of CO\(_2\) in free air (m\(^2\) s\(^{-1}\)) and \( a \) and \( b \) are coefficients obtained by least squares regression.
Procedure to estimate ARQ. The ratio of soil CO₂ efflux to soil O₂ influx, designated as apparent respiratory quotient (ARQ), was estimated following Angert et al.\(^2\):

\[
ARQ = \frac{F_{CO_2}}{F_{O_2}} = -\frac{\rho D_{SCO_2} \frac{\partial c}{\partial z}}{-\rho D_{SO_2} \frac{\partial o}{\partial z}}
\]

simplifying,

\[
ARQ = \frac{-D_{SCO_2} \frac{\partial c}{\partial o}}{-D_{SO_2} \frac{\partial o}{\partial o}} = -0.76 \frac{\partial c}{\partial o}
\]

where the constant “0.76” is derived from the ratio of CO₂/O₂ diffusion coefficients in air, \(\partial c\) is the CO₂ molar fraction gradient calculated using the discrete difference between the atmosphere and the CO₂ value at each depth and \(\partial o\) is the O₂ molar fraction gradient calculated using the difference between atmosphere and the O₂ value at each depth. Consumption of either soil CO₂ or soil O₂ will decrease the ARQ; consumption of soil CO₂ decreases the difference in the numerator (\(\partial c\)) and hence decreases ARQ, whereas consumption of soil O₂ increases the difference represented in the denominator (\(\partial o\)), and hence also decreases ARQ.

ARQ values have previously only been reported by Angert et al.\(^2\), who found that ARQ ranged from 0.14–1.23 across six different experimental sites. Most previous studies have focused either on the respiratory quotient (RQ), defined as the moles of CO₂ produced per mole of O₂ consumed during \(R_{soil}\), or the oxidative ratio (OR), defined as moles of O₂ consumed per mole CO₂ produced (i.e., 1/RQ). Therefore, if we assume that only \(R_{soil}\) drives ARQ, it will be equal to RQ or 1/OR.

The natural biochemical variation in RQ is large depending on the kind of compound undergoing oxidation, ranging from (mean values reported for each biomolecular type) 1.47 for organic acids, 1.00 for carbohydrates, 0.95 for soluble phenolics, 0.88 for proteins and lignins, and 0.73 for lipids (OR values in Randerson et al.\(^2\)). From stoichiometric considerations, mean RQ values were calculated as 0.95 for different types of wood and 0.89 for humic acid and humin (OR values in Severinghaus\(^2\)). In soils, RQ values have been reported to vary from 0.83–0.95 for different biomes inside Biosphere\(^2\), 0.82–1.08 for Boreal, Temperate Subtropical and Mediterranean ecosystems\(^2\), 0.90 in a cool temperate deciduous forest\(^2\), and a mean value of 1 in the Amazonian tropical forest\(^2\). Therefore, based on previous research, an ARQ value of ca. 0.9 ± 0.1 is consistent with \(R_{soil}\) and diffusion processes alone. However, ARQ values below this would indicate removal of CO₂ or O₂ by non-respiratory processes\(^2\). Therefore, assuming both abiotic O₂ removal and autotrophic microorganisms in the soil are negligible, to estimate the \(F_{soil}\) taking into account the CO₂ loss from the soil, one can multiply \(R_{soil}\) (or \(F_{soil}\)) assuming that all \(R_{soil}\) is emitted to the atmosphere by gaseous diffusion processes, and therefore, \(F_{soil} = R_{soil}\) by 0.9 ± 0.1/ARQ, as was done in the current study and previously by Angert et al.\(^2\).

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Author Contributions
G.A.B-G. and J.C. conceived and designed the research. E.P.-C. analysed the data, prepared tables and figures and wrote the draft manuscript. E.P.-C., G.A.B-G. and J.C. wrote the paper.

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