

Using O₂ to study the relationships between soil CO₂ efflux and soil respiration

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Abstract

Soil respiration is the sum of respiration processes in the soil, and is a major flux in the global carbon cycle. It is usually assumed that the CO₂ efflux is equal to the soil respiration rate. Here we challenge this assumption by combining measurements of CO₂ with high-precision measurements of O₂. These measurements were conducted on different ecosystems and soil types, and included measurements of air-samples taken from the soil profile of three Mediterranean sites, a temperate forest, and two alpine forests. Root-free soils from the alpine sites were also incubated in the lab. We found that the ratio between the CO₂ efflux and the O₂ influx (defined as apparent respiratory quotient, ARQ) was in the range of 0.14 to 1.23, and considerably deviated from that of 0.9 ± 0.1 expected from the elemental composition of average plants and soil organic matter. At the Mediterranean sites, these deviations could be explained as a result of CO₂ dissolution in the soil water and transformation to bicarbonate

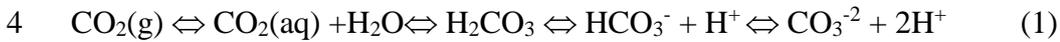
1 ions in these high pH soils, and by carbonates dissolution and precipitation processes. Thus,
2 correct estimate of the short-term, chamber-based biological respiratory flux in such soils can
3 only be made by dividing the measured soil CO₂ efflux by the average (efflux weighted) soil
4 profile ARQ. Applying this approach to a semiarid pine forest resulted in an estimated short-
5 term biological respiration rate that could be 3.8 times higher than the chamber-measured
6 surface CO₂ efflux (8.8 μmol CO₂ m⁻² s⁻¹ instead of 2.3 μmol CO₂ m⁻² s⁻¹, at the time of
7 measurement). The ARQ values often observed in the more acidic soils were unexpectedly
8 low (<0.7). These values could result from the oxidation of reduced iron, which could
9 previously been formed during times of high soil moisture and local anaerobic conditions
10 inside soil aggregates, but requires further research to validate. The results reported here
11 provide direct quantitative evidence for large temporal decoupling between soil gas exchange
12 fluxes and biological soil respiration.

13

14 **1 Introduction**

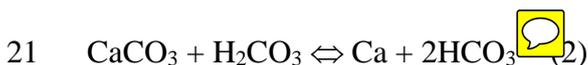
15 Respiration in soils is a major flux in the global carbon cycle, and contributes ~100 Pg C y⁻¹
16 to the atmosphere (Bond-Lamberty and Thomson, 2010). As a result, this process has
17 attracted much attention in recent decades (Davidson et al., 1990; Raich and Potter,
18 1995; Raich and Schlesinger, 1992; Vargas et al., 2011). Soil respiration is defined as the sum
19 of heterotrophic respiration by soil micro-organisms, mostly bacteria and fungi, and
20 autotrophic respiration by living roots. It is usually estimated by measuring the CO₂ efflux
21 from the soil to a chamber placed above it (Davidson et al., 2002), or modelled from the CO₂
22 concentration gradients in the soil profile (Davidson and Trumbore, 1995). Hence, the basic
23 assumption is that the CO₂ efflux is equal to the soil respiration. However, the CO₂ efflux is
24 not necessarily an ideal measure of the respiration rate for the following reasons:

1 First, instead of diffusing through the soil surface, a considerable fraction of the respired CO₂
2 can be dissolved in the soil water, transported in the hydrological system, or take part in
3 reactions of the carbonate system:



5 In a calcareous soil with a pH of ~8 most of the carbon in the soil solution is in the form of
6 bicarbonate (HCO₃⁻). Using the carbonate system equilibrium relationships (Stumm and
7 Morgan, 2012), it can be shown that in such a pH range the storage capacity of dissolved
8 inorganic carbon (mainly bicarbonate) in soil water is considerable. For instance, we
9 calculated given the carbonate system constants (Stumm and Morgan, 2012), that for a soil
10 porosity of 50% which is 50% water filled pores, a soil pCO₂ of 10,000ppm (1%), and a soil
11 pH of ~8, the soil carbon storage capacity would be ~100g carbon m⁻³ soil (mostly as
12 bicarbonate). This DIC storage capacity is large in comparison to typical soil respiration rates,
13 which are in the order of ~2 gC m⁻² d⁻¹. This large storage capacity is particularly important
14 when water is replaced by rain, irrigation, or any other water supply process. In addition,
15 some CO₂ will also be stored in gas-phase in the soil pores. However, with the same soil
16 parameters values as above, the gas phase storage will be only in the order of 1g. Hence, in
17 calcareous soils the gas phase storage is negligible in comparison to the storage of dissolved
18 inorganic carbon, unless large cavities exist below the soil.

19 Second, in addition to the DIC storage, in calcareous soils the CO₂ can also be consumed in
20 calcium carbonate dissolution reaction:



22 or released in the reverse reaction. Such processes have been shown to influence the temporal
23 variation of the soil CO₂ efflux, and to make it different than the biological process of
24 respiration (Benavente et al., 2010;Cuezva et al., 2011;Emmerich, 2003;Eshel et al.,

1 2007;Hastings et al., 2005;Kowalski et al., 2008;Roland et al., 2013;Schlesinger et al.,
2 2009;Serrano-Ortiz et al., 2010;Tamir et al., 2011;Ma et al., 2013;Stevenson and Verburg,
3 2006;Wang et al., 2014).

4 Third, processes within roots may also cause the CO₂ efflux to be different from the actual
5 respiration rate. For example, the CO₂ respired by roots can be dissolved in the xylem water
6 and carried upward in the transpiration stream (Aubrey and Teskey, 2009;Bloemen et al.,
7 2012).

8 Measurement of O₂ uptake rate is an alternative approach to measure respiration, which is
9 routinely applied in studies of aquatic systems. However, making such measurements in air-
10 phase, and especially under field conditions, is challenging since the atmospheric background
11 of O₂ is more than 500 times larger than that of CO₂ (20.95% versus 0.04%). Recently,
12 Angert and Sherer (2011) have demonstrated that the combined measurement of O₂ uptake in
13 addition to the CO₂ efflux can be used to isolate the biological respiration flux in a tree stem.
14 This approach is based on the lower solubility of O₂ in water (28 times lower than that of CO₂
15 at 20°C), and also on the fact that O₂, in contrast to CO₂, does not form additional chemical
16 species by reacting with water. Thus, the O₂ influx may be a better measure of respiration
17 than the widely used CO₂ efflux, as was also suggested previously for plant respiration
18 measurements in the lab (Amthor et al., 2001;Davey et al., 2004).

19 Here we have used high accuracy measurements of O₂ concentrations to study the
20 relationships between soil CO₂ efflux and soil respiration in well-drained soils, and to
21 determine how O₂ measurements can help to better quantify and understand soil respiration.
22 To make our conclusions more general, the study was conducted in different ecosystems, in
23 calcareous and non-calcareous soils, and over ~~wide-range~~ of soil CO₂ and O₂ concentrations.

1 Finally, we demonstrate how O₂ measurements can be used to correct CO₂ measurements for
2 estimating soil respiration flux.

3

4

5 1.1 Expected relationships between O₂ and CO₂ in soils

6 In a one-dimensional model, the change with time of the concentration (C) of a gas in soil is
7 related to the concentration gradient with depth (z), the gas diffusivity in the soil (D) and the
8 rate of net CO₂ production (P). This net rate of CO₂ production integrates the effects of
9 respiration and of CO₂ storage/release discussed above. The one-dimensional model is
10 summarized by the diffusion-production equation (Jury et al., 1991; Stern et al., 1999):

$$11 \quad \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2} - P(z) \quad (3)$$

12 This reaction-diffusion model ignores advection, that can be important in some cases (Maier
13 et al., 2012). For this reason we have conducted all of our experiments under low wind speeds
14 (<4 m sec⁻¹) conditions. For solving Eq. (3), we can for instance assume that CO₂ production
15 rate decreases exponentially with depth such that P(z)=P'exp(-z/z_e), where P' is the rate of
16 CO₂ production at the soil surface and z_e is the depth at which the rate equals P'/e. Then the
17 steady-state solution for the concentration gradient between the soil and the atmosphere (z=0)
18 becomes:

$$19 \quad C(z)-C_{\text{atm}}=(P' * z_e^2 / D)(1-\exp(-z/z_e)) \quad (4) \quad (\text{Hesterberg and Siegenthaler, 1991})$$

20 We noted the difference C(z)-C_{atm} with Δ, and O₂ and CO₂ with the subscripts “O” and “C”
21 (P_O takes negative values since O₂ is consumed). Writing two equations, one for CO₂ and one
22 for O₂, and dividing the first by the second yields:

$$\frac{P_c}{P_o} = \frac{D_c \Delta_c}{D_o \Delta_o} \quad (5)$$

We will define the ratio between the soil CO₂ efflux to O₂ influx as the soil ARQ (Apparent Respiratory Quotient), which is similar to the definition for tree stems (Angert et al., 2012; Angert and Sherer, 2011), so ARQ=-P_C/P_O. If only respiration drives the soil ARQ then it will be equal to the Respiratory Quotient (RQ) or to the inverse of the oxidative ratio (OR which is 1/RQ).

The D_C/D_O term in Eq. (5) can be calculated from the relationship between the diffusivity (D) of a gas in soil and the diffusivity in air (D₀):

$$D=Q \cdot D_0 \quad (6)$$

Where Q is the relative effective diffusivity, that depends on the structure of the air-filled pore spaces (Millington and Shearer, 1971). Hence, we can assume that Q is identical for CO₂ and O₂. As a result, the ratio (D_C/D_O) becomes equal to the ratio of CO₂/O₂ diffusivity in air, which is 0.76 (0.138 cm²sec⁻¹ / 0.182 cm²sec⁻¹ at STP), and is independent of temperature, since for different temperatures both diffusivity coefficients will change by the same factor (Massman, 1998). Thus, Eq. (5) becomes:

$$ARQ=-0.76(\Delta_c/\Delta_o) \quad (7)$$

And the soil ARQ can be calculated from measurements of O₂ and CO₂ concentrations in the soil. It can be shown by a numerical model that Eq. (7) is valid also when other respiration profiles are assumed.

Previous studies have estimated the OR (and hence RQ) of biomass and soils organic material. The RQ of the following plants chemical classes was calculated (Randerson et al., 2006) as: 0.88 for lignin, 0.95 for soluble phenolics, 1.0 for carbohydrates, 1.4 for organic acids, and 0.73 for lipids. In anaerobic respiration RQ>>1 since CO₂ emission is uncoupled

1 from O₂ consumption. Nitrate assimilation by roots will make the RQ values increase above
2 1, since nitrate is used instead of O₂ as electron acceptor (Lambers et al., 2008). On average,
3 and in steady state, the RQ of respiration related to decomposition of soil organic matter, must
4 reflect the stoichiometric ratios found in the soil organic matter. Severinghaus (1995)
5 calculated from elemental abundance data OR values which correspond to RQ values of 0.93
6 for average plant, 0.95 for wood and 0.93 for soil humic acid and humins. Analysis of
7 biomass by elemental composition and by the heat of combustion yielded similar OR values
8 which correspond to RQ of 0.94-1.01 (Masiello et al., 2008). The corresponding RQ values
9 found by ¹³C nuclear magnetic resonance for soil (Hockaday et al., 2009) are 0.82-1.04. These
10 values agree well with the values estimated by Severinghaus (1995) by incubation of various
11 soils in steady-state chambers (and by one in-situ flux measurement) that correspond to RQ
12 values of 0.8-1.0. Hence, if only respiration processes and diffusion drive the concentrations
13 gradients in the soil, the decrease in soil oxygen (-Δ_O) is expected to be equal to, or higher by
14 up to 20% than, the increase in CO₂ concentration gradient, corrected for the lower diffusivity
15 (0.76*Δ_C). However, if CO₂ is removed by non-respiratory processes, such as the chemical
16 processes in the soil, or by dissolution and biological processes within the roots, or if the
17 respiration substrate has different RQ from the values cited above, then the -Δ_O can be far
18 from 0.76*Δ_C and ARQ will be significantly different than 0.9±0.1.

19 **2 Methods**

20 We aimed to provide observational information on the relationships between CO₂ production
21 and O₂ consumptions across a range of soils and seasons. This included soil depth profiles (to
22 about 150 cm) in three Mediterranean sites, and single depth samplings in temperate and
23 alpine sites. These observations were supplemented with laboratory incubations of some of
24 the samples, as well as analysis of the CO₂ and O₂ transport and consumption in sterilized soil
25 columns.

1 **2.1 In situ soil air sampling**

2 To study the CO₂-O₂ relationships in different conditions, we chose to sample soil-air from 6
3 sites from different ecosystems (alpine broadleaf and needle-leaf forests, temperate forest,
4 orchard, and Mediterranean and semi-arid pine forest), with calcareous and non-calcareous
5 soils, and with varying soils and respiration rates, which induce varying gradients in soil CO₂
6 and O₂. Soil air was sampled from stainless steel tubes closed at the bottom end, and
7 perforated near the bottom. The soil air was sampled at six sites:

- 8 1) A citrus orchard located near Kefar-Vitkin, Israel (32°23'N 34°53'E). At this site, the
9 soil is Calcic Vertisol (FAO classification) and changes gradually from clay in the top
10 layers to calcareous sandy clay loam in the deeper ones. This site is irrigated in
11 summer every two weeks. Samples were taken from depths of 30, 60, 90, 120 and
12 150cm, in duplicates. In September 1999, sampling started 10 days after the last
13 irrigation, and in March 2000 it started 3 days after a rain event. Both samplings ended
14 before the next rain/irrigation event.
- 15 2) Yatir forest site, a 45-yr-old Aleppo pine (*Pinus halepensis*) plantation located at the
16 northern edge of the Negev desert, Israel (31°20'N, 35°20'E, elevation 650m). The
17 forest covers an area of 2,800 ha and lies on a Rendzic Leptosol soil (FAO
18 classification, 79 ± 45.7 cm deep), overlying chalk and limestone bedrock. The
19 climate is hot (40-yr average mean annual temperature is 18°C) and dry (40-yr
20 average mean annual precipitation is 280 mm). Monthly soil efflux measurements, soil
21 moisture profiles, and determination of soil characteristics have been routinely carried
22 out at this site (Rotenberg and Yakir, 2010). Samples for ARQ measurements were
23 taken during 2013, from depths of 30, 60, 90, and 120 cm.

- 1 3) A pine grove site located at the Hebrew University Givat Ram campus (31°46'N,
2 35°12'E, elevation 771m) in Jerusalem, on the Judea hills. The climate is semi-humid
3 Mediterranean with mean annual rainfall of 537mm (1981-2010) and an average
4 temperature of 16.8°C. Soil type is Chromic Luvisol (FAO classification) which lies
5 on a carbonate bedrock (Cenomenian dolomite). The vegetation is dominated by *Pinus*
6 *halepensis*. Samples for ARQ measurements were taken from May 2012 to August
7 2013, at 40 cm depth.
- 8 4) A temperate forest site located on the Prospect Hill tract of Harvard Forest, near
9 Petersham, Massachusetts USA (42°32'N, 72°11'W) at 340 m elevation. The mean
10 annual rainfall is 1050mm. This mixed hardwood forest is about 60-yr-old and is
11 dominated by red oak (*Quercus rubra* L.) and red maple (*Acer rubrum* L.), with some
12 stands of hemlock, white pine, and red pine. The sampling site was near the base of
13 the eddy covariance flux tower (Barford et al., 2001). The soil is classified as Dystric
14 Cambisol (FAO classification), the texture is sandy loam, and the soil is well drained.
15 Samples were taken from 85 cm depth, and 10 replicates were taken at each sampling
16 time to ensure sufficient replication necessary due to the small soil-air O₂ gradient in
17 this site. This resulted in standard error in the O₂ concentration measurements of
18 ±0.02%. Samples for ARQ measurements were taken in May and July 2001.
- 19 5) An alpine beech (*Fagus sylvatica* L.) forest in Italy (46°03'N, 11°04'E), with mean
20 annual air temperature of 8.6°C and average annual rainfall of 976 mm. The soil is a
21 Calcaric Cambisol (FAO classification). This site is described in detail in Rodeghiero
22 and Cescatti (2005) (appears there as S6). Soil air was sampled from 30 cm depth for
23 ARQ from one soil tube in June 2011, and from two soil tubes ~3 m apart, during
24 September 2013.

1 6) An alpine Norway spruce (*Picea abies* (L.) Karsten) forest site in Italy (46°02'N,
2 11°03'E), with mean annual air temperature of 5.9° C and average annual rainfall of
3 1015 mm. The soil is a Calcaric Skeletic Cambisol (FAO classification). This site is
4 described in detail by Rodeghiero and Cescatti (2005) (appears there as S8). The soil
5 air was sampled 30 cm depth for ARQ in September 2013, from three soil tubes,
6 which were ~3m apart.

7 **2.2 Diffusion experiments in sterilized soils columns**

8 To study the effects of soil chemistry and gas diffusion separately from biological effects, we
9 conducted a set of experiments with sterilized soils columns. The soil columns were prepared
10 by filling a glass tube, (8 cm long, 0.6 cm outer diameter, 0.4 cm internal diameter) with 2.0-
11 2.4 g loose soil or sand. The soils samples were: 1) Chromatic Luvisols (FAO classification)
12 with clay content of 49%, soil pH=7.6, sampled at a site with natural vegetation and
13 Mediterranean climate in Judean mountains (31°42'N, 35°3'E); 2) Sample from site 5 - clay
14 content 42%, soil pH 7.3; 3) Sample from site 6 - clay content 31%, soil pH 4.9; and 4) Acid-
15 washed sand (Merck) - clay content 0%. The soils were sterilized by gamma radiation from a
16 Cesium-137 source for at least 5 hours. Overnight incubation of the gamma-treated soils
17 showed no CO₂ emission and no O₂ consumption even after re-wetting the soils, which
18 indicates that the sterilization was successful.

19 Plugs made of alumina wool were inserted in both ends of the glass tube to keep the soil in
20 place, while allowing air movement. The soil column was placed horizontally and connected
21 to a 3.6 mL glass flask equipped with a Louwers O-ring high-vacuum-valve. CO₂ and O₂
22 were set to either diffuse out of the flasks, or into it, by either: 1) Connecting a flask with
23 8700 ppm CO₂ in N₂ to one end of the soil column, while leaving the other end open to the
24 outside air, or, 2) Connecting one side of the column to a flask with outside air, and the other

1 end of the soil column to 40 ml flasks filled with the above CO₂-N₂ mixture. Diffusion across
2 the soil columns was allowed for 30-60 minutes before the flasks were closed and CO₂ and O₂
3 concentrations in the flask were then measured as indicated below. Based on the O₂
4 concentrations in the flasks at the end of the experiments, we calculated the expected CO₂
5 concentration, assuming that diffusion was the only process taking place, knowing the ratio
6 between the diffusivities of these two gases (0.76, see introduction). We note that the use of
7 CO₂-N₂ mixtures in the experiments slightly changed the diffusivity ratios, compared to that
8 of air, but the effect was considered to be within the uncertainty of the measurement (~0.02 in
9 the diffusivity ratios) and was not considered in the calculations.

10 **2.3 Soil incubation experiments**

11 To study the effects of heterotrophic respiration, separately from the effects of root respiration
12 and that of gas diffusion in the soil profile, we conducted incubation experiments. To this end,
13 soils were sampled at the alpine sites in September 2013 and were incubated for ~5-44 hours
14 in 60ml glass flasks connected with Swagelok Ultra-Torr tee fittings to two 3.6 mL glass flask
15 equipped with Louwers high-vacuum-valves. Before the incubation, the soils were sieved to
16 2mm to remove roots, and repeated incubations were made with the same soils. Before the
17 last incubation, sucrose (50 μmol g⁻¹ soil) was added to the soils. Soil moisture content and
18 soil pH were measured, and the total dissolved inorganic carbon (DIC) in the soil solution was
19 calculated based on these parameters and the CO₂ concentration using the carbonate systems
20 constants and equations (Stumm and Morgan, 2012). The DIC values were used to calculate
21 “corrected ARQ” that accounts for the fraction of respired CO₂ which is not in the gas phase.

22 **2.4 Gas analysis**

23 Samples of soil air were collected in pre-evacuated ~3.6 mL glass flasks with Louwer™ O-
24 ring high-vacuum valves. Before sampling, the dead volume in the tubing and flask necks was

1 purged with soil air by a plastic syringe equipped with three-way valve. Duplicate samples
2 were taken in all sites, except in the Harvard forest site where 10 replicates were taken (due to
3 the close-to-ambient O₂ concentrations). At sites 1 and 4 oxygen concentrations were
4 calculated from δO₂/Ar values that were measured on a Finnigan Delta-plus mass-
5 spectrometer, assuming that since argon is inert, its concentration is constant (Angert et al.,
6 2001). The standard error in the O₂ concentration measurements was ±0.08% at site 1 and
7 0.02% at site 4. The air used for CO₂ measurements was collected in evacuated blood
8 collection tubes (vacutainers®) at site 1, and in syringes at site 4, and in the same flasks used
9 for O₂ in all other sites. At sites 1 and 4 the CO₂ concentration was measured in the laboratory
10 with a LI-COR-6252 (LI-COR, Lincoln, NE, USA) by the method described in Davidson and
11 Trumbore (1995) with a relative error of ±5%. For the other sites as well as for the diffusion
12 and incubation experiment (see below) the CO₂ and O₂ concentrations were measured on an
13 air circulating system similar to that described in Angert and Sherer (2011). The O₂
14 concentration was measured by a fuel-cell based O₂ analyzer (Sable Systems FC-10) that was
15 in the circulation loop. The analyzer [O₂] reading was corrected for the system's internal
16 pressure and for dilution by water vapor. Water vapor concentrations and CO₂ concentrations
17 were determined by a Li-840A (LI-COR, Lincoln, NE, USA) infra-red-gas-analyzer, through
18 which the air flow in the circulating system passed before entering the oxygen analyzer. The
19 accuracy and precision in [O₂] and [CO₂] determination by this method was ±0.04% for both
20 gases.

21 **3 Results**

22 The derived ARQ values are well beyond the range expected for steady-state respiration (both
23 below and above this range). In temperate and alpine soils we found values that were lower
24 than expected despite the low pH values, which limit DIC storage.

1 Soil depth profiles: The results of soil air in-situ measurements at the Mediterranean sites 1
2 and 2 are presented in Fig 1, 2. The decrease in oxygen ($-\Delta O_2$) was larger than the diffusion
3 corrected increase in carbon dioxide ($0.76\Delta CO_2$) in site 2 in January, and the ARQ value was
4 0.68 at 30cm depth, and ranged between 0.14 to 0.22 at the 60-120 cm depth range. In April
5 the $0.76\Delta CO_2$ value was closer to that of $-\Delta O_2$ and the average ARQ value in the profile was
6 0.79. In site 1 the ARQ values were as low as 0.29 on some dates (10 March, 150 cm depth),
7 but were close to 1.0, or above 1.0 (1.23, for the profile average on 12-Sep) on others.

8 Single point measurements: At the third Mediterranean site (site 3), the decrease in oxygen ($-\Delta O_2$)
9 was larger than the diffusion corrected increase in carbon dioxide ($0.76\Delta CO_2$) during
10 some months, and equal to it within the experimental uncertainty in other months (Fig. 3).
11 The results from the temperate forest site (site 4) and alpine forest sites (sites 5 and 6) are
12 presented in Table 1, which shows ARQ values ranging between 0.23 and 0.96.

13 Diffusion experiments: ~~The diffusion experiments results are presented in Fig. 4.~~ The CO_2
14 concentrations at the end of the experiments with acid-washed sand, and gamma-sterilized
15 alpine soils, agreed well with the values calculated from the O_2 concentration (based on
16 relative rates of O_2 and CO_2 diffusion in air). In contrast, the experiments with Mediterranean
17 calcareous soils fell below the 1:1 line, indicating lower measured CO_2 than that expected
18 from diffusion processes alone.

19 Soil incubation experiments: The incubation experiment with alpine soils (Table 2) gave
20 dissolution corrected ARQ values ranging between 0.60 and 1.24 (0.54-0.92 uncorrected).
21 The results indicate decreasing ARQ values with time since soil sampling, from ~ 0.9 to ~ 0.8
22 and ~ 0.8 to ~ 0.6 in soil samples from the two depths of site 6 over about 140 hours; and from
23 ~ 0.9 to ~ 0.7 in site 5 sample over a similar period. This trend was reversed in later
24 incubations when sucrose was added, with ARQ values of 0.74-1.24.

1

2 **4 Discussion**

3 **4.1 Relationships between CO₂ and soil respiration in calcareous soils**

4 The results from the Mediterranean calcareous soils sites (sites 1, 2 and 3, Fig. 1, 2 and 3)
5 show ARQ values well below 0.9, as well as values slightly above 0.9. These values clearly
6 exceeded the range expected for soil respiration (see ~~Introduction~~). These deviations from the
7 expected RQ value were evident in all three sites, despite an order of magnitude difference in
8 soil CO₂ concentrations. It should be noted that our analysis is based on the assumption of soil
9 air in steady-state, and that due to low wind speeds ($<4 \text{ m sec}^{-1}$) during ~~the~~ sampling, gas
10 exchange was only by diffusion, so that advection could be ignored. In an extreme case in
11 which advection was dominating the gas exchange, the 0.76 factor in Eq. (7) should be
12 omitted, and the low range of our ARQ values would be 0.30 instead of 0.26, which would
13 not significantly affect our interpretation.

14 We hypothesize that the low ARQ values can be explained if in addition to respiration, the
15 soil gases are also involved in reactions in the soil-water, and soil carbonates system. For
16 example, in site 1, during the March sampling, a large portion of the CO₂ in the soil was
17 probably dissolved and much of it transformed into bicarbonate, as a result of the high pH
18 values and the high [CO₂]. In the September sampling at the same site, the ARQ values were
19 slightly above 1.0, which may indicate that the soil solution was releasing carbon stored in
20 soil water-carbonates system, as hypothesized above. This carbon dioxide was probably
21 stored as bicarbonate shortly after irrigation (before the start of the sampling) when CO₂
22 concentration in soil air was higher than during sampling. Thus, the difference between the
23 ARQ values of the March and the September samplings could be attributed to opposing
24 directions of the CO₂ fluxes between the soil air and the soil solution (driven by opposing
25 direction of the gradient between the two). The direction could have been also influenced by

1 the source of the soil water. In March, the source was rainwater that contains very little
2 dissolved carbon, while in September it was irrigated by groundwater that most likely
3 contained high concentration of dissolved inorganic carbon. In a similar way, the January
4 profile in site 2 indicated large uptake of CO₂ by fresh rainwater, and much smaller uptake
5 later in the rainy season when the exchange of soil water slowed down. In addition to CO₂
6 storage and transport in soil water, the dissolved CO₂ can react with the bedrock derived soil
7 carbonate minerals. Such interactions are supported by the high δ¹³C values of around -14‰
8 observed in soil CO₂ and DIC in site 2 (Carmi et al., 2013). These values are significantly
9 higher compared with the δ¹³C values of -21‰ to -23‰ observed in the forest trees (Klein et
10 al., 2005) and may indicate that the dissolved CO₂ and bicarbonate interact with bedrock
11 carbonates (producing δ¹³C value of soil CO₂ in equilibrium with carbonate minerals of -8‰
12 to -9‰). While the isotopes do not indicate net fluxes, they do indicate that the rate of
13 interactions with the soil minerals can be significant even compared to the rapid biological
14 processes.

15 The observed variations in the ARQ values at the three calcareous sites provide direct
16 evidence that in such soils the momentary CO₂ flux from the soil does not represent well the
17 rate of soil respiration. This conclusion is ~~strengthen~~ by the diffusion experiments, which
18 showed that in the calcareous soils, also in the absence of biotic reactions, the resulting CO₂
19 concentrations were lower than expected if diffusion was the only active process. Several
20 previous studies arrived at the same conclusion, based on the mismatch between the observed
21 CO₂ fluxes, and biological models of respiration, or based on geochemical modeling (Eshel et
22 al., 2007;Hastings et al., 2005;Schlesinger et al., 2009;Serrano-Ortiz et al., 2010). However,
23 to the best of our knowledge, this is the first quantification of this effect using O₂ for intact

1 soil profiles. A corrected estimate of soil respiration can be made by dividing the measured
2 CO₂ efflux by the efflux weighted average soil profile ARQ.

3 To demonstrate this correction, we applied it at site 2 (Yatir forest), in which we measured
4 detailed profiles. The diffusivity profile in the soil was calculated from the available soil
5 properties and soil moisture profiles (Klein et al., 2013) after Moldrup et al. (2003). From the
6 diffusivity and the CO₂ concentrations profiles, we calculated the expected net CO₂ efflux
7 from each layer. Note that this calculation assumes steady-state (and hence ignores storage in
8 the gas phase), and neglects non-diffusive transport which in some cases can be important
9 (Maier et al., 2012). In addition, it was shown that this widely used approach is sensitive to
10 the choice of the diffusion model (Pingingtha et al., 2010). Using the estimated respiration flux
11 in each layer, we calculated the flux-weighted average ARQ for the entire profile during this
12 sampling. The resulting weighted average ARQ is 0.26, which indicates that the biological
13 respiration flux at this time of measurements was in fact 3.8 higher than the CO₂ efflux.
14 Hence, the apparent soil respiration flux of 2.3 μmol CO₂ m⁻² s⁻¹, obtained by chamber
15 measurement at the surface, was corrected by the weighted ARQ value to obtain the actual
16 respiration rate of 8.8 μmol CO₂ m⁻² s⁻¹. This value is consistent with previously observed
17 rates of ~8 to ~15 μmol CO₂ m⁻² s⁻¹ at this site during the wet season (October to April;
18 Grünzweig et al., 2009)). The chemical interactions of respired CO₂ with the soil solution
19 and minerals can thus considerably bias the estimates of short-term dynamics of soil
20 respiration at the hourly and daily measurements made by soil chambers or even by eddy
21 covariance flux measurements. At these time scales, the soil CO₂ efflux will not be a good
22 indicator for the biological process of respiration in such sites. However, on longer time
23 scales this effect is expected to be canceled out, since during soil drying, CO₂ will be emitted
24 out of the soil in higher rate than the actual respiration flux yielding high ARQ values, as

1 evident in site 1 during the September experiment. This is because drying increases the soil
2 solution DIC concentrations, and the respired CO₂ that was consumed in dissolution (Eqs. (1),
3 (2)) will be re-emitted during drying associated re-precipitation of carbonate. Only DIC
4 removal by drainage represents a permanent CO₂ loss. However, such drainage is low in
5 Mediterranean soils in general, and in dry environments in particular. For example, in site 2,
6 over 95% of the rainfall is accounted for by evapo-transpiration (Raz-Yaseef et al., 2010).

7 The soil CO₂ efflux measurements are usually reported and interpreted as soil respiration.
8 Upscaling point measurements, on particular dates, to the entire year and entire region, are
9 usually done by fitting the efflux data to some temperature and soil moisture functions -
10 assuming that the efflux is controlled only by the biological response of respiration. Based on
11 the data we show here, it seems important in calcareous soils to correct the efflux to non-
12 biological processes. Accurate O₂ measurements, which are relatively fast and inexpensive,
13 were ~~lately~~ developed (Hilman and Angert, manuscript in preparation), ~~were used here,~~ and
14 could facilitate similar studies in the future. We recommend that such future studies will also
15 include incubations of detached roots for ARQ measurements, to improve the method
16 accuracy by having direct measurements of the root respiration component. A previous study
17 found the same RQ values for detached and intact roots: 0.80 to 0.95, which is within the
18 range we assumed here.

19 **4.2 Relationships between CO₂ and O₂ in non-calcareous soils**

20 The low ARQ values found at the in-situ measurements in temperate forest (site 4, ARQ
21 range: 0.58 to 0.70) and alpine forest (sites 5,6, ARQ range: 0.23 to 0.96) are surprising. At
22 soil pH of 4.3 and 4.9 (sites 4 and 6, respectively) almost no dissolved carbon in the soil
23 solution can be in the form of bicarbonate or carbonate. Since the amount of carbon that can
24 be dissolved in the form of CO₂(aq) is limited, the overall storage will be small. For example,

1 at pH 4 and [CO₂] in soil air of 7000ppm only 1gC can be stored in the solution that is present
2 in 1m³ of soil (assuming that the solution occupies 25% of the volume). Since summer
3 respiration rates in these sites are in the order of few g m⁻² d⁻¹, it is obvious that the water
4 entering the soil during a rain event cannot absorb CO₂ for more than a few hours and thus
5 will not remove significant fraction of the respiratory production. Hence, the low ARQ in
6 these soils is probably not driven by carbonate chemistry. This assertion is supported by the
7 soil incubation experiments.

8 In these incubation experiments of alpine soils (Table 2), the soil pH and water content were
9 measured, and the ARQ was corrected accordingly, assuming equilibrium between the
10 headspace and the soil water. This correction was small, as can be expected, in the acidic
11 soils. The non-geochemical control on ARQ in the alpine soils was also demonstrated by the
12 diffusion experiments in gamma-sterilized soils. In the alpine soils the measured CO₂ was as
13 expected based on O₂ and the ratio of diffusivities of the two gases (0.76, same as used for the
14 in-situ profiles ARQ calculations), as it was in the experiments with acid-washed sand (Fig.
15 3). The incubation experiments, performed on roots-screened soils, also indicated that the low
16 ARQ measured in the soils profile occurs with no presence of roots, and thus, processes
17 within roots are not the sole driver of the ARQ<1.0. The DIC corrected ARQ during
18 incubation showed values as low as 0.60, with an average of 0.78. The ARQ values of the
19 incubated soils also showed a decrease with time since sampling. However, the ARQ
20 increased to 0.92 following the addition of sucrose. The incubation results indicate that the
21 low ARQ values found in the in-situ measurements in the acidic and neutral soils are real (e.g.
22 not an artifact of the soil air profile sampling or modeling), and need to be explained.

23 A Similar decrease in incubated soil RQ with time (up to 100 days) since sampling was
24 observed for incubation of soils from grassland sites (Severinghaus, 1995). This study also

1 reported values that correspond to ARQ of 0.59-0.78 for “Biosphere 2” soils, which may have
2 resulted from carbonate reactions during the incubation. However, since the alkalinity or pH
3 was not measured, this could not be confirmed. Other soils incubated in that research in an
4 open system with no CO₂ build-up gave values that correspond to ARQ of 0.83-0.95, and 0.84
5 for in-situ soil-chamber experiment, which are within the expected range of 0.9±0.1. Seibt et
6 al. (2004) reported values in a forest soil chamber which correspond to RQ of 1.5, (and to
7 1.06 after removing one data point which was considered to be an outlier) which is higher
8 than the 0.90 value reported recently for a soil chamber at a forest in Japan (Ishidoya et al.,
9 2013). Soil profile RQ values of 1.0 were found at Amazonian tropical forest in Peru (Angert
10 et al., 2012). In contrast, low RQ values were reported for the incubation of acidic soils from
11 Argentina (0.27-0.65) (Aon et al., 2001), and from Germany (Dilly, 2001) (<0.5 for some
12 soils). In the latter soils the RQ increased to ~1.0 immediately after glucose addition, and
13 reached ~1.3 with time. The low RQ in these soils (before glucose addition) was explained to
14 be substrate related. This explanation may also fit our incubation results from sites 4, 5 and 6.
15 The decrease of ARQ with time since soil sampling can be explained as the result of the
16 exhaustion of labile sugars and organic acids supplied by the root exudates, while the re-
17 supply of sucrose supported the increase in ARQ towards 1.0.

18 While the exhaustion of labile substrate hypothesis seems to fit nicely the result of these
19 experiments and of previous ones, it leaves open the question of the non-labile substrates -
20 what are they and why do they produce low RQ? The literature values correspond to RQ of
21 0.93 for average plant matter, 0.95 for wood and 0.93 for soil humic acid and humins
22 (Severinghaus, 1995). Another estimate for average plant RQ is 0.95-0.98 (Randerson et al.,
23 2006), and the RQ of the following plants chemical classes was calculated as: 0.88 for lignin,
24 0.95 for soluble phenolics, 1.0 for carbohydrates, 1.4 for organic acids, and 0.73 for lipids.
25 Since only lipids are associated with low RQ, and since they are ~10% of the soil carbon

1 (Ziegler, 1989), a straight forward explanation would be that lipids are the non-labile
2 substrates responsible for the low RQ. However, since this will imply that almost 100% of the
3 respiration in some of our incubation experiments is using lipids as substrates, we do not find
4 this explanation very plausible.

5 The low RQ values cannot be explained by nitrification (ammonium oxidation). Indeed, this
6 process lowers the RQ, since it consumes oxygen but does not emit CO₂. However, the
7 elemental composition based RQ values cited above, already account for the content of
8 reduced nitrogen and hence for nitrification. Moreover, it does not seem likely that this
9 process will become more important with incubation time, since ammonium stocks will
10 probably be depleted.

11 We speculate that the most likely process that can explain the low RQ values in non-
12 calcareous soils is the oxidation of Fe²⁺ (and another reduced species), which consumes O₂
13 but does not release CO₂. While the soils we studied were well-aerated, it was previously
14 shown that even in such soils, anoxic microsites might be present inside soil aggregates (von
15 Fischer and Hedin, 2002). The Fe²⁺ can be formed inside the soil aggregates when the soil is
16 wet (or when respiration rates are very high, like after sucrose addition), and as the soil dries
17 up (or sucrose stock depletes) oxygen can diffuse into the soil aggregate and react with the
18 Fe²⁺. Under this explanation, the RQ will be above 1.0 when the aggregates are anoxic, since
19 CO₂ will be produced but Fe³⁺ and not O₂ will be the oxygen acceptor. Since the soil
20 diffusivity in this step is low, there will be limited gas exchange between the aggregate and its
21 surrounding, and this high RQ value will be hard to measure. As the soil dries up, the RQ will
22 drop below 1.0, since oxygen will be consumed by Fe²⁺ with no CO₂ production. Thus, on
23 long-term average the RQ should match the value expected from the elemental composition
24 of plants to keep ecosystem stoichiometry balanced. This suggested mechanism should be

1 tested in future studies. If $\text{Fe}^{2+}/\text{Fe}^{3+}$ redox reactions are found to be common and
2 quantitatively important with respect to oxygen fluxes in soils, this would provide another
3 mechanism by which the instantaneous respiration rate is decoupled from the gas fluxes. In
4 previous studies (Hall et al., 2013; Hall and Silver, 2013) highland soils with mean bulk soil-
5 air O_2 of 19%, were found to have over $6\text{mg g}^{-1}(\text{soil})$ of Fe^{2+} . Such concentration of Fe^{2+} can
6 sustain many days of oxidation at the rates we measured in our soil incubation experiments.

7 Final word of caution: The ratio between oxygen consumption to CO_2 release (OR, the
8 inverse of RQ) in soil respiration is an important parameter in estimates of global carbon
9 sinks from atmospheric O_2 measurements (Keeling et al., 1996). Small deviations in the
10 global soil respiration OR from the assumed value, can introduce considerable error to such
11 estimates (Randerson et al., 2006). In this study we report large deviation measured in RQ
12 (and hence OR), but such effects might be temporal, or local fluctuations, and cannot be used
13 to infer the global annual average value before a more systematic measurement program is
14 applied.

15

16 **5 Conclusions**

17 Our results demonstrate that in contrast to the common assumption, soil ARQ (and RQ)
18 values are rarely 1.0, and often deviate from this value considerably. In calcareous soils this is
19 most likely due to chemical reactions with the soil solution and minerals, which need to be
20 accounted for during attempts to estimate the biological CO_2 efflux on short time-scales, such
21 as weekly to seasonal. This can be done by introducing measurements of the weighted
22 average ARQ in the soil profile, as done here, and then dividing the measured CO_2 efflux by
23 the observed ARQ. Such measurements become less important on annual and longer time
24 scales when the effects of CO_2 storage and release are probably canceled out. In acidic and

1 neutral soils, the variations in RQ are probably related to substrates and process that are not
2 well understood at present and warrant further research.

3

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7

1 References

- 2 Amthor, J. S., Koch, G. W., Willms, J. R., and Layzell, D. B.: Leaf O₂ uptake in the dark is
3 independent of coincident CO₂ partial pressure, *Journal of Experimental Botany*, 52, 2235-
4 2238, 10.1093/jexbot/52.364.2235, 2001.
- 5 Angert, A., Luz, B., and Yakir, D.: Fractionation of oxygen isotopes by respiration and
6 diffusion in soils and its implications for the isotopic composition of atmospheric O₂, *Global*
7 *Biogeochemical Cycles*, 15, 871-881, 2001.
- 8 Angert, A., and Sherer, Y.: Determining the relationship between tree-stem respiration and
9 CO₂ efflux by dO₂/Ar measurements, *Rapid Communications in Mass Spectrometry*, 25,
10 1752–1756, 2011.
- 11 Angert, A., Muhr, J., Negron-Juarez, R., Alegria-Muñoz, W., Kraemer, G., Ramirez-Santillan,
12 J., Barkan, E., Mazeh, S., Chambers, J. Q., and Trumbore, S. E.: Internal respiration of
13 Amazon tree stems greatly exceeds external CO₂ efflux, *Biogeosciences*, 9, 4979-4991, 2012.
- 14 Aon, M. A., Sarena, D. E., Burgos, J. L., and Cortassa, S.: Interaction between gas exchange
15 rates, physical and microbiological properties in soils recently subjected to agriculture, *Soil*
16 *and Tillage Research*, 60, 163-171, [http://dx.doi.org/10.1016/S0167-1987\(01\)00191-X](http://dx.doi.org/10.1016/S0167-1987(01)00191-X), 2001.
- 17 Aubrey, D. P., and Teskey, R. O.: Root-derived CO₂ efflux via xylem stream rivals soil CO₂
18 efflux, *New Phytol*, 184, 35-40, 10.1111/j.1469-8137.2009.02971.x, 2009.
- 19 Barford, C. C., Wofsy, S. C., Goulden, M. L., Munger, J. W., Pyle, E. H., Urbanski, S. P.,
20 Hutyyra, L., Saleska, S. R., Fitzjarrald, D., and Moore, K.: Factors Controlling Long- and
21 Short-Term Sequestration of Atmospheric CO₂ in a Mid-latitude Forest, *Science*, 294, 1688-
22 1691, 2001.
- 23 Benavente, J., Vadillo, I., Carrasco, F., Soler, A., Linan, C., and Moral, F.: Air Carbon
24 Dioxide Contents in the Vadose Zone of a Mediterranean Karst, *Vadose Zone Journal*, 9, 126-
25 136, 10.2136/vzj2009.0027, 2010.
- 26 Bloemen, J., McGuire, M. A., Aubrey, D. P., Teskey, R. O., and Steppe, K.: Transport of
27 root-respired CO₂ via the transpiration stream affects aboveground carbon assimilation and
28 CO₂ efflux in trees, *New Phytol.* , 197, 555-565 10.1111/j.1469-8137.2012.04366.x., 2012.
- 29 Bond-Lamberty, B., and Thomson, A.: Temperature-associated increases in the global soil
30 respiration record, *Nature*, 464, 579-582, 2010.
- 31 Carmi, I., Yakir, D., Yechieli, Y., Kronfeld, J., and Stiller, M.: Variations in soil CO₂
32 concentrations and isotopic values in a semi-arid region due to biotic and abiotic processes in
33 the unsaturated zone, *Radiocarbon*, 55, 932-942, 2013.
- 34 Cuezva, S., Fernandez-Cortes, A., Benavente, D., Serrano-Ortiz, R., Kowalski, A. S., and
35 Sanchez-Moral, S.: Short-term CO₂(g) exchange between a shallow karstic cavity and the
36 external atmosphere during summer: Role of the surface soil layer, *Atmospheric*
37 *Environment*, 45, 1418-1427, 10.1016/j.atmosenv.2010.12.023, 2011.
- 38 Davey, P. A., Hunt, S., Hymus, G. J., DeLucia, E. H., Drake, B. G., Karnosky, D. F., and
39 Long, S. P.: Respiratory oxygen uptake is not decreased by an instantaneous elevation of
40 [CO₂], but is increased with long-term growth in the field at elevated [CO₂], *Plant*
41 *Physiology*, 134, 520-527, 10.1104/pp.103.030569, 2004.

- 1 Davidson, E. A., and Trumbore, S. E.: Gas diffusivity and production of CO₂ in deep soils of
2 the eastern Amazon, *Tellus B*, 47, 550-565, 10.1034/j.1600-0889.47.issue5.3.x, 1995.
- 3 Davidson, E. A., Belk, E., and Boone, R. D.: Soil water content and temperature as
4 independent or confounded factors controlling soil respiration in a temperate mixed hardwood
5 forest, *Global Change Biology*, 4, 217-227, 10.1046/j.1365-2486.1998.00128.x, 1998.
- 6 Davidson, E. A., Savage, K., Verchot, L. V., and Navarro, R.: Minimizing artifacts and biases
7 in chamber-based measurements of soil respiration, *Agricultural and Forest Meteorology*,
8 113, 21-37, 2002.
- 9 Dilly, O.: Microbial respiratory quotient during basal metabolism and after glucose
10 amendment in soils and litter, *Soil Biology & Biochemistry*, 33, 117-127, 10.1016/s0038-
11 0717(00)00123-1, 2001.
- 12 Emmerich, W. E.: Carbon dioxide fluxes in a semiarid environment with high carbonate soils,
13 *Agricultural and Forest Meteorology*, 116, 91-102, Pii s0168-1923(02)00231-9
14 10.1016/s0168-1923(02)00231-9, 2003.
- 15 Eshel, G., Fine, P., and Singer, M. J.: Total soil carbon and water quality: An implication for
16 carbon sequestration, *Soil Science Society of America Journal*, 71, 397-405,
17 10.2136/sssaj2006.0061, 2007.
- 18 Grünzweig, J. M., Hemming, D., Maseyk, K., Lin, T., Rotenberg, E., Raz-Yaseef, N.,
19 Falloon, P. D., and Yakir, D.: Water limitation to soil CO₂ efflux in a pine forest at the
20 semiarid “timberline”, *Journal of Geophysical Research*, 114, G03008, 2009.
- 21 Hall, S. J., McDowell, W. H., and Silver, W. L.: When Wet Gets Wetter: Decoupling of
22 Moisture, Redox Biogeochemistry, and Greenhouse Gas Fluxes in a Humid Tropical Forest
23 Soil, *Ecosystems*, 16, 576-589, DOI 10.1007/s10021-012-9631-2, 2013.
- 24 Hall, S. J., and Silver, W. L.: Iron oxidation stimulates organic matter decomposition in
25 humid tropical forest soils, *Global Change Biology*, 19, 2804-2813, 10.1111/gcb.12229, 2013.
- 26 Hastings, S. J., Oechel, W. C., and Muhlia-Melo, A.: Diurnal, seasonal and annual variation
27 in the net ecosystem CO₂ exchange of a desert shrub community (Sarcocaulis) in Baja
28 California, Mexico, *Global Change Biology*, 11, 927-939, 10.1111/j.1365-
29 2486.2005.00951.x, 2005.
- 30 Hesterberg, R., and Siegenthaler, U.: Production and Stable Isotopic Composition of Co₂ in a
31 Soil near Bern, Switzerland, *Tellus B*, 43, 197-205, 1991.
- 32 Hockaday, W. C., Masiello, C. A., Randerson, J. T., Smernik, R. J., Baldock, J. A., Chadwick,
33 O. A., and Harden, J. W.: Measurement of soil carbon oxidation state and oxidative ratio by
34 ¹³C nuclear magnetic resonance, *Journal of Geophysical Research: Biogeosciences*, 114,
35 G02014, 10.1029/2008jg000803, 2009.
- 36 Ishidoya, S., Murayama, S., Takamura, C., Kondo, H., Saigusa, N., Goto, D., Morimoto, S.,
37 Aoki, N., Aoki, S., and Nakazawa, T.: O₂:CO₂ exchange ratios observed in a cool temperate
38 deciduous forest ecosystem of central Japan, *Tellus Series B-Chemical and Physical
39 Meteorology*, 65, Article Number: 21120 10.3402/tellusb.v65i0.21120, 2013.
- 40 Jury, W. A., Gardner, W. R., and Gardner, W. H.: *Soil Physics*, Wiley, 1991.
- 41 Keeling, R. F., Piper, S. C., and Heimann, M.: Global and hemispheric CO₂ sinks deduced
42 from changes in atmospheric O₂ concentration, *Nature*, 381, 1996.

- 1 Klein, T., Hemming, D., Lin, T. B., Grunzweig, J. M., Maseyk, K., Rotenberg, E., and Yakir,
2 D.: Association between tree-ring and needle delta C-13 and leaf gas exchange in *Pinus*
3 *halepensis* under semi-arid conditions, *Oecologia*, 144, 45-54, 10.1007/s00442-005-0002-y,
4 2005.
- 5 Klein, T., Shpringer, I., Fikler, B., Elbaz, G., Cohen, S., and Yakir, D.: Relationships between
6 stomatal regulation, water-use, and water-use efficiency of two coexisting key Mediterranean
7 tree species, *For. Ecol. Manage.*, 302, 34-42, 10.1016/j.foreco.2013.03.044, 2013.
- 8 Kowalski, A. S., Serrano-Ortiz, P., Janssens, I. A., Sanchez-Moral, S., Cuezva, S., Domingo,
9 F., Were, A., and Alados-Arboledas, L.: Can flux tower research neglect geochemical CO2
10 exchange?, *Agricultural and Forest Meteorology*, 148, 1045-1054,
11 10.1016/j.agrformet.2008.02.004, 2008.
- 12 Lambers, H., Chapin, F. S., III, and Pons, T. L.: *Plant Physiological Ecology*, 2nd ed.,
13 Springer, 610 pp., 2008.
- 14 Ma, J., Wang, Z. Y., Stevenson, B. A., Zheng, X. J., and Li, Y.: An inorganic CO2 diffusion
15 and dissolution process explains negative CO2 fluxes in saline/alkaline soils, *Sci Rep-Uk*, 3,
16 2013.
- 17 Maier, M., Schack-Kirchner, H., Aubinet, M., Goffin, S., Longdoz, B., and Parent, F.:
18 Turbulence Effect on Gas Transport in Three Contrasting Forest Soils, *Soil Sci. Soc. Am. J.*,
19 76, 1518-1528, 10.2136/sssaj2011.0376, 2012.
- 20 Masiello, C. A., Gallagher, M. E., Randerson, J. T., Deco, R. M., and Chadwick, O. A.:
21 Evaluating two experimental approaches for measuring ecosystem carbon oxidation state and
22 oxidative ratio, *Journal of Geophysical Research: Biogeosciences*, 113, G03010,
23 10.1029/2007jg000534, 2008.
- 24 Massman, W. J.: A review of the molecular diffusivities of H2O, CO2, CH4, CO, O3, SO2,
25 NH3, N2O, NO, and NO2 in air, O2 and N2 near STP, *Atmospheric Environment*, 32, 1111-
26 1127, 1998.
- 27 Millington, R. J., and Shearer, R. C.: Diffusion in aggregated porous media, *Soil Science*,
28 111, 372-378, 1971.
- 29 Moldrup, P., Olesen, T., Komatsu, T., Yoshikawa, S., Schjonning, P., and Rolston, D. E.:
30 Modeling diffusion and reaction in soils: X. A unifying model for solute and gas diffusivity in
31 unsaturated soil, *Soil Science*, 168, 321-337, 2003.
- 32 Pingingtha, N., Leclerc, M. Y., BEASLEY Jr, J., Zhang, G., and Senthong, C.: Assessment of
33 the soil CO2 gradient method for soil CO2 efflux measurements: comparison of six models in
34 the calculation of the relative gas diffusion coefficient, *Tellus B*, 62, 47-58, 2010.
- 35 Raich, J. W., and Schlesinger, W. H.: The global carbon dioxide flux in soil respiration and its
36 relationship to vegetation and climate, *Tellus B*, 44, 81-99, 10.1034/j.1600-0889.1992.t01-1-
37 00001.x, 1992.
- 38 Raich, J. W., and Potter, C. S.: Global patterns of carbon dioxide emissions from soils, *Global*
39 *Biogeochem. Cycles*, 9, 23-36, 10.1029/94gb02723, 1995.
- 40 Randerson, J. T., Masiello, C. A., Still, C. J., Rahn, T., Poorter, H., and Field, C. B.: Is carbon
41 within the global terrestrial biosphere becoming more oxidized? Implications for trends in
42 atmospheric O2, *Global Change Biology*, 12, 260-271, 10.1111/j.1365-2486.2006.01099.x,
43 2006.

- 1 Raz-Yaseef, N., Yakir, D., Rotenberg, E., Schiller, G., and Cohen, S.: Ecohydrology of a
2 semi-arid forest: partitioning among water balance components and its implications for
3 predicted precipitation changes, *Ecohydrology*, 3, 143-154, 10.1002/eco.65, 2010.
- 4 Rodeghiero, M., and Cescatti, A.: Main determinants of forest soil respiration along an
5 elevation/temperature gradient in the Italian Alps, *Global Change Biology*, 11, 1024-1041,
6 10.1111/j.1365-2486.2005.00963.x, 2005.
- 7 Roland, M., Serrano-Ortiz, P., Kowalski, A. S., Godderis, Y., Sanchez-Canete, E. P., Ciais,
8 P., Domingo, F., Cuezva, S., Sanchez-Moral, S., Longdoz, B., Yakir, D., Van Grieken, R.,
9 Schott, J., Cardell, C., and Janssens, I. A.: Atmospheric turbulence triggers pronounced diel
10 pattern in karst carbonate geochemistry, *Biogeosciences*, 10, 5009-5017, 10.5194/bg-10-
11 5009-2013, 2013.
- 12 Rotenberg, E., and Yakir, D.: Contribution of Semi-Arid Forests to the Climate System,
13 *Science*, 327, 451-454, 10.1126/science.1179998, 2010.
- 14 Schlesinger, W. H., Belnap, J., and Marion, G.: On carbon sequestration in desert ecosystems,
15 *Global Change Biology*, 15, 1488-1490, 10.1111/j.1365-2486.2008.01763.x, 2009.
- 16 Seibt, U., Brand, W. A., Heimann, M., Lloyd, J., Severinghaus, J. P., and Wingate, L.:
17 Observations of O₂:CO₂ exchange ratios during ecosystem gas exchange, *Global*
18 *Biogeochemical Cycles*, 18, GB4024, 10.1029/2004gb002242, 2004.
- 19 Serrano-Ortiz, P., Roland, M., Sanchez-Moral, S., Janssens, I. A., Domingo, F., Godderis, Y.,
20 and Kowalski, A. S.: Hidden, abiotic CO₂ flows and gaseous reservoirs in the terrestrial
21 carbon cycle: Review and perspectives, *Agricultural and Forest Meteorology*, 150, 321-329,
22 10.1016/j.agrformet.2010.01.002, 2010.
- 23 Severinghaus, J. P.: Studies of the terrestrial O₂ and carbon cycles in sand dune gases and in
24 biosphere 2, Columbia University, 1995.
- 25 Stern, L., Baisden, W. T., and Amundson, R.: Processes controlling the oxygen isotope ratio
26 of soil CO (sub 2) ; analytic and numerical modeling, *Geochimica et Cosmochimica Acta*, 63,
27 799-814, 1999.
- 28 Stevenson, B. A., and Verburg, P. S. J.: Effluxed CO₂-C-13 from sterilized and unsterilized
29 treatments of a calcareous soil, *Soil Biology & Biochemistry*, 38, 1727-1733, 2006.
- 30 Stumm, W., and Morgan, J. J.: Aquatic chemistry: chemical equilibria and rates in natural
31 waters, John Wiley & Sons, 2012.
- 32 Tamir, G., Shenker, M., Heller, H., Bloom, P. R., Fine, P., and Bar-Tal, A.: Can Soil
33 Carbonate Dissolution Lead to Overestimation of Soil Respiration?, *Soil Science Society of*
34 *America Journal*, 75, 1414-1422, 10.2136/sssaj2010.0396, 2011.
- 35 Vargas, R., Carbone, M., Reichstein, M., and Baldocchi, D.: Frontiers and challenges in soil
36 respiration research: from measurements to model-data integration, *Biogeochemistry*, 102, 1-
37 13, 10.1007/s10533-010-9462-1, 2011.
- 38 von Fischer, J. C., and Hedin, L. O.: Separating methane production and consumption with a
39 field-based isotope pool dilution technique, *Global Biogeochemical Cycles*, 16, 1034
40 10.1029/2001gb001448, 2002.
- 41 Wang, W. F., Chen, X., Luo, G. P., and Li, L. H.: Modeling the contribution of abiotic
42 exchange to CO₂ flux in alkaline soils of arid areas, *J Arid Land*, 6, 27-36, 2014.

1 Ziegler, F.: Changes of lipid content and lipid composition in forest humus layers derived
2 from Norway spruce, *Soil Biology and Biochemistry*, 21, 237-243,
3 [http://dx.doi.org/10.1016/0038-0717\(89\)90100-4](http://dx.doi.org/10.1016/0038-0717(89)90100-4), 1989.

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1 Table 1. The [CO₂], [O₂], and ARQ (average values of replicates) for in-situ measurements in
 2 acidic and neutral soils in temperate and alpine forest sites (sites 4, 5, 6). Apparent
 3 respiratory quotient (ARQ) values different from the 0.9±0.1 expected for respiration
 4 (based on plant composition) were observed in these soils.

date	site	description	soil pH	depth (cm)	CO ₂ %	O ₂ %	ARQ
30/05/2001	4	Temperate forest	4.5	85	0.46	20.40	0.58±0.05
31/07/2001	4	Temperate forest	4.5	85	0.73	20.20	0.70±0.05
07/06/2011	5	Alpine forest	7.3	40	0.62	19.06	0.23±0.04
09/09/2013	5	Alpine forest	7.3	30	0.28	20.67	0.64±0.06
09/09/2013	6	Alpine forest	4.9	30	0.26	20.77	0.96±0.24

5

1 Table 2. Results of the soil incubation experiments with alpine forest soils. The soils were
 2 sieved to remove roots before incubation. The apparent respiratory quotient (ARQ)
 3 values declined with time since sampling, and increase following the addition of
 4 sucrose, with good agreement between pair measurements. For calculating the
 5 “dissolution corrected ARQ” the dissolved inorganic carbon in the soil solution was
 6 calculated, given the CO₂ partial pressure, the temperature, and the soil solution pH.

Depth	Site	Start time		CO ₂ (%)	O ₂ (%)	ARQ	dissolution corrected ARQ
		after sampling (h)	Incubation time (h)				
5-20cm	6	3.95	16.45	2.43	18.19	0.87	0.88
5-20cm	6	3.95	32.78	3.85	16.49	0.85	0.87
5-20cm	6	147.40	25.60	1.17	19.49	0.77	0.79
5-20cm	6	147.40	44.80	2.08	18.35	0.78	0.80
5-20cm	6	337 +sucrose	5.15	4.22	16.32	0.90	0.92
5-20cm	6	337 +sucrose	22.37	15.21	2.37	0.82	0.83
30-40cm	6	7.65	12.75	1.3	19.42	0.82	0.85
30-40cm	6	7.65	29.08	2.3	18.15	0.81	0.83
30-40cm	6	36.73	18.02	1.57	18.3	0.58	0.60
30-40cm	6	36.73	45.62	2.86	16.36	0.61	0.63
30-40cm	6	147.40	25.60	0.71	19.88	0.63	0.65
30-40cm	6	147.40	44.80	1.16	19.22	0.65	0.67
30-40cm	6	337 +sucrose	5.15	4.39	14.88	0.72	0.74
30-40cm	6	337 +sucrose	22.37	14.78	2.18	0.79	0.81
5-20cm	5	6.78	5.25	1.36	19.1	0.71	0.93
5-20cm	5	6.78	12.75	2.48	17.36	0.68	0.89
5-20cm	5	146.53	25.60	1.1	18.99	0.54	0.71
5-20cm	5	146.53	44.80	2.01	17.54	0.58	0.76
5-20cm	5	337 +sucrose	5.15	1.38	19.06	0.71	0.95
5-20cm	5	337 +sucrose	22.37	7.63	12.72	0.92	1.24

7

1 **Figure captions**

2

3 Figure 1. Temporal variations in depth profiles of $-\Delta O_2$ (open blue squares, O_2 decrease from
4 ambient) and $0.76\Delta CO_2$ (red diamonds, CO_2 increase above ambient corrected for lower gas
5 diffusivity compared to O_2) profiles in the soil of site 1 (citrus orchard). The March
6 experiment started 3 days after a rain event, while the September experiment started 10 days
7 after irrigation. Error bars are smaller than the markers.

8 Figure 2. The $-\Delta O_2$ (open blue squares, O_2 decrease from ambient) and $0.76\Delta CO_2$ (red
9 diamonds, CO_2 increase above ambient corrected for lower gas diffusivity compared to O_2)
10 profiles in the soil of site 2 (semi-arid pine forest) in January (a) and April (b). The values are
11 in percent (and in order of magnitude lower than in Fig. 1). Some error bars are smaller than
12 the markers.

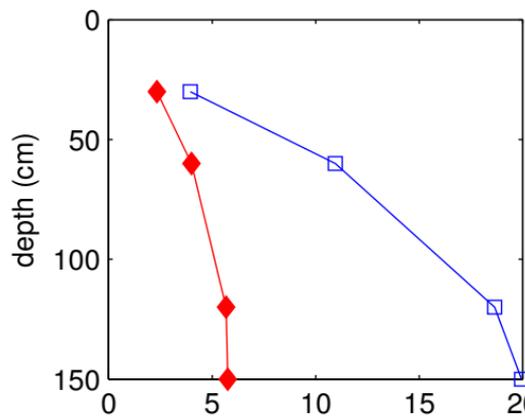
13 Figure 3. Temporal changes in $-\Delta O_2$ (open blue squares, O_2 decrease from ambient) and
14 $0.76\Delta CO_2$ (red diamonds, CO_2 increase above ambient corrected for lower gas diffusivity
15 compared to O_2) at the soil of site 3 (pine stand; 40cm depth) from May 2012 to August 2013.
16 Most error bars are smaller than the markers.

17 Figure 4. Diffusion in gamma-sterilized soils. For sand and alpine soils the measured CO_2
18 agrees well with the values calculated from O_2 concentration and the known diffusivity ratio, but
19 this was not the case for Mediterranean soils, where measured CO_2 concentrations were lower
20 than expected from O_2 measurements.

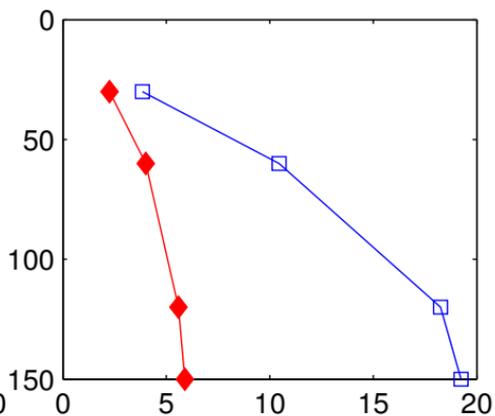
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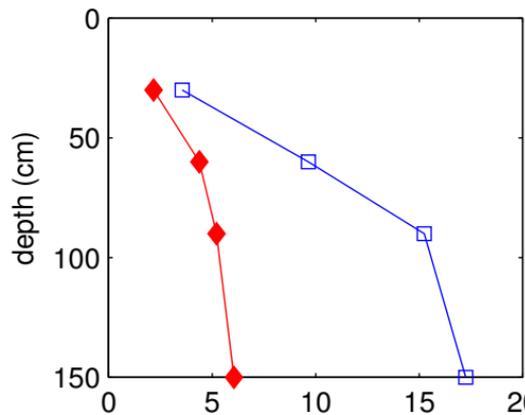
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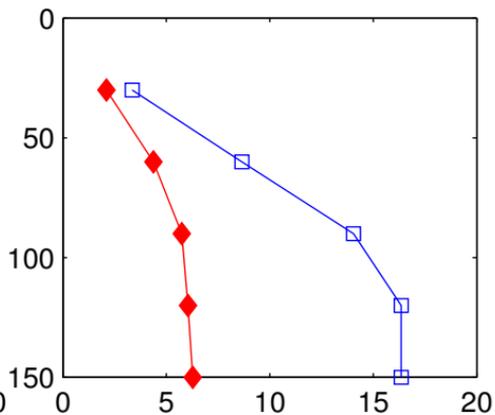
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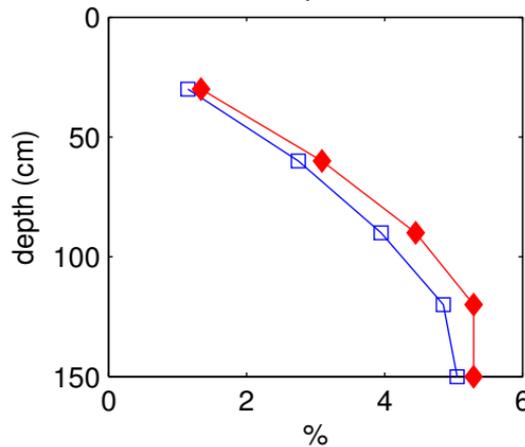
March 14



March 17



Sep 10



Sep 12

