Using O_2 to study the relationships between soil CO_2 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 CO_2 efflux is equal to the soil respiration rate. Here we challenge this assumption by combining measurements of CO_2 with high-precision measurements of O_2. 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 CO_2 efflux and the O_2 influx (defined as apparent respiratory quotient, ARQ) was in the range of 0.14 to 1.23, and considerably deviated from the value of 0.9±0.1 expected from the elemental composition of

average plants and soil organic matter. At the Mediterranean sites, these deviations are explained as a result of CO_2 dissolution in the soil water and transformation to bicarbonate ions in these high pH soils, and by carbonate minerals dissolution and precipitation processes. Thus, correct estimate of the short-term, chamber-based biological respiratory flux in such soils can only be made by dividing the measured soil CO_2 efflux by the average (efflux weighted) soil profile ARQ. Applying this approach to a semiarid pine forest resulted in an estimated short-term biological respiration rate that is 3.8 times higher than the chamber-measured surface CO_2. The ARQ values often observed in the more acidic soils were unexpectedly low (<0.7). These values probably result from the oxidation of reduced iron, which have been previously formed during times of high soil moisture and local anaerobic conditions inside soil aggregates. The results reported here provide direct quantitative evidence for large temporal decoupling between soil gas exchange fluxes and biological soil respiration.

1 Introduction

Respiration in soils is a major flux in the global carbon cycle, and contributes ~100 Pg C y^{-1} to the atmosphere (Bond-Lamberty and Thomson, 2010). As a result, this process has attracted much attention in recent decades (Davidson et al., 1998; Raich and Potter, 1995; Raich and Schlesinger, 1992; Vargas et al., 2011). Soil respiration is defined as the sum of heterotrophic respiration by soil micro-organisms, mostly bacteria and fungi, and autotrophic respiration by living roots. It is usually estimated by measuring the CO_2 efflux from the soil to a chamber placed above it (Davidson et al., 2002), or modelled from the CO_2 concentration gradients in the soil profile (Davidson and Trumbore, 1995). Hence, the basic assumption is that the CO_2 efflux is

equal to the soil respiration. However, the CO_2 efflux is not necessarily an ideal measure of the respiration rate for the following reasons:

First, instead of diffusing through the soil surface, a considerable fraction of the respired CO_2 can be dissolved in the soil water, transported in the hydrological system, or take part in reactions of the carbonate system:

$$CO_2(g) \Leftrightarrow CO_2(aq) + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{-2} + 2H^+ \qquad (1)$$

In a calcareous soil with a pH of ~8 most of the carbon in the soil solution is in the form of bicarbonate (HCO_3^-). Using the carbonate system equilibrium relationships (Stumm and Morgan, 2012), it can be shown that in such a pH range the storage capacity of dissolved inorganic carbon (mainly bicarbonate) in soil water is considerable. For instance, we calculated given the carbonate system constants (Stumm and Morgan, 2012), that for a soil porosity of 50% which is 50% water filled pores, a soil pCO_2 of 10,000ppm (1%), and a soil pH of ~8, the soil carbon storage capacity would be ~100g carbon m^{-3} soil (mostly as bicarbonate). This DIC storage capacity is large in comparison to typical soil respiration rates, which are in the order of ~2 gC m^{-2} d^{-1}. This large storage capacity is particularly important when water is replaced by rain, irrigation, or any other water supply process. In addition, some CO_2 will also be stored in gas-phase in the soil pores. However, with the same soil parameters values as above, the gas phase storage will be only in the order of 1g. Hence, in calcareous soils the gas phase storage is negligible in comparison to the storage of dissolved inorganic carbon, unless large cavities exist below the soil.

Second, in addition to the DIC storage, in calcareous soils the CO_2 can also be consumed in calcium carbonate dissolution reaction:

$$CaCO_3 + H_2CO_3 \Leftrightarrow Ca^+ + 2HCO_3^- \qquad (2)$$

or released in the reverse reaction. Such processes have been shown to influence the temporal variation of the soil CO_2 efflux, and to make it different than the biological process of respiration (Benavente et al., 2010; Cuezva et al., 2011; Emmerich, 2003; Eshel et al., 2007; Hastings et al., 2005; Kowalski et al., 2008; Roland et al., 2013; Schlesinger et al., 2009; Serrano-Ortiz et al., 2010; Tamir et al., 2011; Ma et al., 2013; Stevenson and Verburg, 2006; Wang et al., 2014).

Third, processes within roots may also cause the CO_2 efflux to be different from the actual respiration rate. For example, the CO_2 respired by roots can be dissolved in the xylem water and carried upward in the transpiration stream (Aubrey and Teskey, 2009; Bloemen et al., 2012).

Measurement of O_2 uptake rate is an alternative approach to measure respiration, which is routinely applied in studies of aquatic systems. However, making such measurements in air-phase, and especially under field conditions, is challenging since the atmospheric background of O_2 is more than 500 times larger than that of CO_2 (20.95% versus 0.04%). Recently, Angert and Sherer (2011) have demonstrated that the combined measurement of O_2 uptake in addition to the CO_2 efflux can be used to isolate the biological respiration flux in a tree stem. This approach is based on the lower solubility of O_2 in water (28 times lower than that of CO_2 at 20°C), and also on the fact that O_2, in contrast to CO_2, does not form additional chemical species by reacting with water. Thus, the O_2 influx may be a better measure of respiration than the widely used CO_2 efflux, as was also suggested previously for plant respiration measurements in the lab (Amthor et al., 2001; Davey et al., 2004).

The ratio between the soil CO_2 efflux to O_2 influx was seldom studied. Values of 0.59-0.78 were reported (Severinghaus, 1995) for lab incubation of “Biosphere 2” soils, which may have resulted from carbonate reactions during the incubation.

However, since the alkalinity or pH was not measured, this could not be confirmed. Other soils incubated in that research in an open system with no CO_2 build-up gave values of 0.83-0.95, and 0.84 for in-situ soil-chamber experiment. Seibt et al. (2004) reported values in a forest soil chamber which correspond to RQ of 1.5, (and to 1.06 after removing one data point which was considered to be an outlier) which is higher than the 0.90 value reported recently for a soil chamber at a forest in Japan (Ishidoya et al., 2013). A average value of 1.0 for soil profiles were found at Amazonian tropical forest in Peru (Angert et al., 2012).

Here we have used high accuracy measurements of O_2 concentrations to study the relationships between soil CO_2 efflux and soil respiration in well-drained soils, and to determine how O_2 measurements can help to better quantify and understand soil respiration. To make our conclusions more general, the study was conducted in different ecosystems, in calcareous and non-calcareous soils, and over a wide range of soil CO_2 and O_2 concentrations. Finally, we demonstrate how O_2 measurements can be used to correct CO_2 measurements for estimating soil respiration flux.

1.1 Expected relationships between O_2 and CO_2 in soils

In a one-dimensional model, the change with time of the concentration (C) of a gas in soil is related to the concentration gradient with depth (z), the gas diffusivity in the soil (D) and the rate of net CO_2 production (P). This net rate of CO_2 production integrates the effects of respiration and of CO_2 storage/release discussed above. The one-dimensional model is summarized by the diffusion-production equation (Jury et al., 1991; Stern et al., 1999):

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

This reaction-diffusion model ignores advection, that can be important in some cases (Maier et al., 2012). For this reason we have conducted all of our experiments under low wind speeds (<4 m sec^{-1}) conditions. For solving Eq. (3), we can for instance assume that CO_2 production rate decreases exponentially with depth such that $P(z)=P'exp(-z/z_e)$, where P' is the rate of CO_2 production at the soil surface and z_e is the depth at which the rate equals P'/e. Then the steady-state solution for the concentration gradient between the soil and the atmosphere (z=0) becomes:

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

We noted the difference $C(z)-C_{atm}$ with Δ, and O_2 and CO_2 with the subscripts "O" and "C" (P_O takes negative values since O_2 is consumed). Writing two equations, one for CO_2 and one for O_2, and dividing the first by the second yields:

$$\frac{P_C}{P_O}=\frac{D_C}{D_O}\frac{\Delta_C}{\Delta_O} \qquad (5)$$

We will define the ratio between the soil CO_2 efflux to O_2 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 (D_0):

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

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 CO_2 and O_2. As a result, the ratio (D_C/D_O) becomes equal to the ratio of

CO_2/O_2 diffusivity in air, which is 0.76 (0.138 cm^2sec^{-1} / 0.182 cm^2sec^{-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 O_2 and CO_2 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 CO_2 emission is uncoupled from O_2 consumption. Nitrate assimilation by roots will make the RQ values increase above 1, since nitrate is used instead of O_2 as electron acceptor (Lambers et al., 2008). On average, and in steady state, the RQ of respiration related to decomposition of soil organic matter, must reflect the stoichiometric ratios found in the soil organic matter. Severinghaus (1995) calculated from elemental abundance data OR values which correspond to RQ values of 0.93 for average plant, 0.95 for wood and 0.93 for soil humic acid and humins. Analysis of biomass by elemental composition and by the heat of combustion yielded similar OR values which correspond to RQ of 0.94-1.01 (Masiello et al., 2008). The corresponding RQ values found by ^{13}C nuclear magnetic resonance for soil (Hockaday et al., 2009) are 0.82-1.04. These values agree well with the values estimated by Severinghaus (1995) by incubation of various soils in steady-state chambers (and by one in-situ flux measurement) that correspond to RQ values of 0.8-1.0. Hence, if only respiration processes and diffusion drive the concentrations gradients in the soil, the decrease in

soil oxygen ($-\Delta_O$) is expected to be equal to, or higher by up to 20% than, the increase in CO_2 concentration gradient, corrected for the lower diffusivity ($0.76*\Delta_C$). However, if CO_2 is removed by non-respiratory processes, such as chemical processes in soil, or by dissolution and biological processes within the roots, or if the respiration substrate has different RQ from the values cited above, then the $-\Delta_O$ can be far from $0.76*\Delta_C$ and ARQ will be significantly different than 0.9±0.1.

2 Methods

We aimed to provide observational information on the relationships between CO_2 production and O_2 consumptions across a range of soils and seasons. This included soil depth profiles (to about 150 cm) in three Mediterranean sites, and single depth samplings in temperate and alpine sites. These observations were supplemented with laboratory incubations of some of the samples, as well as analysis of the CO_2 and O_2 transport and consumption in sterilized soil columns.

2.1 In situ soil air sampling

To study the CO_2-O_2 relationships in different conditions, we chose to sample soil-air from 6 sites from different ecosystems (alpine broadleaf and needle-leaf forests, temperate forest, orchard, and Mediterranean and semi-arid pine forest), with calcareous and non-calcareous soils, and with varying soils and respiration rates, which induce varying gradients in soil CO_2 and O_2. Soil air was sampled from stainless steel tubes closed at the bottom end, and perforated near the bottom. The soil air was sampled at six sites:

1) A citrus orchard located near Kefar-Vitkin, Israel (32°23'N 34°53'E). At this site, the soil is Calcic Vertisol (FAO classification) and changes gradually from clay in the top layers to calcareous sandy clay loam in the deeper ones. This site is irrigated in summer every two weeks. Samples were taken from depths of 30, 60, 90,120 and 150cm, in duplicates. In September 1999,

sampling started 10 days after the last irrigation, and in March 2000 it started 3 days after a rain event. Both samplings ended before the next rain/irrigation event.

2) Yatir forest site, a 45-yr-old Aleppo pine (*Pinus halepensis*) plantation located at the northern edge of the Negev desert, Israel (31°20'N, 35°20'E, elevation 650m). The forest covers an area of 2,800 ha and lies on a Rendzic Leptosol soil (FAO classification, 79 ± 45.7 cm deep), overlying chalk and limestone bedrock. The climate is hot (40-yr average mean annual temperature is 18°C) and dry (40-yr average mean annual precipitation is 280 mm). Monthly soil efflux measurements, soil moisture profiles, and determination of soil characteristics have been routinely carried out at this site (Rotenberg and Yakir, 2010). Samples for ARQ measurements were taken during 2013, from depths of 30, 60, 90, and 120 cm.

3) A pine grove site located at the Hebrew University Givat Ram campus (31˚46'N, 35˚12'E, elevation 771m) in Jerusalem, on the Judea hills. The climate is semi-humid Mediterranean with mean annual rainfall of 537mm (1981-2010) and an average temperature of 16.8˚C. Soil type is Chromic Luvisol (FAO classification) which lies on a carbonate bedrock (Cenomenian dolomite). The vegetation is dominated by *Pinus halepensis*. Samples for ARQ measurements were taken from May 2012 to August 2013, at 40 cm depth.

4) A temperate forest site located on the Prospect Hill tract of Harvard Forest, near Petersham, Massachusetts USA (42°32'N, 72°11'W) at 340 m elevation. The mean annual rainfall is 1050mm. This mixed hardwood forest is about 60-yr-old and is dominated by red oak (*Quercus rubra* L.) and red maple (*Acer*

rubrum L.), with some stands of hemlock, white pine, and red pine. The sampling site was near the base of the eddy covariance flux tower (Barford et al., 2001). The soil is classified as Dystric Cambisol (FAO classification), the texture is sandy loam, and the soil is well drained. Samples were taken from 85 cm depth, and 10 replicates were taken at each sampling time to ensure sufficient replication necessary due to the small soil-air O_2 gradient in this site. This resulted in standard error in the O_2 concentration measurements of ±0.02%. Samples for ARQ measurements were taken in May and July 2001.

5) An alpine beech (*Fagus sylvatica* L.) forest in Italy (46°03′N, 11°04'E), with mean annual air temperature of 8.6°C and average annual rainfall of 976 mm. The soil is a Calcaric Cambisol (FAO classification). This site is described in detail in Rodeghiero and Cescatti (2005) (appears there as S6). Soil air was sampled from 30 cm depth for ARQ from one soil tube in June 2011, and from two soil tubes ~3 m apart, during September 2013.
6) An alpine Norway spruce (*Picea abies* (L.) Karsten) forest site in Italy (46°02′N, 11°03′E), with mean annual air temperature of 5.9° C and average annual rainfall of 1015 mm. The soil is a Calcaric Skeletic Cambisol (FAO classification). This site is described in detail by Rodeghiero and Cescatti (2005) (appears there as S8). The soil air was sampled 30 cm depth for ARQ in September 2013, from three soil tubes, which were ~3m apart.

2.2 Diffusion experiments in sterilized soils columns

To study the effects of soil chemistry and gas diffusion separately from biological effects, we conducted a set of experiments with sterilized soils columns. The soil columns were prepared by filling a glass tube, (8 cm long, 0.6 cm outer diameter, 0.4 cm internal diameter) with 2.0-2.4 g loose soil or sand. The soils samples were: 1)

Chromatic Luvisols (FAO classification) with clay content of 49%, soil pH=7.6, sampled at a site with natural vegetation and Mediterranean climate in Judean mountains (31º42'N, 35º3'E); 2) Sample from site 5 - clay content 42%, soil pH 7.3; 3) Sample from site 6 - clay content 31%, soil pH 4.9; and 4) Acid-washed sand (Merck) - clay content 0%. The soils were sterilized by gamma radiation from a Cesium-137 source for at least 5 hours. Overnight incubation of the gamma-treated soils showed no CO_2 emission and no O_2 consumption even after re-wetting the soils, which indicates that the sterilization was successful.

Plugs made of alumina wool were inserted in both ends of the glass tube to keep the soil in place, while allowing air movement. The soil column was placed horizontally and connected to a 3.6 mL glass flask equipped with a Louwers O-ring high-vacuum-valve. CO_2 and O_2 were set to either diffuse out of the flasks, or into it, by either: 1) Connecting a flask with 8700 ppm CO_2 in N_2 to one end of the soil column, while leaving the other end open to the outside air, or, 2) Connecting one side of the column to a flask with outside air, and the other end of the soil column to 40 ml flasks filled with the above CO_2-N_2 mixture. Diffusion across the soil columns was allowed for 30-60 minutes before the flasks were closed and CO_2 and O_2 concentrations in the flask were then measured as indicated below. Based on the O_2 concentrations in the flasks at the end of the experiments, we calculated the expected CO_2 concentration, assuming that diffusion was the only process taking place, knowing the ratio between the diffusivities of these two gases (0.76, see introduction). We note that the use of CO_2-N_2 mixtures in the experiments slightly changed the diffusivity ratios, compared to that of air, but the effect was considered to be within the uncertainty of the measurement (~0.02 in the diffusivity ratios) and was not considered in the calculations.

2.3 Soil incubation experiments

To study the effects of heterotrophic respiration, separately from the effects of root respiration and that of gas diffusion in the soil profile, we conducted incubation experiments. To this end, soils were sampled at the alpine sites in September 2013 and were incubated for ~5-44 hours in 60ml glass flasks connected with Swagelok Ultra-Torr tee fittings to two 3.6 mL glass flask equipped with Louwers high-vacuum-valves. Before the incubation, the soils were sieved to 2mm to remove roots, and repeated incubations were made with the same soils. Before the last incubation, sucrose (50μmol g^{-1} soil) was added to the soils. Soil moisture content and soil pH were measured, and the total dissolved inorganic carbon (DIC) in the soil solution was calculated based on these parameters and the CO_2 concentration using the carbonate systems constants and equations (Stumm and Morgan, 2012). The DIC values were used to calculate "corrected ARQ" that accounts for the fraction of respired CO_2 which is not in the gas phase.

2.4 Gas analysis

Samples of soil air were collected in pre-evacuated ~3.6 mL glass flasks with Louwer™ O-ring high-vacuum valves. Before sampling, the dead volume in the tubing and flask necks was purged with soil air by a plastic syringe equipped with three-way valve. Duplicate samples were taken in all sites, except in the Harvard forest site where 10 replicates were taken (due to the close-to-ambient O_2 concentrations). At sites 1 and 4 oxygen concentrations were calculated from $\delta O_2/Ar$ values that were measured on a Finnigan Delta-plus mass-spectrometer, assuming that since argon is inert, its concentration is constant (Angert et al., 2001). The standard error in the O_2 concentration measurements was ±0.08% at site 1 and 0.02% at site 4. The air used for CO_2 measurements was collected in evacuated blood collection tubes

(vacutainers®) at site 1, and in syringes at site 4, and in the same flasks used for O_2 in all other sites. At sites 1 and 4 the CO_2 concentration was measured in the laboratory with a LI-COR-6252 (LI-COR, Lincoln, NE, USA) by the method described in Davidson and Trumbore (1995) with a relative error of ±5%. For the other sites as well as for the diffusion and incubation experiment (see below) the CO_2 and O_2 concentrations were measured on an air circulating system similar to that described in Angert and Sherer (2011). The O_2 concentration was measured by a fuel-cell based O_2 analyzer (Sable Systems FC-10) that was in the circulation loop. The analyzer [O_2] reading was corrected for the system's internal pressure and for dilution by water vapor. Water vapor concentrations and CO_2 concentrations were determined by a Li-840A (LI-COR, Lincoln, NE, USA) infra-red-gas-analyzer, through which the air flow in the circulating system passed before entering the oxygen analyzer. The accuracy and precision in [O_2] and [CO_2] determination by this method was ±0.04% for both gases.

3 Results

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

Soil depth profiles: The results of soil air in-situ measurements at the Mediterranean sites 1 and 2 are presented in Fig 1, 2. The decrease in oxygen ($-\Delta O_2$) was larger than the diffusion corrected increase in carbon dioxide ($0.76\Delta CO_2$) in site 2 in January, and the ARQ value was 0.68 at 30cm depth, and ranged between 0.14 to 0.22 at the 60-120 cm depth range. In April the $0.76\Delta CO_2$ value was closer to that of $-\Delta O_2$ and the average ARQ value in the profile was 0.79. In site 1 the ARQ values were as low as

0.29 on some dates (10 March, 150 cm depth), but were close to 1.0, or above 1.0 (1.23, for the profile average on 12-Sep) on others.

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

It should be noted that our analysis is based on the assumption of soil air in steady-state, and that due to low wind speeds (<4 m sec^{-1}) during the sampling, gas exchange was only by diffusion, so that advection could be ignored. In an extreme case in which advection was dominating the gas exchange, the 0.76 factor in Eq. (7) should be omitted, and the low range of our ARQ values would be 0.30 instead of 0.26, which would not significantly affect our interpretation.

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

Soil incubation experiments: The incubation experiment with alpine soils (Table 2) gave dissolution corrected ARQ values ranging between 0.60 and 1.24 (0.54-0.92 uncorrected). The results indicate decreasing ARQ values with time since soil sampling, from ~0.9 to ~0.8 and ~0.8 to ~0.6 in soil samples from the two depths of

site 6 over about 140 hours; and from ~0.9 to ~0.7 in site 5 sample over a similar period. This trend was reversed in later incubations when sucrose was added, with ARQ values of 0.74-1.24.

4 Discussion

Based on the variations in the CO_2/O_2 concentrations in soil profiles we demonstrated widespread temporal decoupling between soil gas exchange fluxes and biological respiration. CO_2 dissolution in soil water and a-biotic interactions with the carbonate systems (calcareous soils) and oxidation of reduced iron (acidic soils) could explain most of these decoupling.

4.1 Relationships between CO_2 and soil respiration in calcareous soils

The ARQ values measured in the Mediterranean calcareous soils sites (sites 1, 2 and 3; Fig. 1, 2 and 3) clearly exceeded the range expected for soil respiration (Hockaday et al., 2009; Masiello et al., 2008; Severinghaus, 1995) being well below and above 0.9. These deviations from the expected RQ values were evident in all three calcareous soil sites, despite an order of magnitude difference in soil CO_2 concentrations. The low ARQ values can be explained if in addition to respiration, the soil gases also react with the soil water. In addition to the processes of CO_2 storage and transport in soil water, the dissolved CO_2 can react with the bedrock-derived soil carbonate minerals.

A possible evidence of such reactions is the high $\delta^{13}C$ values of around -14‰ observed in soil CO_2 and DIC in site 2 (Carmi et al., 2013). These values are significantly higher than the $\delta^{13}C$ values of -21‰ to -23‰ observed in the forest trees (Klein et al., 2005) and may indicate that the dissolved CO_2 and bicarbonate interact with bedrock carbonates (producing $\delta^{13}C$ value of soil CO_2 in equilibrium with

carbonate minerals of -8‰ to -9‰). While the isotopes do not indicate net fluxes, they do indicate that the rate of interactions with the soil minerals can be significant even compared to the rapid biological processes.

The observed variations in the ARQ values at the three calcareous soil sites provide direct evidence that the momentary CO_2 flux is not representative of the rate of soil respiration. This conclusion is strengthened by the results obtained from the diffusion experiments, which showed that in sterilized calcareous soils, the resulting CO_2 concentrations were lower than expected if diffusion was the only active process. Several previous studies arrived at the same conclusions by noticing the mismatch between the measured and modelled CO_2 fluxes, or even based on the results of geochemical modeling (Eshel et al., 2007; Hastings et al., 2005; Schlesinger et al., 2009; Serrano-Ortiz et al., 2010). However, to the best of our knowledge, this study provides the first confirmation and quantification of this effect by O_2 monitoring in intact soil profiles.

A correct estimate of soil respiration should therefore account for the above described processes and can be made by dividing the measured CO_2 efflux by the efflux-weighted average soil profile ARQ. As an example, we applied the correction to the data collected at site 2 (Yatir forest), in which we measured detailed CO_2 profiles and derived the diffusivity profile from the available soil properties and soil moisture data (Klein et al., 2013) after Moldrup et al. (2003). From the diffusivity and the CO_2 concentration profiles, we calculated the expected net CO_2 efflux from each layer. Note that this transport calculation assumes steady-state (and hence ignores storage in the gas phase); neglects non-diffusive transport which in some cases can be important (Maier et al., 2012); and is sensitive to the choice of the diffusion model (Pingintha et al., 2010). The resulting weighted average ARQ was 0.26, which indicates that the

biological respiration flux at this time of measurements was in fact 3.8 higher than the CO_2 efflux.

The chemical interactions of respired CO_2 with the soil solution and minerals can thus bias estimates of hourly and daily soil respiration measured by soil chambers, or ecosystem respiration measured by eddy covariance flux. At these time scales, the soil CO_2 efflux will not be a good indicator for the biological process of respiration in such sites. However, on longer time scales this effect is expected to be canceled out, since during soil drying, CO_2 will be emitted out of the soil at higher rate than the actual respiration flux, yielding high ARQ values as noticed in site 1 during the September experiment. This can be explained if we consider that drying increases the soil solution DIC concentrations, and the respired CO_2 that was consumed in dissolution (Eqs. (1), (2) will be re-emitted during drying-associated re-precipitation of carbonate. Therefore, only DIC removal by drainage represents a permanent CO_2 loss. However, such drainage is low in Mediterranean soils in general and in dry environments in particular. For example, in site 2, over 95% of the rainfall is accounted for by evapo-transpiration (Raz-Yaseef et al., 2010).

The soil CO_2 efflux measurements are usually reported and interpreted as soil respiration. Upscaling point measurements, on particular dates, to the entire year and entire region, is usually done by fitting the efflux data to some temperature and soil moisture functions - assuming that the efflux is controlled only by the biological response of respiration. Based on the data we showed here, it seems important in calcareous soils to correct the CO_2 efflux for non-biological processes. Relatively fast and inexpensive O_2 measurement were recently performed by Hilman and Angert (manuscript in preparation), and could facilitate similar studies in the future. We recommend that such future studies will also include ARQ measurements (by

incubations chambers) of detached roots, in order to improve the method accuracy by direct estimate of the root respiration ARQ at the study site. A previous study found the same RQ values for detached and intact roots: 0.80 to 0.95 (Lipp and Andersen, 2003), which are within the range we assumed here.

4.2 Relationships between CO_2 and O_2 in low pH soils

The low ARQ values found at the in-situ measurements in temperate forest (site 4, ARQ range: 0.58 to 0.70) and alpine forest (sites 5, 6, ARQ range: 0.23 to 0.96) were unexpected. At soil pH of 4.3 and 4.9 (sites 4 and 6, respectively) almost no dissolved carbon in the soil solution can be in the form of bicarbonate or carbonate, and since the amount of carbon that can be dissolved in the form of CO_2(aq) is limited, the overall storage will be also small. As an example, with a pH of 4 and [CO_2] in soil air of 7000ppm only 1gC can be stored in the solution that is present in $1m^3$ of soil (assuming that the solution occupies 25% of the volume). Since the summer respiration rates in these sites are in the order of few g m^{-2} d^{-1}, the water entering the soil during a rain event cannot absorb CO_2 for more than a few hours and thus will not remove significant fraction of the respiratory production. As a result, the low ARQ in these soils is probably not driven by carbonate chemistry.

The non-geochemical control on ARQ in the alpine sites was also demonstrated by the diffusion experiments in gamma-sterilized soils where the measured CO_2 was as expected based on O_2 measurements and the ratio of diffusivities of the two gases (0.76, same as used for the in-situ profiles ARQ calculations; Fig. 3). The observed low ARQ also occurs with no roots present, and thus, processes within roots are not the sole driver of the ARQ<0.9. The DIC corrected ARQ during incubation showed values as low as 0.60, with an average of 0.78. The ARQ values of the incubated soils

also showed a decrease with time since sampling, and an increase following the addition of sucrose. The incubation results indicate that the low ARQ values found in the in-situ measurements in the acidic and neutral soils are real (e.g. not an artifact of the soil air profile sampling or modeling), and need to be explained.

A similar decrease in incubated soil RQ with time (up to 100 days) since sampling was observed for incubation of soils from grassland sites (Severinghaus, 1995). Low RQ values were reported for the incubation of acidic soils from Argentina (0.27-0.65) (Aon et al., 2001), and from Germany (Dilly, 2001) (<0.5 for some soils). In the latter soils the RQ increased to ~1.0 immediately after glucose addition, and reached ~1.3 with time. The low RQ noted in these soils (before glucose addition) was explained to be substrate related and this hypothesis may also fit our incubation results from sites 4, 5 and 6. The decrease of ARQ with time since soil sampling can be explained as the result of the exhaustion of labile sugars and organic acids supplied by the root exudates, while the re-supply of sucrose supported the increase in ARQ towards 1.0.

While hypothesis of labile substrate exhaustion seems to fit nicely the result of these experiments and of previous ones, it leaves open the question of the non-labile substrates: what is their nature and why do they turn in a low RQ? Literature reports RQ values of 0.93 for average plant matter, 0.95 for wood and 0.93 for soil humic acid and humins (Severinghaus, 1995). Values of 0.95-0.98 were measured (Randerson et al., 2006) for average plant RQ, whereas when considering single plant chemical classes RQ was calculated as: 0.88 for lignin, 0.95 for soluble phenolics, 1.0 for carbohydrates, 1.4 for organic acids, and 0.73 for lipids. Since only lipids are associated with low RQ, and since they are ~10% of the soil carbon (Ziegler, 1989), one may suggest that lipids are the non-labile substrates responsible for the soil low RQ. However, since this would imply that almost 100% of the respiration in some of

our incubation experiments derived from lipid substrates, we do not find this explanation very plausible.

Nor can the low ARQ values be explained by nitrification (ammonium oxidation); this process lowers the RQ, since it consumes oxygen, but does not emit CO_2. However, the elemental composition based RQ values cited above, already account for the content of reduced nitrogen and hence for nitrification. Moreover, it does not seem likely that this process will become more important with incubation time, since ammonium stocks will probably be depleted.

Thus, we suggest that the oxidation of Fe^{2+} (and another reduced species) could be the most likely process that can explain the low RQ values in non-calcareous soils given that it consumes O_2 but does not release CO_2. While the soils we studied were well-aerated, it was previously shown that even in such soils, anoxic microsites might be present inside soil aggregates (von Fischer and Hedin, 2002). The Fe^{2+} can be formed inside the soil aggregates when the soil is wet (or when respiration rates are very high, like after sucrose addition), and as the soil dries (or sucrose stock depletes) oxygen can diffuse into the soil aggregate and react with the Fe^{2+}. Under this explanation, the RQ will be above 1.0 when the aggregates are anoxic, since CO_2 will be produced but Fe^{2+} and not O_2 will be the oxygen acceptor. Since the soil diffusivity in this step is low, there will be limited gas exchange between the aggregate and its surrounding, and this high RQ value will be hard to measure. As the soil dries, the RQ will drop below 1.0, since oxygen will be consumed by Fe^{2+} with no CO_2 production. Indeed, recent soil incubations we made showed a decrease in Fe^{2+} and a drop in ARQ during soil drying. In previous studies (Hall et al., 2013; Hall and Silver, 2013) highland soils with mean bulk soil-air O_2 of 19%, were found to have over 6mg g^{-1}(soil) of Fe^{2+}, which can sustain oxidation, at the rates we measured in our soil incubation

experiments, over few days. On long-term average the RQ should match the value expected from the elemental composition of plants to keep ecosystem stoichiometry balanced. However, such Fe^{2+}/Fe^{3+} redox reactions provide another mechanism by which the instantaneous respiration rate is decoupled from the gas fluxes.

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

5 Conclusions

Our results demonstrate that in contrast to the common assumption, soil ARQ (and RQ) values are rarely 1.0, and often deviate from this value considerably. In calcareous soils this is most likely due to chemical reactions with the soil solution and minerals, which need to be accounted for during attempts to estimate the biological CO_2 efflux on short time-scales, such as weekly to seasonal. This can be done by introducing measurements of the weighted average ARQ in the soil profile, as done here, and then dividing the measured CO_2 efflux by the observed ARQ. Such measurements become less important on annual and longer time scales when the effects of CO_2 storage and release are probably canceled out. In acidic and neutral soils, the variations in RQ are probably related to substrates and process that are not well understood at present and warrant further research.

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Table 1. The [CO_2], [O_2], and ARQ (average values of replicates) for in-situ measurements in acidic and neutral soils in temperate and alpine forest sites (sites 4, 5, 6). Apparent respiratory quotient (ARQ) values different from the 0.9±0.1 expected for respiration (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

Table 2. Results of the soil incubation experiments with alpine forest soils. The soils were sieved to remove roots before incubation. The apparent respiratory quotient (ARQ) values declined with time since sampling, and increase following the addition of sucrose, with good agreement between pair measurements. For calculating the "dissolution corrected ARQ" the dissolved inorganic carbon in the soil solution was calculated, given the CO_2 partial pressure, the temperature, and the soil solution pH.

Depth	Site	Start time after sampling (h)	Incubation duration (h)	CO_2 (%)	O_2 (%)	ARQ	dissolution corrected ARQ
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**

Figure captions

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

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

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

Figure 4. Diffusion in gamma-sterlized soils. For sand and alpine soils the measured CO_2 agrees well with the values caluclated from O_2 concetration and the know diffusivity ratio, but this was not the case for Mediterranean soils, where measured CO_2 concentrations were lower than expected from O_2 measurements.