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# 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 at the lab. We found that the ratio between the $CO_2$ efflux to the $O_2$ influx (which we defined as apparent respiratory quotient, ARQ) was in the range of 0.14 to 1.23, which strongly deviates from $0.9 \pm 0.1$, which is the ratio expected from the elemental composition of average plants and soil organic matter. At the Mediterranean sites these deviations were explained as a result of $CO_2$ dissolution in the soil water and transformation to bi-carbonate in these high pH soils, and by carbonates 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 $CO_2$ efflux by the average (efflux weighted) soil profile ARQ. We demonstrated that applying this approach to a semiarid pine forest resulted in estimated short-term respiration rate 3.8 times higher than the chamber-measured surface $CO_2$ efflux ($8.8\,\mu mol\,CO_2\,m^{-2}\,s^{-1}$ instead of $2.3\,\mu mol\,CO_2\,m^{-2}\,s^{-1}$, at the time of measurement). The ARQ values that were often found for the more acidic soils were lower than 0.7, and hence surprising. These values might be the result of the oxidation of reduced iron, which could previously form during times of high soil moisture and local anaerobic conditions inside aggregates. Further research is needed to confirm that low ARQ found in non-calcareous soils, is the result of this process, which can cause additional temporal decoupling between gas fluxes and soil respiration.





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## 1 Introduction

Respiration in soils is a major flux in the global carbon cycle, and contributes ~ 100 Pg C $yr^{-1}$ 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, a considerable fraction of the respired $CO_2$ instead of diffusing through the soil surface can be dissolved in the soil water, transported in the hydrological system, or take part in reactions of the carbonate system:

$$CO_2\,(g) \Leftrightarrow CO_2\,(aq) + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{-2} + 2H^+ \quad \text{(R1)}$$

In a calcareous soil with a pH of ~ 8 most of the carbon in the system 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, given the carbonate system constants (Stumm and Morgan, 2012), for a soil porosity of 50 % which is 50 % water filled pores, a soil $pCO_2$ of 10 000 ppm (1 %), and a soil pH of ~ 8, the soil carbon storage capacity would be ~ 100 g 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 few g C $m^{-2}$ $d^{-1}$. This large storage capacity is particularly significant when water is replaced by rain, irrigation, or any other water transport process. In addition, some $CO_2$ will be also stored in gas-phase in the soil pores. However, with the same soil parameters values as above, the gas





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phase storage will be only in the order of 1 g. Hence, in calcareous soils the gas phase storage is negligible in comparison to the storage of dissolved inorganic carbon, unless large cavities exists 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:

$$CaCO_3 + H_2CO_3 \Leftrightarrow Ca + 2HCO_3^- \quad \text{(R2)}$$

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 to be 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).

Third, processes within roots may also cause the $CO_2$ efflux to be different than the actual respiration rate. Roots respired $CO_2$ 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 % vs. 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).

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. To make





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our conclusions more general, the study was conducted in different ecosystems, in calcareous and non-calcareous soils, and over 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 respiration ($R$) according to the diffusion-production equation (Jury et al., 1991; Stern et al., 1999):

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - R(z) \quad (1)$$

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 ($< 4\,\mathrm{m\,s^{-1}}$) conditions. For solving Eq. (1), we can for instance assume that respiration rate decreases exponentially with depth such that $R(z) = R' \exp(-z/z_e)$, where $R'$ is the rate at the soil surface and $z_e$ is the depth at which the rate equals $R'/e$, then the steady-state solution for the concentration gradient between the soil and the atmosphere ($z = 0$) becomes:

$$C(z) - C_{\mathrm{atm}} = \left(R' \cdot z_e^2/D\right)(1 - \exp(-z/z_e)) \quad \text{(Hesterberg and Siegenthaler, 1991)} \quad (2)$$

Noting the difference $C(z) - C_{\mathrm{atm}}$ with $\Delta$, and $O_2$ and $CO_2$ with the subscripts "O" and "C", and replacing the actual respiration rates with the soil emission rates ($E$, where $E_O$ takes negative values) Eq. (2) becomes:

$$\frac{E_C}{E_O} = \frac{D_C}{D_O}\frac{\Delta_C}{\Delta_O} \quad (3)$$





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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 = $-E_C/E_O$. 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).

The $D_C/D_O$ term in Eq. (3) can be calculated from the relationship between the diffusivity ($D$) of a gas in soil and the diffusivity in air ($D_0$):

$$D = Q \cdot D_0 \quad (4)$$

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 ($0.138\,cm^2\,s^{-1}/0.182\,cm^2\,s^{-1}$ at STP), and is independent of temperature, since for different temperatures both diffusivity coefficients will change by the same factor (Massman, 1998). Thus, Eq. (3) becomes:

$$ARQ = -0.76(\Delta_C/\Delta_O) \quad (5)$$

And the soil ARQ can be calculated from measurements of $O_2$ and $CO_2$ concentrations in the soil. It can be shown numerically that Eq. (5) is valid also under other respiration profiles.

Previous studies have estimated the OR (and hence RQ) of biomass and soils organic material. 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





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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 ($0.76 \cdot \Delta_C$). However, if $CO_2$ is removed by non-respiratory processes, such as the chemical processes in the soil, or by dissolution and biological processes within the roots, or if the respiration substrate has different RQ than the values cited above, then the $-\Delta_O$ can be far from $0.76 \cdot \Delta_C$ and ARQ will be significantly different than $0.9 \pm 0.1$.

## 2 Methods

### 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. Samples of the soil air were collected in duplicates in pre-evacuated ~ 3.6 mL glass flasks with a Louwers™ O-ring valve. 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. 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 150 cm, in duplicates. In September 1999, sampling started 10 days





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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 *P. halepensis* plantation located at the northern edge of the Negev desert, Israel (31°20′ N, 35°20′ E, elevation 650 m). The forest covers an area of 2800 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 771 m) in Jerusalem, on the Judea hills. The climate is semi-humid Mediterranean with mean annual rainfall of 537 mm (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 1050 mm. 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





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$O_2$ 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 (labeled as 5a, 5b) ∼ 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 (labeled as 6a, 6b, 6c) which were ∼ 3 m 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 (31°42′ N, 35°3′ 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 h. 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.





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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 min 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).

### 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 h in 60 mL 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 2 mm to remove roots, and repeated incubations were made with the same soils. Before the last incubation, sucrose (50 µmol $g^{-1}$ 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 account for the fraction of respired $CO_2$ which is not in the gas phase.





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## 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. Duplicate samples were taken in all sites, except in the Harvard forest site were 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 results provided observational information on the relationships between $CO_2$ production and $O_2$ consumptions across a range of soils and sampling dates. This included soil depth profiles (to about 150 cm) in three Mediterranean sites, and single





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

Soil depth profiles: the results of soil air in-situ measurements at the Mediterranean sites 1 and 2 are presented in Figs. 1 and 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 30 cm 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 September) 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 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 1.17.

Diffusion experiments: the diffusion experiments results are presented in Fig. 4. The $CO_2$ concentrations at the end of the 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). 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.

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,





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

## 4 Discussion

### 4.1 Relationships between $CO_2$ and soil respiration in calcareous soils

The results from the Mediterranean calcareous soils sites (sites 1–3, Figs. 1–3) show ARQ values well below 0.9, as well as values slightly above 0.9. These values clearly exceeded the range expected for soil respiration (see Introduction). This deviation from the expected RQ value were evident in all three sites, despite an order of magnitude difference in soil $CO_2$ concentrations. 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 ($< 4\,m\,s^{-1}$) during the sampling, gas exchange was caused 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. (5) 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. We hypothesize that the low ARQ values can be explained if in addition to respiration, the soil gases are also involved in reactions in the soil-water, and soil carbonates system. For example, in site 1, during the March sampling, a large portion of the $CO_2$ in the soil was probably dissolved and much of it transformed into bicarbonate, as a result of the high pH and the high [$CO_2$]. This $CO_2$ storage could have caused the low ARQ ratios in these samplings. In the September sampling at the same site, the ARQ values were slightly above 1.0, which may indicate that the soil solution was releasing carbon stored in soil water-carbonates system, as hypothesized above. This carbon was probably stored as bicarbonate shortly after irrigation (before the start of the sampling) when $CO_2$ concentration in soil air was higher than during sampling.





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Thus, the difference between the ARQ values of the March and the September samplings could be attributed to opposing directions of the $CO_2$ fluxes between the soil air and the soil solution (driven by opposing direction of the gradient between the two). The direction could have been also influenced by the source of the soil water. In March the source was rainwater that contains very little dissolved carbon, while in September it was irrigated by groundwater that most likely contained high concentration of dissolved inorganic carbon. In a similar way, the January profile in site 2 indicated large uptake of $CO_2$ by fresh rainwater, and much smaller uptake later in the rainy season when the exchange of soil water slowed down. In addition to $CO_2$ storage and transport in soil water, the dissolved $CO_2$ can react with the bedrock derived soil carbonate minerals. Such interactions are supported by 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 compared with 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 (with $\delta^{13}C$ value of soil carbonate minerals around −8 ‰). 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 sites provide direct evidence that in such soils the momentary $CO_2$ flux from the soil does not represent well the rate of soil respiration. This conclusion is strengthen by the diffusion experiments, which showed that in the calcareous soils, also in the absence of biotic reactions, the resulting $CO_2$ concentrations were lower than expected if diffusion was the only active process. Several previous studies arrived at the same conclusion, based on the mismatch between the observed $CO_2$ fluxes, and biological models of respiration, or based on 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 is the first report of directly observing this discrepancy, based on $O_2$ measurements. A corrected estimate of soil respiration can be made by dividing the measured $CO_2$ efflux by the efflux weighted average soil profile ARQ.





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This could be demonstrated for site 2 where data are available to estimate the expected distribution of $CO_2$ efflux along the soil profile. The diffusivity profile in the soil was calculated from the available soil properties and soil moisture profiles (Klein et al., 2013) after Moldrup et al. (2003). From the diffusivity and the $CO_2$ concentrations profiles, we calculated the expected net $CO_2$ efflux from each layer. Note that this calculation assumes steady-state (and hence ignores storage in the gas phase), and neglects non-diffusive transport which in some cases can be important (Maier et al., 2012). In addition, it was shown that this widely used approach is sensitive to the choice of the diffusion model (Pingintha et al., 2010). Using the estimated respiration flux in each layer, we calculated the flux-weighted average ARQ for the entire profile during this sampling. The resulting weighted average ARQ is 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. Hence, the apparent soil respiration flux of 2.3 $\mu mol\, CO_2\, m^{-2}\, s^{-1}$, obtained by chamber measurement at the surface, was corrected by the weighted ARQ value to obtain the actual respiration rate of 8.8 $\mu mol\, CO_2\, m^{-2}\, s^{-1}$. This value is consistent with previously observed rates of $\sim 8$ to $\sim 15\, \mu mol\, CO_2\, m^{-2}\, s^{-1}$ at this site during the wet season (October to April; Grünzweig et al., 2009). The chemical interactions of respired $CO_2$ with the soil solution and minerals can thus considerably bias the estimates of short-term dynamics of soil respiration at the hourly and daily measurements made by soil chambers or even by eddy covariance flux measurements. 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 in higher rate than the actual respiration flux yielding high ARQ values, as evident in site 1 during the September experiment. This is because drying increases the soil solution DIC concentrations, and the respired $CO_2$ that was consumed in dissolution (Reactions 1 and 2) will be re-emitted during drying associated re-precipitation of carbonate. 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,





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in site 2, over 95 % of the rainfall is accounted for by evapo-transpiration (Raz-Yaseef et al., 2010).

### 4.2 Relationships between $CO_2$ and $O_2$ in non-calcareous 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 1.17) are surprising. 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. Since the amount of carbon that can be dissolved in the form of $CO_2$ (aq) is limited, the overall storage will be small. For example, at pH 4 and [$CO_2$] in soil air of 7000 ppm only 1 g C can be stored in the solution that is present in 1 $m^3$ of soil (assuming that the solution occupies 25 % of the volume). Since summer respiration rates in these sites are in the order of few g $m^{-2}$ $d^{-1}$, it is obvious that 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. Hence, the low ARQ in these soils is probably not driven by carbonate chemistry. This assertion is supported by the soil incubation experiments.

In these incubation experiments of alpine soils (Table 2), the soil pH and water content were measured, and the ARQ was corrected accordingly, assuming equilibrium between the headspace and the soil water. This correction was small, as can be expected, in the acidic soils. The non-geochemical control on ARQ in the alpine soils was also demonstrated by the diffusion experiments in gamma-sterilized soils. In the alpine soils the measured $CO_2$ was as expected based on $O_2$ and the ratio of diffusivities of the two gases (0.76, same as used for the in-situ profiles ARQ calculations), as it was in the experiments with acid-washed sand (Fig. 3). The incubation experiments, performed on roots-screened soils, also indicated that the low ARQ measured in the soils profile occurs with no presence of roots, and thus, processes within roots are not the sole driver of the ARQ < 1.0. 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





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showed a decrease with time since sampling. However, the ARQ increased to 0.92 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). This study also reported values which correspond to ARQ of 0.59–0.78 for "Biosphere 2" soils, which may have resulted from carbonate reactions during the incubation, but 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 which correspond to ARQ of 0.83–0.95, and 0.84 for in-situ soil-chamber experiment, which are within the expected range of $0.9 \pm 0.1$. 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). Soil profile RQ values of 1.0 were found at Amazonian tropical forest in Peru (Angert et al., 2012). In contrast, 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 $\sim 1.0$ immediately after glucose addition, and reached with time $\sim 1.3$. The low RQ in these soils (before glucose addition) was explained to be substrate related. This explanations may also fit our incubation results from sites 4–6. 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 the exhaustion of labile substrate hypothesis seems to fit nicely the result of these and of previous experiments, it leaves open the question of what are the non-labile substrates, and why they produce low RQ? The literature values correspond to RQ of 0.93 for average plant matter, 0.95 for wood and 0.93 for soil humic acid and humins (Severinghaus, 1995). Another estimate for average plant RQ is 0.95–0.98





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(Randerson et al., 2006), and the RQ of the following plants chemical classes 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), a straight forward explanation would be that lipids are the non-labile substrates responsible for the low RQ. However, since this will imply that almost 100 % of the respiration in some of our incubation experiments is using lipids as substrates, we do not find this explanation very plausible.

The low RQ values cannot be explained by nitrification (ammonium oxidation). Indeed, 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 processes will become more important with incubation time, since ammonium stocks will probably be depleted.

We speculate that the most likely process that can explain the low RQ values in non-calcareous soils is the oxidation of $Fe^{2+}$ (and another reduced species), which 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 up (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 $Fe^{3+}$ 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 up, the RQ will drop below 1.0, since oxygen will be consumed by $Fe^{2+}$ with no $CO_2$ production. Thus, on long-term average the RQ should match the value expected from the elemental composition of plants to keep ecosystem stoichiometry balanced. This suggested mechanism should be tested in future studies. If $Fe^{2+}/Fe^{3+}$ redox reactions are found to be common





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and quantitatively important with respect to oxygen fluxes in soils, this would provide another mechanism by which the instantaneous respiration rate is decoupled from the gas fluxes.

Final word of caution: 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 the 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 weekly and seasonal time scales. This can be done by measuring the weighted average ARQ in the soil profile, as done here, and then dividing the measured $CO_2$ efflux by the ARQ. On annual time scale these effects are probably canceled out. In acidic and neutral soils the variations in RQ are probably substrate and process related, and as discussed above, not well understood and warrant further research.

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**Table 1.** Summary of the in-situ measurements in acidic and neutral soils in temperate and alpine forest sites (sites 4–6). Apparent respiratory quotient (ARQ) values different from the $0.9 \pm 0.1$ 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 May 2001 | 4 | Temperate forest | 4.5 | 85 | 0.46 | 20.40 | 0.58 |
| 31 Jul 2001 | 4 | Temperate forest | 4.5 | 85 | 0.73 | 20.20 | 0.70 |
| 7 Jun 2011 | 5 | Alpine forest | 7.3 | 40 | 0.62 | 19.06 | 0.23 |
| 9 Sep 2013 | 5a | Alpine forest | 7.3 | 30 | 0.25 | 20.69 | 0.60 |
| 9 Sep 2013 | 5b | Alpine forest | 7.3 | 30 | 0.30 | 20.66 | 0.68 |
| 9 Sep 2013 | 6a | Alpine forest | 4.9 | 30 | 0.31 | 20.75 | 1.01 |
| 9 Sep 2013 | 6b | Alpine forest | 4.9 | 30 | 0.27 | 20.80 | 1.17 |
| 9 Sep 2013 | 6c | Alpine forest | 4.9 | 30 | 0.22 | 20.76 | 0.70 |





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**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 time (h) | $CO_2$ (%) | $O_2$ (%) | ARQ | dissolution corrected ARQ |
|---|---|---|---|---|---|---|---|
| 5–20 cm | 6 | 3.95 | 16.45 | 2.43 | 18.19 | 0.87 | 0.88 |
| 5–20 cm | 6 | 3.95 | 32.78 | 3.85 | 16.49 | 0.85 | 0.87 |
| 5–20 cm | 6 | 147.40 | 25.60 | 1.17 | 19.49 | 0.77 | 0.79 |
| 5–20 cm | 6 | 147.40 | 44.80 | 2.08 | 18.35 | 0.78 | 0.80 |
| 5–20 cm | 6 | 337 + sucrose | 5.15 | 4.22 | 16.32 | 0.90 | 0.92 |
| 5–20 cm | 6 | 337 + sucrose | 22.37 | 15.21 | 2.37 | 0.82 | 0.83 |
| 30–40 cm | 6 | 7.65 | 12.75 | 1.3 | 19.42 | 0.82 | 0.85 |
| 30–40 cm | 6 | 7.65 | 29.08 | 2.3 | 18.15 | 0.81 | 0.83 |
| 30–40 cm | 6 | 36.73 | 18.02 | 1.57 | 18.3 | 0.58 | 0.60 |
| 30–40 cm | 6 | 36.73 | 45.62 | 2.86 | 16.36 | 0.61 | 0.63 |
| 30–40 cm | 6 | 147.40 | 25.60 | 0.71 | 19.88 | 0.63 | 0.65 |
| 30–40 cm | 6 | 147.40 | 44.80 | 1.16 | 19.22 | 0.65 | 0.67 |
| 30–40 cm | 6 | 337 + sucrose | 5.15 | 4.39 | 14.88 | 0.72 | 0.74 |
| 30–40 cm | 6 | 337 + sucrose | 22.37 | 14.78 | 2.18 | 0.79 | 0.81 |
| 5–20 cm | 5 | 6.78 | 5.25 | 1.36 | 19.1 | 0.71 | 0.93 |
| 5–20 cm | 5 | 6.78 | 12.75 | 2.48 | 17.36 | 0.68 | 0.89 |
| 5–20 cm | 5 | 146.53 | 25.60 | 1.1 | 18.99 | 0.54 | 0.71 |
| 5–20 cm | 5 | 146.53 | 44.80 | 2.01 | 17.54 | 0.58 | 0.76 |
| 5–20 cm | 5 | 337 + sucrose | 5.15 | 1.38 | 19.06 | 0.71 | 0.95 |
| 5–20 cm | 5 | 337 + sucrose | 22.37 | 7.63 | 12.72 | 0.92 | 1.24 |





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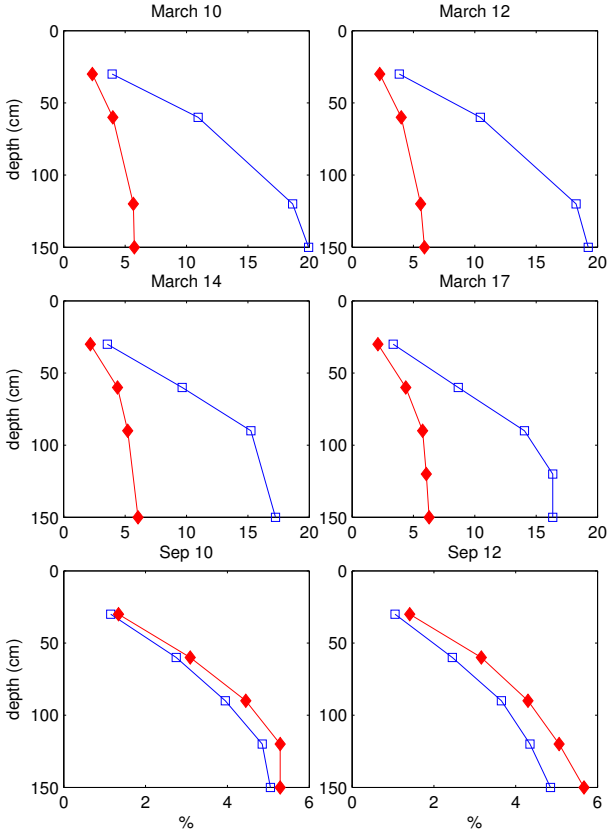


**Figure 1.** Temporal variations in depth profiles of $-\Delta O_2$ (open blue squares, $O_2$ decrease from ambient) and $0.76\Delta CO_2$ (red diamonds, $CO_2$ increase above ambient corrected for lower gas diffusivity compared to $O_2$) profiles in the soil of site 1 (citrus orchard). The March experiment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





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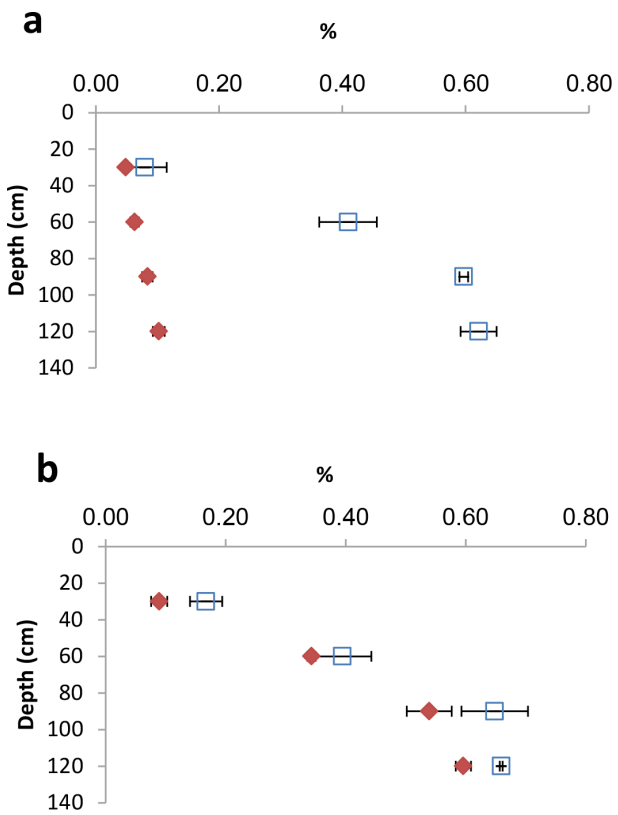


**Figure 2.** The $-\Delta O_2$ (open blue squares, $O_2$ decrease from ambient) and $0.76\Delta CO_2$ (red diamonds, $CO_2$ increase above ambient corrected for lower gas diffusivity compared to $O_2$) profiles in the soil of site 2 (semi-arid pine forest) in January **(a)** and April **(b)**. The values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





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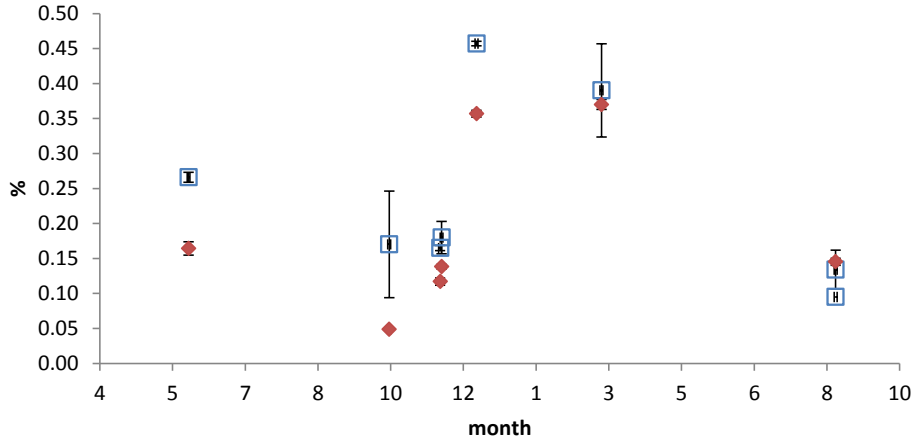


**Figure 3.** Temporal changes in $-\Delta O_2$ (open blue squares, $O_2$ decrease from ambient) and $0.76\Delta CO_2$ (red diamonds, $CO_2$ increase above ambient corrected for lower gas diffusivity compared to $O_2$) at the soil of site 3 (pine stand; 40 cm depth) from May 2012 to August 2013. Most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





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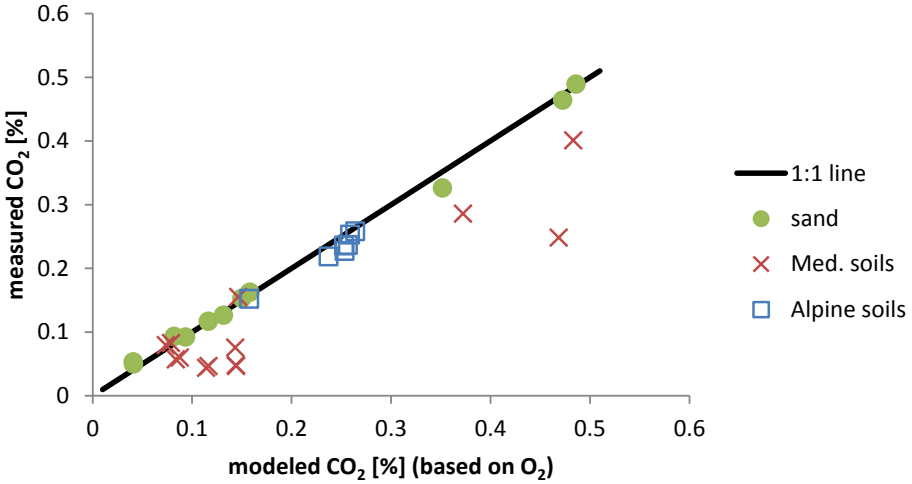


**Figure 4.** Diffusion in gamma-sterlized soils. For sand and alpine soils the measured $CO_2$ agrees well with the values caluclated from $O_2$ concetration and the know diffusivity ratio, but this was not the case for Mediterranean soils, where measured $CO_2$ concentrations were lower than expected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





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