Studying Soil and Tree Stem Respiration in Mediterranean oak forest using the Respiratory Quotient

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Abstract

Forests exchange CO and O with the atmosphere at similar molar ratios. Correspondingly, the apparent respiratory quotient (CO/O flux ratio, ARQ) is expected to be [?]1 given the stoichiometry of organic substrates in soils and plants. However, measured ARQ values often deviate from [?]1, and it is still unclear how CO and O fluxes are balanced among ecosystem components, and what are the sources of ARQ variability. Here we measured ARQ of soil pore space air (ARQ), and in headspace air from incubations of bulk-soil (ARQ), tree stem-cores (ARQ) and roots in 10 measurement campaigns over 15 months in a Mediterranean oak forest. Mean (range) values were: ARQ = 0.76 (0.60-0.92), ARQ = 0.75 (0.53-0.90), and ARQ = 0.39 (0.19-0.70). As expected, ARQ was usually higher than ARQ and lower than the ARQ of incubated roots (range of 0.73-0.96). Variability in ARQ was correlated with soil moisture parameters. Temperature positively correlated with ARQ and ARQoutside the growing season. Abiotic O uptake by Fe was demonstrated to reduce ARQ, but this effect would be significant under field conditions only if respiration rates are very low. We hypothesize that low measured ARQ values likely result from selective decomposition of reduced compounds and physical protection of oxidized compounds. ARQ, measured at two stem positions, was lower than expected from oxidation of any possible substrate, indicating partial retention of respired C. The overall ARQ <1 reveals an imbalance of stem-soil CO and O fluxes that is unexpected at the ecosystem level.

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Key Points:

- The ratio of CO_2/O_2 fluxes in respiration (ARQ) depends mainly on the oxidation state of the substrate
- We measured remarkably lower than expected ARQ in tree stems and soil respiration
- Selective decomposition of reduced compounds and physical protection of oxidized compounds are plausible explanations for low soil ARQ

Abstract

Forests exchange CO_2 and O_2 with the atmosphere at similar molar ratios. Correspondingly, the apparent respiratory quotient (CO_2/O_2 flux ratio, ARQ) is expected to be ≈1 given the stoichiometry of organic substrates in soils and plants. However, measured ARQ values often deviate from ≈1, and it is still unclear how CO_2 and O_2 fluxes are balanced among ecosystem components, and what are the sources of ARQ variability. Here we measured ARQ of soil pore space air (ARQ_{sa}), and in headspace air from incubations of bulk-soil (ARQ_{bs}), tree stem-cores (ARQ_{ts}) and roots in 10 measurement campaigns over 15 months in a Mediterranean oak forest. Mean (range) values were: ARQ_{sa} = 0.76 (0.60-0.92), ARQ_{bs} = 0.75 (0.53-0.90), and ARQ_{ts} = 0.39 (0.19-0.70). As expected, ARQ_{sa} was usually higher than ARQ_{bs} and lower than the ARQ of incubated roots (range of 0.73-0.96). Variability in ARQ_{sa} was correlated with soil moisture parameters. Temperature positively correlated with ARQ_{bs} and ARQ_{sa} outside the growing season. Abiotic O_2 uptake by Fe^{2+} was demonstrated to reduce ARQ_{bs}, but this effect would be significant under field conditions only if respiration rates are very low. We hypothesize that low measured ARQ_{bs} values likely result from selective decomposition of reduced compounds and physical protection of oxidized compounds. ARQ_{ts}, measured at two stem positions, was lower than expected from oxidation of any possible substrate, indicating partial retention of respired C. The overall ARQ <1 reveals an imbalance of stem-soil CO_2 and O_2 fluxes that is unexpected at the ecosystem level.

Plain Language Summary

Respiration by plants and soils are among the most important processes in terrestrial ecosystems, both oxidizing organic compounds using O_2 and emitting the resulting CO_2 to the atmosphere. However, our understanding of this process is still incomplete. Here we measured the ratio of CO_2 released to O_2 consumed, termed the apparent respiration quotient (ARQ), to investigate respiration in tree stems and soils in a Mediterranean forest. ARQ measurements are rarely made, but can provide valuable information about the chemistry of the respiratory substrates, and about additional processes that involve CO_2 and O_2. The expected substrates in tree stems and soils yield ARQ ≈1; however, we measured considerably lower values. Soil respiration is mainly the sum of respiration by roots and by the microbes that decompose the soil organic carbon. The low ARQ values in the soil can be explained if microbes decompose preferentially compounds with low amounts of oxygen, which is surprising. No substrates can produce low ARQ values as those we measured in stem core incubations, indicating another process at work. CO_2 and the O_2 fluxes in the stem-soil system were not balanced as expected, which means we do not fully understand the respiration processes in different ecosystem components.

1 Introduction

The oxidative ratio (OR) is commonly used to describe the net O_2/CO_2 molar exchange between the terrestrial biosphere and the atmosphere. Direct estimates of OR using gas measurements in and above forests canopies are rare. In temperate forests OR averaged over diel and annual cycles range between 0.94 and 1.10 [*M O Battle et al.*, 2019; *Ishidoya et al.*, 2013; *Seibt et al.*, 2004; *Stephens et al.*, 2007]. The OR of an ecosystem is stoichiometrically related to the oxidation state of the organic C (C_{ox}) in the system [*Masiello et al.*, 2008], and meta-analysis of C_{ox} of soil organic carbon (SOC) and vegetation estimated the OR of the terrestrial biosphere at 1.04 ±0.03 [*Worrall et al.*, 2013]. In global scale, the OR is important for terrestrial C sink estimations that are based on the relative changes of O_2 and CO_2 concentrations in the atmosphere [*M Battle et al.*, 2000; *Keeling and Shertz*, 1992].

The inverse term of the OR is the respiratory quotient (RQ = 1/OR), the ratio of CO_2 produced to O_2 consumed during ecosystem processes associated with respiration and decomposition (microbial and soil heterotroph respiration). The RQ value of a given substrate is determined from its stoichiometry (C_{ox}) required for complete respiration. The more oxidized (higher C_{ox}) the compound, the fewer moles of O_2 are consumed per mole of CO_2 released, and the RQ is higher. Accordingly, the expected RQ values for representative chemical groups are 0.73 for lipids, 0.88 for lignin and amino acids, 0.95 for soluble phenolics, 1.0 for carbohydrates, and 1.4 for organic acids [*Masiello et al.*, 2008]. The apparent RQ (ARQ) has been defined as the ratio between CO_2 efflux and O_2 influx in isolated components of the larger ecosystem such as soils and tree stems [*A. Angert and Sherer*, 2011; *A Angert et al.*, 2015]. The term 'apparent' is used since processes other than respiration may exert control on the measured fluxes. In contrast to the "average" ecosystem that exchanges CO_2 and O_2 with the atmosphere at similar rates (i.e. OR ~1), ARQ determined for soil and tree stems usually have values that demonstrate an imbalance CO_2 and O_2 fluxes.

Several studies have reported a range of ARQ values, with many <1.0. For example, ARQ has been estimated from the difference in the ratio of CO_2/O_2 in soil air pore space compared to overlying air, corrected for diffusivity differences. Soil air ARQ (ARQ_{sa}) ranged from 0.58-0.70 in a temperate forest, 0.70-0.89 in a Mediterranean forest, 0.83-1.14 in a tropical forest, while in alpine and non-calcareous semi-arid soils lower values of 0.23-0.30 were measured [*A. Angert et al.*, 2012; *A Angert et al.*, 2015; *Hicks Pries et al.*, 2019; *Sanchez-Canete et al.*, 2018]. This large observed variability is attributed to the fact that a number of processes influence the soil pore space CO_2 and O_2 and therefore the ARQ_{sa} value. These include: heterotrophic respiration, that can be approximated by incubating bulk root-free soil (ARQ_{bs}), root/rhizosphere respiration (ARQ_{root}), and additional processes in the soil that incorporate CO_2 and/or O_2 like abiotic O_2 uptake, oxidation of organic matter using alternative electron acceptors like Fe^{+3}, and CO_2 dissolution/degassing.

The ARQ of soil heterotrophic respiration (ARQ_{bs}) is expected to range between 0.77-1.11 based on the meta-analysis of soil organic matter C_{ox} [*Worrall et al.*, 2013]. However, values of 0.27-0.94 measured previously from a variety of natural ecosystems and agricultural lands are mostly below these expected values [*A Angert et al.*, 2015; *Aon et al.*, 2001a; b; *O. Dilly*, 2001; 2003; *Oliver Dilly and Zyakun*, 2008; *Severinghaus*, 1995]. Carbohydrates with ARQ = 1.0 are the main substrate in plant respiration [*Hoch et al.*, 2003; *Masiello et al.*, 2008; *Plaxton and Podestá*, 2007], and ARQ_{root} values reported range is between 0.79 and 1.4 [*Hawkins et al.*, 1999; *Rachmilevitch et al.*, 2006; *Shane et al.*, 2004]. ARQ_{root} values greater

than 1.0 were explained by nitrate assimilation that consumes electrons otherwise delivered to O_2 [*Bloom et al.*, 1989; *Lambers et al.*, 2008; *Rachmilevitch et al.*, 2006], or by protein and lipid synthesis in the roots themselves or in the associated mycorrhiza, since the conversion of carbohydrates to more reduced compounds result in ARQ >1.0 [*De Vries et al.*, 1974; *Hawkins et al.*, 1999; *Shane et al.*, 2004]. The ARQ associated with respiration in the rhizosphere depends on the composition of the root exudates, which vary greatly [*Bais et al.*, 2006]; ARQ will be above 1.0 when exudates are dominated by organic acids and below 1.0 when dominated by amino acids.

Lower than expected ARQ_{bs} values might be explained by preferential respiration of more reduced compounds if they are cycled faster than the bulk SOC. However, simple thermodynamic calculations suggest that more oxidized compounds should release energy more easily and therefore more favorable for decomposition [*LaRowe and Van Cappellen*, 2011]. Processes other than respiration taking place in soils can also affect ARQ_{bs} and ARQ_{sa} values. Enhanced O_2 uptake derived from abiotic oxidation of reduced species like Fe^{2+} and Mn^{2+} increases the denominator of the ARQ ratio and thus decreases its value. The opposite effect on ARQ is expected during anoxic conditions when oxidized Fe^{3+} and Mn^{3+} are used as an alternative electron acceptors. In that case, CO_2 is respired without any O_2 uptake, and the numerator of the ARQ ratio increases. Anoxic conditions may exist within soil aggregates even in aerated soils [*Druschel et al.*, 2008; *Hall and Silver*, 2013; *Sexstone et al.*, 1985], but become more important after soil wetting as diffusion in water is slower by orders of magnitude than diffusion in air, and when respiration rates are high and O_2 replenishment in microsites cannot meet respiratory needs. Storage of respired CO_2 as dissolved inorganic carbon (DIC) in the soil water can also lower the measured ARQ_{sa}, with greater effect when soil water has high pH. However, if the DIC does not leach, the CO_2 is expected to degas back to the soil pore space when the soil is dried. In calcareous soils, mainly in arid and semi-arid regions, large ARQ_{sa} deviations are expected due to precipitation and dissolution of carbonates [*A Angert et al.*, 2015; *Benavente et al.*, 2010; *Cuezva et al.*, 2011; *Emmerich*, 2003; *Ma et al.*, 2013]. Reduction in ARQ_{sa} can also be the result of dissolution of root-respired CO_2 in the xylem water and its transport to above ground tissues [*Aubrey and Teskey*, 2009]. Dark fixation of CO_2 by the microbial community is another process that can lower ARQ in the soil, but with maximum fixation rates of ~5% of total respiration it not likely large enough to be a significant effect [*Miltner et al.*, 2005].

The ARQ for tree stem tissues (ARQ_{ts}) is expected to be 1.0 since local respiration is assumed to utilize mainly carbohydrates. However, the mean ARQ_{ts} measured as fluxes at the stem surface of tropical, temperate, and Mediterranean trees was found to be 0.59 [*A. Angert et al.*, 2012; *Hilman et al.*, 2019]. Dissolution and transport of respired CO_2 via the xylem water stream is thought to influence the CO_2 efflux measured from tree stems [*Teskey et al.*, 2008] and, as O_2 is much less soluble, should result in low ARQ values in the same way as for dissolution in soils. However, CO_2 transport was found to have only a minor role in explaining low ARQ_{ts} [*Hilman et al.*, 2019]. An alternative hypothesis for lower than expected ARQ_{ts} values is non-phototrophic CO_2 fixation by the enzyme phosphoenolpyruvate carboxylase (PEPC) [*Hilman et al.*, 2019], which was found to be highly abundant in young tree stems [*Daniel Berveiller and Damesin*, 2007; *D. Berveiller et al.*, 2007]. PEPC is involved in biosynthesis of compounds more oxidized than carbohydrates e.g. organic acids [*Lambers et al.*, 2008]. According to the hypothesis, the fact that ARQ_{ts} never exceeded the value of 1.0 (the result of catabolism of

oxidized compounds) is the export of the oxidized compounds, potentially as root exudates to the soil in which organic acids are important constituent.

Recently, *Hicks Pries et al.* [2019] found strong seasonality in ARQ_{sa} in western US forest conifer stand with summer vs. winter values of 0.89 ±0.01 and 0.70 ±0.02, respectively. The seasonal variation was assumed to reflect changes in respiratory substrates, with switching dominance between root-based respiration of more oxidized compounds during summer and bulk-soil-based respiration of more reduced compounds during winter. In order to better resolve the source of variability in ARQ_{sa} we performed seasonal measurements in ~1.5 months intervals of ARQ_{sa}, ARQ_{bs}, and ARQ_{ts} in a Mediterranean oak forest with soil pH <7. In such seasonal measurements the abiotic oxidation of reduced species and temporal storage of CO_2 as DIC, which are expected to lower ARQ_{sa} temporarily, should be mirrored by high ARQ during anoxia and CO_2 release from the soil water DIC. Therefore, the mean ARQ_{sa} value over one year of measurements should provide a better estimate of the respiration-related ARQ and the gas exchange with the atmosphere.

To test the degree to which ARQ_{sa} reflects root respiration and decomposition sources, we used incubations of excised roots and root-free bulk soil. We expected ARQ_{sa} to be higher than ARQ_{bs} as was hypothesized by [*Hicks Pries et al.*, 2019]. We further predicted that if low ARQ_{ts} values are the result of organic acid production and their export to the soil as root exudates, ARQ_{ts} will vary with stem height, and have lower values close to the soil surface. Further, we predicted that ARQ_{sa} will be inversely related to ARQ_{ts}. To test this, ARQ_{ts} was measured near the ground (20 cm) and at breast height (130 cm).

Apart from the seasonal observations, we conducted additional experiments to investigate the three following questions: 1) what is the potential of Fe^{2+} and Mn^{2+} oxidation to reduce ARQ_{bs}? 2) Are compounds with lower ARQ_{bs} decomposed preferentially because of lower energy requirements than higher ARQ_{bs} compounds? And 3) Can ARQ be used for partitioning the contributions of soil organic matter decomposition and root respiration? To address question 1) we conducted two experiments. In the first we compared ARQ_{bs} and the concentrations of the reduced species Fe^{2+} and Mn^{2+} under anaerobic conditions and after re-oxygenation. We also conducted a drying-rewetting experiment where changes in ARQ_{bs} and $[Fe^{2+}]$ were tracked. For answering 2) we performed soil incubations at different temperatures. According to the 'C quality theory' [*Bosatta and Ågren*, 1999], we expected that at lower temperatures (lower available energy) the compounds with more accessible chemical energy will be decomposed preferably. The same theory predicts that 'recalcitrant' compounds with less accessible energy are more sensitive to temperature and have higher values of the temperature coefficient Q_{10} [*Bosatta and Ågren*, 1999; *Fierer et al.*, 2005]. To address 3) we compared ARQ_{sa} with ARQ_{bs} and ARQ_{root}, expecting ARQ_{sa} value to fall in between the ARQ values of the two main components of soil respiration.

2 Materials and Methods

2.1 Study site

The study was conducted in Odem Forest, located 950 m a.s.l, 33°13' N, 35°45' E. The climate is Mesic Mediterranean with a mean annual precipitation of 950 mm and summer and winter mean temperature of 21.3° C and 7.3°C, respectively. The dominant tree species are the evergreen *Quercus calliprinos* Webb (about 75% of the woody cover area) and the winter-

deciduous *Quercus boissieri* Reut. (15%) [Kaplan and Gutman, 1996]. *Q. calliprinos* is the dominant tree in the Mediterranean scrub in Israel, while *Q. boissieri* grows above altitudes of 500 m a.s.l [Kaplan and Gutman, 1996]. The soil was formed on basaltic bedrock and is classified as Eutric Lithosol in the FAO classification system and as Lithic Xerorthent in the USDA classification system. The soil pH is 6.6 and the organic C content is 12% [Gross and Angert, 2017].

2.2 Experimental design

2.2.1 Seasonal measurements

Seasonal sampling took place in ten campaigns between February 2017 and May 2018. Soil air was sampled from 1/2" (OD) stainless steel tubes closed at the bottom end, and perforated near the bottom, that were hammered into the soil. The samples of soil air were collected from a depth of 15 ±4 cm 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 a two-way valve. A total of 120 samples were taken near each tree species (2 replicates x 2 samples x 3 trees x 10 campaigns). Since sampling caused some disturbance to the soil and the stem (see below), every tree that was sampled was marked so that each tree was only sampled once. ARQ_{sa}, the CO_2 efflux/O_2 uptake in soil respiration, was calculated from the measured gases concertation using the following equation [*A Angert et al.*, 2015]:

$$ARQ = 0.76 \times \Delta CO_2/\Delta O_2 \quad (1)$$

where ΔCO_2 and ΔO_2 are the difference in [CO_2] and [O_2] between the soil and the atmosphere. The term 0.76, the CO_2/O_2 diffusivity ratio in air [*Massman*, 1998], corrects to the CO_2 diffusional-enrichment in the soil that is expected in the assumed steady-state conditions. The 0.76 term will cause over-correction and too low ARQ_{sa} when advection of atmospheric air into the soil pore space is dominant. For this reason we avoided sampling in days with high wind speeds.

Surface soil from a depth of 0-10 cm was collected with a trowel and stored in a plastic bag. A total of 30 samples were taken near each tree species by pooling from two places near each tree (3 trees x 10 campaigns). Soil moisture was measured gravimetrically on ~3 g subsamples (available only for the last 6 campaigns). For bulk soil incubation experiments, the soil was sieved to 2 mm (except on January 2018 sampling, when the soil was too wet and sticky to allow sieving), and a subsample of 3 g was incubated overnight in 6 mL glass, 12 mm OD test tubes connected to ~3.6 mL glass flasks by Ultra-Torr fittings (Swagelok, Solon, OH, USA). The gas in the headspace had initial mean atmospheric values (20.95% O_2, 0.04% CO_2). Incubations were conducted usually two days after soil collection, at room temperature. In March 2018 samples of *Q. calliprinos* coarse roots (< 1 cm in diameter) were incubated under the same conditions as the soil.

For estimating ARQ_{ts} we performed stem tissue incubations. This method was shown to give similar ARQ values as the stem-chamber method for the oak Quercus ilex and for two tropical tree species [Hilman et al., 2019]. We decided to incubate only the phloem and cambium tissues, since they are the most metabolically active tissues in the stem [*Bowman et al.*, 2005; *M. L. Pruyn et al.*, 2002a; *Michele L. Pruyn et al.*, 2002b], and since transport in the phloem is the

pathway for C to flow from the stem to the roots. Cores of the outermost stem layers were extracted using a 1.0 cm diameter cork borer, at 20 cm and 130 cm above the soil surface. A total of 60 samples were taken from each tree species (2 stem positions x 3 trees x 10 campaigns). We removed from the cores the outer bark and sapwood sieves, and further cut the cores to fit into the 3.6 mL glass flask neck. For the incubations, we plugged the neck with a rubber stopper to create a gas-tight headspace with initial mean atmospheric values. The incubations started immediately after harvesting and lasted 3-4 hours at environmental temperatures. Metabolism in stem cores changes rapidly after harvesting; in a previous study, an increase of ARQ_{ts} within 32 h from 0.4 to values closer to 1.0 while respiration rates were maintained was interpreted as evidence for gradual inhibition of PEPC activity by its own products [Hilman et al., 2019]. To observe temporal change in ARQ_{ts} the tissues were re-incubated 24 hours after harvesting (ARQ_{ts24}) for the same duration at room temperature. After their collection, the stem tissues were wrapped with moist gauze cloth to avoid desiccation and kept in the dark to prevent possible photosynthesis.

The ARQ_{bs}, ARQ_{ts}, and ARQ_{ts24} were calculated by the ratio between $[CO_2]$ and $[O_2]$ net percent changes in the incubation headspace. Bulk soil O_2 uptake (nmole O_2 g.DW-1 min-1) was calculated using the equation:

$$O_2 \text{ uptake} = \frac{\Delta O_2 \times V_{HS} \times BP}{t \times M \times I_t \times 8.314 \times 10^{-3}} \quad (2)$$

where ΔO_2 is net percent decrease in $[O_2]$ during the incubation, V_{HS} is the volume of the headspace (mL), BP is the local barometric pressure (hPa), t is the temperature (k), M is the soil dry weight (g), I_t is the incubation time (min), and 8.314×10^{-3} is the ideal gas constant (mL hPa k^{-1} $nmol^{-1}$). Soil samples were oven-dried (105^{o}C, 24 h) for dry weights. Soil temperature was measured by a thermocouple, and for barometric pressure we used data from nearby stations.

We also report ARQ_{bs} values corrected for CO_2 dissolution since the large volume of water in the bulk soil samples, especially in soils collected during winter, and the fairly high pH value for non-calcareous soil (6.6) are expected to cause to some of the respired CO_2 to convert into DIC. For calculating the absolute amount of DIC we used the $[CO_2]$ in the headspace, the soil pH, the carbonate system equilibrium constants for fresh water, and the amount of water in the sample. When soil moisture data were unavailable, we estimated its value from the relation between the available soil moisture data and rainfall in the last 3 weeks. We assumed the DIC at the beginning of the incubation was in steady-state with atmospheric $[CO_2]$ of 0.04%. The net change in the calculated DIC was converted to gaseous CO_2 and added to the measured $[CO_2]$.

2.2.2 Evaluation of temperature effect on bulk soil ARQ and comparison with roots

During January 2019 we conducted additional sampling at the site for comparing ARQ_{sa}, ARQ_{bs}, and ARQ_{root}, and for estimating temperature sensitivity of ARQ_{bs} and bulk soil respiration. For that purpose, soils near three additional trees from each species were sampled. Soil temperatures at the site ranged between 6-8^{o}C. Fine roots (<2 mm), which are known to have the highest respiration rates among root diameters [*Chen et al.*, 2010; *Desrochers et al.*, 2002; *Pregitzer et al.*, 1998], were excavated from each tree. Soil was washed thoroughly from one subsample of roots before incubation, while a second subsample was incubated with the surrounding soil intact, to test the effect of the surface microbial communities on the respiratory

fluxes. Roots were incubated shortly after harvesting in the dark in a set-up of two 3.6 mL glass flasks connected by Ultra-Torr fitting, and kept at ~7^{o}C to represent field respiration rates. Since we expected low respiration rates incubations lasted 24 h. Bulk soil incubations were conducted at temperatures of 6, 22, and 30^{o}C and lasted 68-90 h. The Q_{10}, the factor by which respiratory flux rises with a 10^{o}C increase, was calculated using the function Q_{10} from the R package respirometry that fits the measured fluxes (R) at given temperatures (T) with the equation $R=a\times e^{(b\times T)}$ and then calculates $Q_{10}=e^{(10\times b)}$. We also present ARQ_{bs} values for soils sampled in March and May 2018, when soil temperatures were 1°C and 22^{o}C, respectively.

2.2.3 Evaluation of the effect of abiotic O_2 uptake on bulk soil ARQ

Two additional soil incubation experiments were undertaken to investigate the potential for abiotic O_2 uptake to affect ARQ. In the first experiment we tested the response to temporary anaerobic conditions with un-screened soils (for maintaining their structure) sampled in the same campaigns. Three 1 L Mason jars with a small volume of soil (~150 ml) and three jars with large soil volume (~550 ml) were incubated for 13 days, to create low O_2 concentrations. Headspace [O_2] was measured by the end of the incubation, and soils were sampled for [Fe^{2+}] determination. The soils were then ventilated for 1.5 hours, before an overnight incubation. Air and soil samples were taken again at the end of this incubation for headspace [O_2], [CO_2], and [Fe^{2+}] and [Mn^{2+}] concentrations in the soil. The soil moisture during this experiment was 31% by weight. The soil [Fe^{2+}] was measured by the Ferrozine method [*Liptzin and Silver*, 2009]. The soil samples were sieved to 2 mm, and extracted by 0.5 M HCl immediately at the end of the incubation experiments. The soil [Mn^{2+}] was measured by assuming that HCl-extractable Mn, which was quantified by ICP (7500cx Agilent technologies, Santa Clara, CA, USA), predominantly represents Mn^{2+} [*Keiluweit et al.*, 2018].

In a second experiment we tracked ARQ_{bs} during a wetting-drying cycle, and measured [Fe^{2+}] and soil moisture during the soil drying. Ultra-Torr Tee fittings (Swagelok, Solon, OH, USA) were used for the incubation, connecting a test-tube with soil, a test-tube with a drying-agent (magnesium perchlorate), and a 3.6 ml flask equipped with Louwer™ O-ring high-vacuum valve. We incubated 2 mm sieved soil and un-sieved soil. After each incubation the flask was closed and removed, the system was ventilated for 1 hour and then new flask was attached. The first incubation was used to determine the basal ARQ_{bs} and respiration rate (O_2 uptake). The soil was then dried for 17 days, wetted, and dried again for 26 days. Soil wetting was roughly equivalent to a rainfall event of 20 mm. The destructive Fe^{2+} and soil moisture measurements during the soil drying were done for the sieved soil, after re-wetting it to the same degree. We report the relative respiration rate (RR) as the ratio between the O_2 uptake in each incubation to the basal rate.

2.3 Gas analysis

The [O_2] and [CO_2] of the air samples were measured in the laboratory by a closed system (The *Hampadah* [*Hilman et al.*, 2019]). This system is based on two analyzers: an infra-red gas analyzer (IRGA) for CO_2 measurement (LI 840A LI-COR; Lincoln, NE, USA) and a fuel-cell based analyzer (FC-10; Sable Systems International, Las Vegas, NV, USA) for measuring O_2, and is fully automated.

For measuring [CO_2] and [O_2] from the Mason jars we equipped each lid with a septum. Air from the headspace was sampled by plastic syringe with needle and injected to a flow-

316 through CO_2 (K33 ICB 30% CO_2 Sensor, CO_2 Meter, Inc) and O_2 (Fibox 3, PreSens-Precision
317 Sensing) sensors, connected by plastic tubing. The O_2 sensor is a quenching based optical fiber
318 (optode) that reads the fluorescence from a sensing "spot". We placed the “spot” in a 3 mm clear
319 plastic aperture in an opaque lid of a custom-made 2-cm diameter flow-through cell, which made
320 from 4 mm thick aluminum base (to stabilized the temperature). From the outside of the aperture
321 a connector for the optical fiber that reads the "spot" fluorescence was fixed. The same air was
322 injected to pre-evacuated ~3.6 mL glass flasks for comparison with the "*Hampadah*" method.

323 **2.3 Statistical analysis**

324 For comparison of ARQ and O_2 uptake rate values between the two tree species in tree
325 stem, roots, and soil, as well as between sampling heights in tree stems and soil moisture, we
326 performed One-way analysis of variance (ANOVA) and a *t*-test, after assuring homogeneity of
327 variances using Bartlett’s test. For unequal variances, we used a Welch’s test and nonparametric
328 comparisons with Wilcoxon method. Significant differences were determined at $P < 0.05$. In
329 addition, we tested the relations between ARQ_{sa}, ARQ_{bs}, and bulk-soil O_2 uptake rate with
330 meteorological data (soil temperature at 10 cm depth and precipitation courtesy of El Rom
331 metrological station www.meteo-tech.co.il/golan/golan_en.asp) and soil moisture, using
332 backward selection technique for multiple regressions, including estimates of the interactions
333 between each two factors. We used linear regressions not only to evaluate the relationship of
334 dependent and independent variables, but also to describe correlations between ARQ_{ts} values
335 measured in different stem positions and with ARQ_{sa}. All statistical analysis was done using JMP
336 (JMP®, JMP Pro 13, SAS Institute Inc., Cary, NC, USA).

337 **3 Results**

338 **3.1 Results of the seasonal measurements**

339 The seasonal ARQ measurements are presented in Figure 1. The overall mean ±SE values
340 (range of means per species per date) of ARQ_{sa}, ARQ_{bs} (raw values, i.e without correction for
341 CO_2 dissolution in water), ARQ_{ts}, and ARQ_{ts24} were respectively 0.76 ±0.02 (0.60-0.92), 0.65
342 ±0.02 (0.47-0.80), 0.39 ±0.03 (0.19-0.70), and 0.68 ±0.04 (0.42-1.08). The dissolution correction
343 for the ARQ_{bs} values increased the mean value and range to 0.72 (0.51-0.88), and weighted mean
344 of the corrected ARQ_{bs} (using O_2 uptake rates for weighting) increased the mean value and range
345 even more to 0.75 (0.53-0.90). From this point on in the paper ARQ_{bs} values will refer to values
346 corrected for CO_2 dissolution.

347

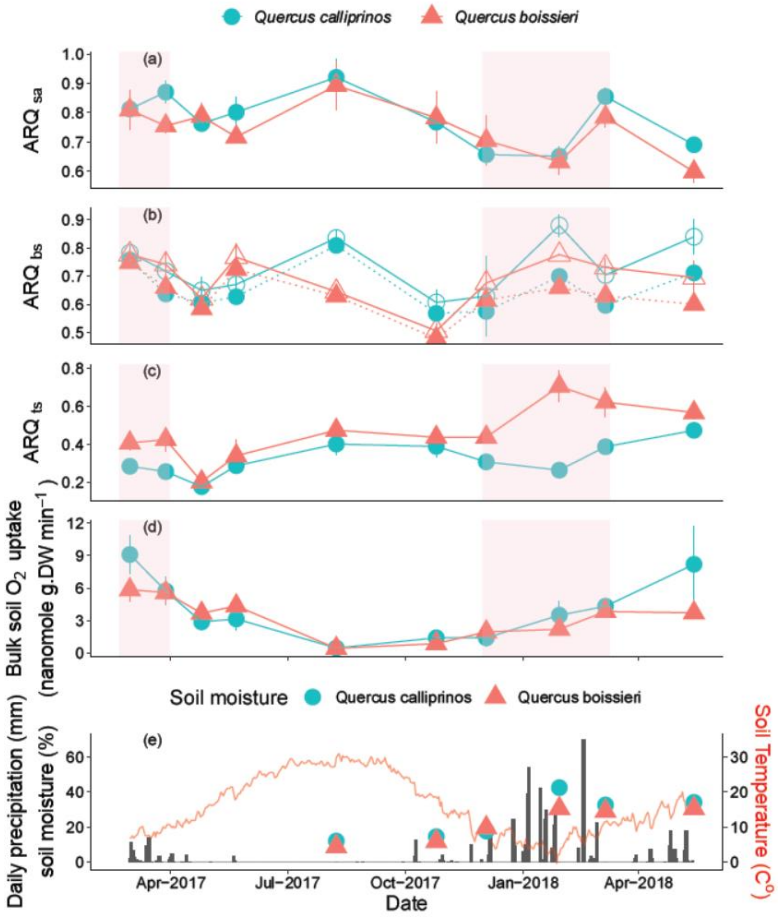

Figure 1 - Time series of (a) ARQ (the ratio of CO_2 efflux/O_2 uptake) measured for soil air in depth of 15 ±4 cm (ARQ_{sa}), (b) ARQ measured from bulk soil incubation (ARQ_{bs}, empty markers; dashed lines are the dissolution corrected values), (c) the mean ARQ measured for incubated stem cores containing the tissues phloem and cambium (ARQ_{ts}) sampled 20 and 130 cm above ground, (d) the O_2 uptake rate of the incubated bulk soils, (e) daily precipitation (black bars) and soil temperature (blue line) measured by adjacent meteorological station and the soil moisture in the site. Shaded periods indicate winter dormancy of the deciduous *Q. boissieri*. Soil sampling was conducted underneath the trees. Error bars represents standard errors.

Differences in ARQ_{ts} between the tree species were observed (Fig. 1c). The ARQ_{ts} ±SE values of the deciduous *Q. boissieri* during winter exfoliation were higher than the evergreen *Q. calliprinos* values in both 20 cm (0.47 ±0.04 vs. 0.32 ±0.02) and 130 cm (0.56 ±0.05 vs. 0.27 ±0.02) above the soil (P = 0.0193 and 0.0102, respectively, t test), while in the foliated period no significant differences were observed between species at 20 cm (0.39 ±0.04 vs. 0.38 ±0.04) and 130 cm (0.43 ±0.04 vs. 0.31 ±0.04) above the soil, respectively (P = 0.9261 and 0.2345, t test). Averaged for the whole sampling period, ARQ_{ts} was higher in *Q. boissieri* than *Q. calliprinos* at 130 cm (0.49 ±0.04 vs. 0.29 ±0.02) according to the Wilcoxon test (P = 0.0072). ARQ in the tree stems increased with incubation time (Fig. 2); immediately after harvesting the overall ARQ_{ts} means ±SE were 0.46 ±0.02 and 0.32 ±0.02 for the *Q. boissieri* and *Q. calliprinos*, respectively, while after 24 h, ARQ_{ts24} values had increased to 0.68 ±0.04 and 0.67 ±0.03, respectively.

Correlations were found in ARQ_{ts} and ARQ_{ts24} between the two stem positions (Fig. 2). The slopes of the relations were closely maintained in the later incubation and differed between species, where the slopes of the *Q. boissieri* were 1.1 and those of *Q. calliprinos* were 0.65-0.69 (Fig. 2).

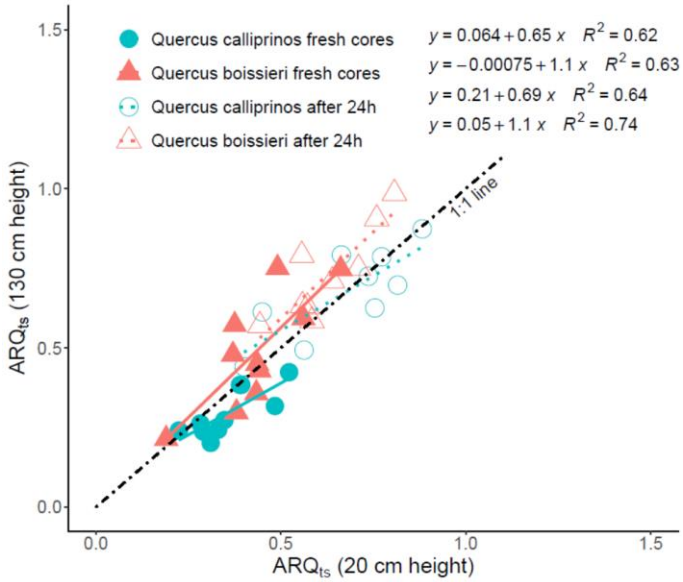

Figure 2. Scatter plot of ARQ (ratio of CO2 efflux/O2 influx) measured from incubated stem cores containing the tissues phloem and cambium (ARQ_{ts}) sampled 130 and 20 cm above ground at the main trunks of the tree species *Quercus calliprinos* and *Quercus boissieri*. Each point represents the mean of three trees measured in each campaign. Filled symbols represent values of incubations started immediately after harvesting, and empty symbols represent incubation started 24 h after harvesting. The *P* values of the correlations are 0.0068, 0.0064, 0.00972, and 0.0028 ordered as appears in the legend.

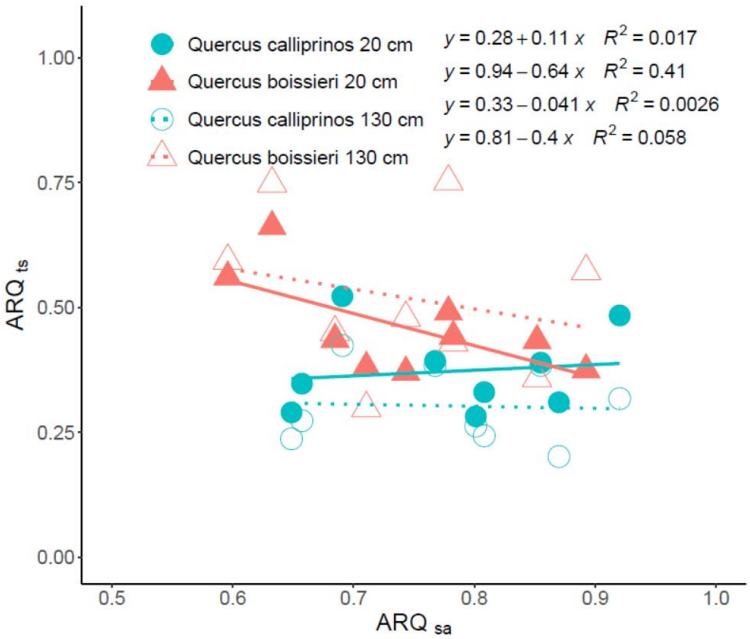

Figure 3. Scatter plot of stem ARQ (ratio of CO2 efflux/O2 influx) measured from incubated stem cores containing phloem and cambium tissues (ARQ_{ts}) sampled 20 (filled symbols) and 130 cm (hollow symbols) above ground from the main trunks of the tree species *Quercus calliprinos* and *Quercus boissieri*, against soil air ARQ measured below the same trees. Each point represents the mean value of three trees (stems and underlying soils) measured in each campaign. The *P* values of the correlations are 0.7393, 0.0618, 0.8973, and 0.533 ordered as appears in the legend.

No significant difference was found using the *t* test between species in ARQ_{ts24} (P = 0.6645), ARQ_{sa} (P = 0.457) and in ARQ_{bs} (P = 0.232), but the weighted mean ARQ_{bs} of the *Q. calliprinos* was higher than *Q. boissieri* (0.77 vs. 0.72; P = 0.0593). Inverse correlation with marginal significance was found between the *Q. boissieri* ARQ_{ts} at 20 cm above the ground and ARQ_{sa} (R^2=0.41, P = 0.0618) after excluding 1 outlier point out of 10 (measured in April 2017 when ARQ_{ts} was minimal, Fig. 3).

Concentrations of CO_2 and O_2 in the soils in single tube samplings ranged from 0.17 - 2.25% and 20.79 - 18.14%, respectively. The lowest O_2 concentrations were measured during January 2018 after 163 mm of precipitation over the previous 3 weeks. For ARQ_{sa}, the water related parameters of soil moisture (M, available for the last 6 out of 10 campaigns), the number of days elapsed since the last rain event (D), and accumulated rain in the 3 weeks prior to sampling (R) were found to have the strongest effects in the backward selection technique for multiple regression. A reciprocal effect was found between the last two factors. The statistical model is defined by the equation:

$$\mathrm{ARQ}_{sa} = 0.471\mathrm{M} + 0.023\mathrm{D} + 0.004\mathrm{R} + (\mathrm{D} - 18) \times (\mathrm{R} - 58.543) \times 3 \times 10^{-4} + 0.241 \quad (3)$$

With P = 0.0002 using F test, with an overall R^2 of 0.94 for the correlation between the actual and predicted soil ARQ. ARQ_{sa} increased with soil temperature, but the effect of this parameter is relatively small testing the whole sampling period and its addition to the prediction formula had a minor contribution to the coefficient of determination. The individual effects of M and T on ARQ_{sa}, and their inter-correlation, are presented in Figure 4. However, when omitting from the analysis data collected during late winter and spring and including only data from May 2017 – Jan 2018, ARQ_{sa} is found to be strongly dependent on temperature (R^2 = 0.92, P < 0.0001). The relation is given by the linear equation: $ARQ_{sa} = 0.01 \times T + 0.6$. No correlations were found between the physical parameters tested and ARQ_{bs}. We did observe a trend of higher ARQ_{bs} with higher bulk-soil O_2 uptake rates, especially during the months when growth is highest (February-May, Fig. 5).

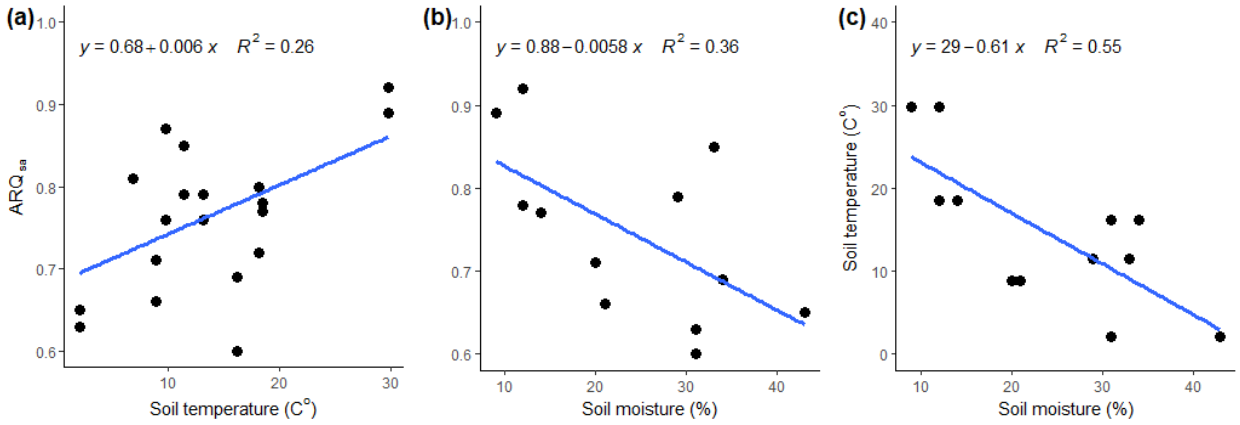

Figure 4. Linear regressions of ARQ (ratio of CO_2 efflux/O_2 influx) measured in soil air (ARQ_{sa}) with (a) soil temperature (°C) and (b) gravimetric soil moisture (%). ARQ_{sa} values were measured 15 ±4 cm deep in soils underneath *Quercus calliprinos* and *Quercus boissieri* trees. Each point represents mean ARQ_{sa} measured from three trees from the same species in one date. (c) linear regression between soil temperature and soil moisture. *P* values are 0.021, 0.039, and 0.006 respectively.

Figure 5. Scatter plot of ARQ (the ratio of CO_2 efflux/O_2 uptake) measured from bulk soil incubations (ARQ_{bs}) and the O_2 uptake rate of the incubated bulk soils, grouped by the month of sampling.

The bulk-soil O_2 uptake rate showed a strong seasonal cycle, with maximal rates during spring (March-May) and minimal rates during the end of the summer (August-October) (Fig. 1d). The uptake rates of the two species were linearly correlated (R^2 = 0.80, *P* = 0.001), and no significant difference was found between the species (*P* = 0.766, *t* test). A reciprocal effect on

435 bulk-soil O_2 uptake rate (nanomole O_2 g.DW s-1) was found between M and T. The effect is
436 described by the following equation:

437 $$O_2 \text{ uptake} = 0.291\text{M} + 1.5 \times 10^{-3}\,\text{T} + (\text{T} - 14.5) \times \big((\text{M} - 0.241) \times 0.001\big) - 0.047 \quad (4)$$

438 Equation (4) predicts actual respiration rates well (R^2 = 0.94; $P < 0.0001$). A significant
439 linear relation was found also between bulk-soil O_2 uptake and the number of days elapsed since
440 the last rain event (R^2 = 0.4, P = 0.005). Adding this effect to the prediction formula does not
441 improve R^2 which remains 0.94 (no reciprocal effect was found in relation to this parameter). A
442 correlation coefficient R^2 of 0.75 (P = 0.0003) was calculated while assuming M is the only
443 driving factor of bulk-soil O_2 uptake.

444 **3.2 Bulk soil ARQ increases with temperature**

445 We incubated soils sampled underneath both tree species at 6°, 22°C and 30°C. The soils
446 sampled underneath the *Q. calliprinos* were moister than under the *Q. boissieri* (P = 0.0001, *t*
447 test, Fig. 5d), and had higher ARQ_{bs} values at 22°C (0.82 ±0.01 vs. 0.77 ±0.04) and 30°C (0.82
448 ±0.01 vs. 0.74 ±0.02, P = 0.0089 and 0.0006 respectively, *t* test), but not at 6°C (0.60 ±0.05 vs.
449 0.58 ±0.08, P = 0.3553, *t* test, Fig. 5a). The *Q. calliprinos* soils had higher CO_2 and O_2 fluxes
450 than the *Q. boissieri* soils at 6°C (P = 0.0076 for both in *t* test), 22°C (P = 0.0023 and 0.0020,
451 Welch test), and 30°C (P = 0.0001 for both in *t* test), and had greater sensitivity to temperature
452 (higher Q_{10} values, Fig. 5b,c). The relation of ARQ_{bs} and temperature according to results of both
453 species was best explained by a logarithmic fit (R^2 = 0.78) with the equation:

454 $$\text{ARQ}_{\text{si}} = 0.13 \times \ln(\text{t}_{\text{incubation}}) + 0.36 \quad (5)$$

455 Where $t_{incubation}$ is the temperature in which the incubation took place (C°). Additional
456 incubations at 1°C yielded average ARQ_{bs} of 0.40 versus 0.65 for the same soils 22°C (Fig. 5a).

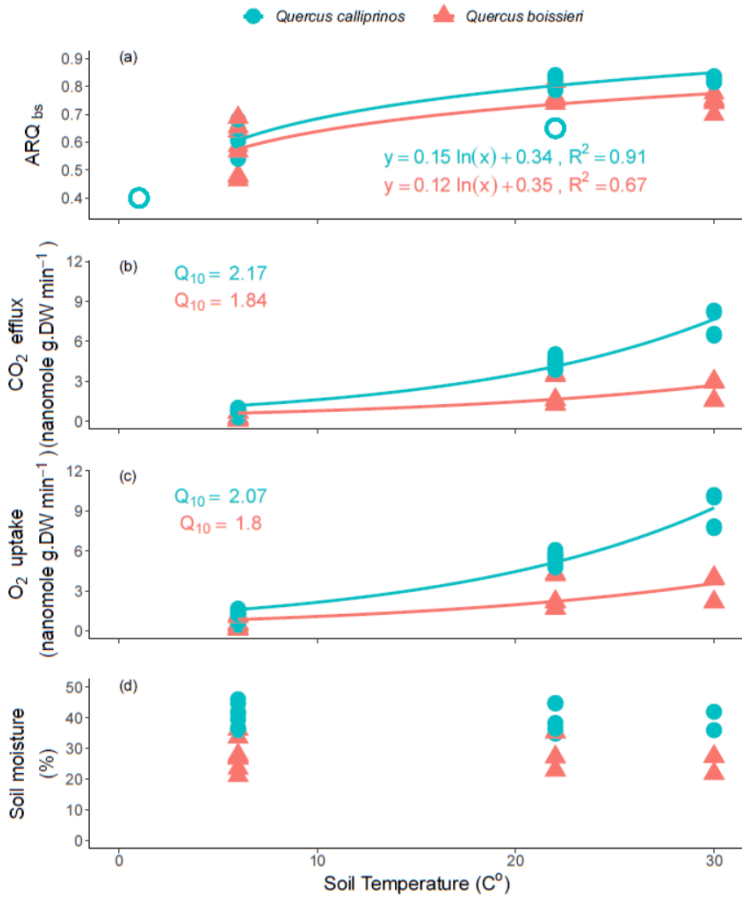

Figure 6. Results from bulk soil incubations at different temperatures. Filled symbols represent soils collected on January 29th 2019 underneath three trees from each species with two soil samples per tree (n=6). Empty symbols represent soils collected on March 6th and May 14th 2018. (a) ARQ_{bs} (ratio of CO_2 efflux/O_2 uptake) with logarithmic fit, (b) the CO_2 efflux rates (after CO_2 dissolution correction) and the calculated temperature coefficient Q_{10}, (c) the O_2 uptake rates and the calculated Q_{10} values, and (d) the gravimetric moisture of the soils.

3.3 Comparison of ARQ in bulk soil, roots, and soil air

No difference in ARQ was found between the washed and non-washed roots collected in January 2019 (P = 0.9863, t test) therefore we polled all root data together. The mean ARQ_{root} per species per date ranged between 0.73-0.96 (Fig 7a). In the direct comparisons, ARQ_{sa} values were always lower than ARQ_{root} and above ARQ_{bs} (Fig. 7a). Assuming that root and microbial respiration are the only end members affecting the soil pore space, their relative contributions could be estimated using a simple mixing mode, where $ARQ_{sa} = X \times ARQ_{root} + (1 - X) \times ARQ_{bs}$ (Fig. 7a). The mean and maximal contributions of roots to the total respiration based on this calculation are 44% and 65%, respectively. With the mixing model we further calculated ARQ_{root} for the seasonal measurements assuming fixed root respiration contributions of 44% and 65%, according to the measured ARQ_{sa} and temperature-corrected ARQ_{bs} (Fig. 7b). Since soil incubations in the seasonal sampling were conducted in room temperature, we added an empirical correction that takes into account the soil temperature in the field, on top of the CO_2

dissolution correction. For the calculation we averaged values of both species. First the intercept term b from Eq. 5 was modified:

$$b = 0.36 - (ARQ_{t=room} - ARQ_{bs\_measured}) \quad (6)$$

Where 0.36 is the calculated intercept as appears in Eq. 5, $ARQ_{t=room}$ is the expected ARQ according to Eq. 5 and the room temperature (varied slightly between measurements), and $ARQ_{bs\_measured}$ is the measured ARQ in the bulk-soil incubation, corrected to CO_2 dissolution. The bulk soil ARQ values reported in Figure 7b were calculated with the equation:

$$ARQ = 0.13 \times ln(t_{field}) + b \quad (7)$$

Where t_{field} is the temperature measured in the field.

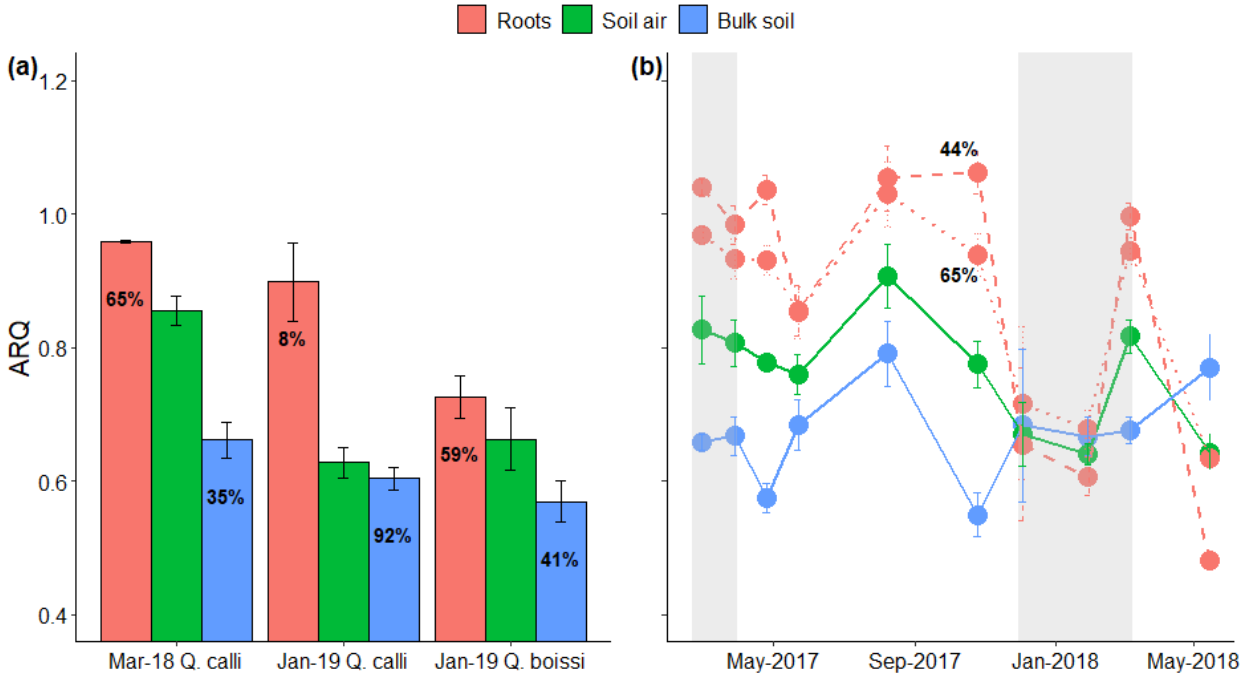

Figure 7. (a) A comparison of ARQ (ratio of CO_2 efflux/O_2 uptake) measured from root incubations (n=2, 6, 6), soil air (n=3), and bulk soil incubations (n=3), corrected for dissolution of CO_2 in soil water. Error bars are standard errors. The x axis indicates the date of sampling and the tree species. The relative contributions of roots and bulk-soil respiration to the total soil respiration are indicated. The contributions were calculated using the equation $ARQ_{sa} = X \times ARQ_{root} + (1 - X) \times ARQ_{bs}$. (b) The seasonal course of ARQ means of both tree species, where the bulk soil values are temperature corrected, and the roots values are calculated with the above equation assuming root contributions of 44% and 65% (mean and maximum of panel a). Shaded periods indicate winter dormancy of the deciduous *Q. boissieri*. Error bars are standard errors.

3.4 Relation between ARQ_{bs} and Fe^{2+}

The $[CO_2]$ and $[O_2]$ determined by the sensors in the jars experiment were highly consistent with the *Hampadah* measurement (R^2 of 0.997 and 0.975 in linear regression with slopes of 1.01 and 1.01, respectively). After the first 13-days of incubation the average $[O_2]$ ±SD

of the incubation jars with the large and small soil volumes were 0.90 ±0.44% and 7.25 ±0.07%, respectively (n = 3). In agreement, [Fe^{2+}] in the jars with large soil volume was higher than measured for the small soil volume jars (0.89 ±0.24 vs. 0.05 ±0.01 mg g^{-1} soil, respectively). In the subsequent incubation, performed after ventilation of 1.5 hours aimed to increase the [O_2] in the jars, a sharp decrease in [O_2] was observed in the large soil volume jars from an ambient value of 20.95% to value of 4.77 ±0.21%, while ARQ was 0.37 ±0.01. The [Fe^{2+}] dropped from 0.89 ±0.24 to 0.21±0.04 mg g^{-1} soil. The [Mn^{2+}] was 1.27 mg g^{-1} soil, and did not significantly change during this incubation. In the small soil volume jars the [O_2] decreased from 20.95% to 19.80 ±0.26%, ARQ was 0.74 ±0.02, and [Fe^{2+}] did not change from the initial value of 0.05 mg g^{-1} soil. Taking into account the different soil volumes, the rate of O_2 uptake was 2.9-fold faster in the large soil volume jars than in the small soil volume jars.

The soil wetting-drying experiment induced variations in ARQ_{bs}, RR, and [Fe^{2+}] (Fig. 8). RR peaked in the day of soil wetting and then gradually decreased. Following the soil wetting ARQ_{bs} increased during 11 days from 0.63-0.69 to 0.79-0.80 and then decreased during 15 days to 0.46. [Fe^{2+}] values of ~0.14 mg g^{-1} were measured during the first 13 days after soil wetting, at soil moisture values of 14.4%-7.7%. After the 13th day [Fe^{2+}] decreased to 0.10 mg g^{-1} in the 34th day and to 0.05 mg g^{-1} in the 46th day.

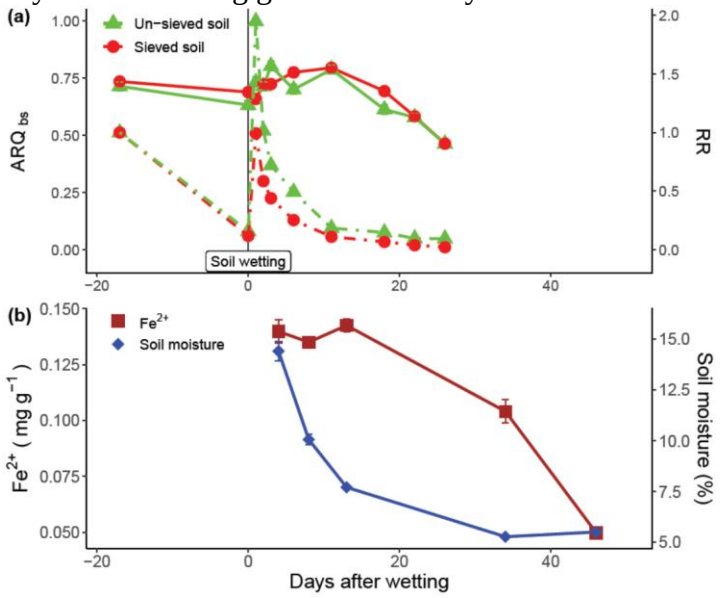

Figure 8. Results from soil drying-rewetting experiment. The day of the rewetting is day 0. (a) ARQ_{bs} (ratio of CO_2 efflux/O_2 uptake) in solid lines and relative respiration rate (RR) in dashed lines for un-sieved and sieved (2 mm) soils. Each data point represents one measurement without replicates. (b) The concentration of Fe^{2+} (mg g^{-1}) and the gravimetric moisture of the sieved soil. Following the experiment presented in panel a, the same sieved soil was wetted to the same moisture. Each data point represents mean of duplicate sub-samples taken from the drying soil. Error bars are the standard deviations.

4 Discussion

4.1 Bulk soil ARQ is affected by redox only at low respiration rates

Soil incubations are often used to isolate and study the heterotrophic (or microbial) contribution to soil respiration. The overall mean for our bulk soil incubations (ARQ_{bs}) was affected by dissolution of CO_2 in soil water, increasing from 0.65 (uncorrected) to 0.72 (corrected). The greatest corrections occurred during the second and rainier winter, when soil moisture was higher and therefore the storage capacity of the DIC in the soil was higher (Fig. 1b). Thus, even for soils with pH of 6.6, dissolution of CO_2 in the soil water can be significant for CO_2 flux calculations. In addition, the sensitivity of ARQ_{bs} to temperature (Fig. 6) indicates that care needs to be taken to either make incubations at the field temperature, or use an empirical temperature correction.

The overall weighted mean of dissolution-corrected ARQ_{bs} is 0.75, with mean values per species per campaign ranging between 0.53 to 0.90, well within the 0.27-0.94 range of previous ARQ_{bs} and equivalent assessments [*A Angert et al.*, 2015; *Aon et al.*, 2001a; b; *O. Dilly*, 2001; 2003; *Severinghaus*, 1995]. Once corrected for dissolution effect, the ARQ_{bs} value is primarily controlled by the elemental composition of the SOM consumed in respiration, although additional effects from anaerobic respiration and abiotic oxidation of reduced species, were also assessed.

The anaerobic/aerobic jar incubations confirmed that abiotic oxidation of Fe^{2+} in the soil can reduce measured ARQ_{bs}. In soils recovering from anaerobic conditions ($[O_2]$ ~1%) ARQ_{bs} was 0.37 ±0.01 while the value for the control soils ($[O_2]$ ~7%) was 0.74 ±0.02, similar to the seasonal temperature-corrected ARQ_{bs} values of 0.68-0.77 measured at the time of soil sampling (March and May 2018, Fig 7b). The concentrations of $[Fe^{2+}]$ decreased sharply in the soils recovering from anoxia, in parallel to enhanced O_2 uptake. In contrast, in the control soils $[Fe^{2+}]$ value was 0.05 mg g^{-1} throughout the experiment. The same concentration was measured at the end of the drying-rewetting experiment, suggesting that 0.05 mg g^{-1} is the basal level of $[Fe^{2+}]$ in the site's soil. Mn^{2+} oxidation did not play a role in the studied soils. For the soils recovering anaerobic conditions, the stoichiometry for the overall oxidation of Fe^{2+} ions by O_2, $O_{2(aq)} + 4Fe^{2+} + 6H_2O \leftrightarrow 4FeOOH_{(s)} + 8H^+$ [*Burke and Banwart*, 2002], explains 27% of the drop in $[O_2]$, another third of the O_2 uptake can be explained by faster oxidation of soil organic matter that usually follows anaerobic conditions (e.g. [*Keiluweit et al.*, 2017]), while the last third can be explained by the same microbial respiration as in the control soils. As the low O_2 jars in our experiments were nearly anoxic, the ARQ_{bs} reduction from 0.74 to 0.37 seems to represent the maximal effect of Fe^{2+} oxidation for the site. However, the important question is: how important this Fe^{2+} oxidation effect is under field conditions?

In the soil drying-rewetting experiment the decrease in ARQ_{bs} values in the 11^{th} day after soil wetting seems to be the result of Fe^{2+} oxidation that occurred with similar timing (Fig. 8). However, the ARQ_{bs} decrease occurred when respiration rates were slow. We estimated that the amount of O_2 decrease due to Fe^{2+} oxidation, which is equivalent to the amount of alternative oxidants during anaerobic respiration, is less than 10% of the O_2 flux when respiration rates were higher. Thus we conclude that abiotic O_2 consumption is significant at this site only at low respiration rates. Indeed, the lowest ARQ_{bs} values were measured for incubations with the lowest O_2 uptake rates (Fig. 1, 8a).

4.2 Bulk soil ARQ indicate that more reduced compounds dominate microbial respiration sources

The weighted mean value of ARQ_{bs} (0.75) probably averaged over much of the seasonal anaerobic/abiotic O_2 effects, and therefore it provides good estimate for the mean microbial substrate in Odem forest. According to SOC-C_{ox} values summarized in a meta-analysis study, the mean value of ARQ_{bs} we measured (0.75) is appreciably below 0.95, the median value, and slightly below 0.77, the minimum value measured for humic substances in mollisols [*Worrall et al.*, 2013]. The striking difference between the observed and expected values indicates that the microbial metabolism in the site relies on more reduced compounds than the mean SOM. In agreement, Rock-Eval indices show increase in C_{ox} (higher oxygen index and lower hydrogen index) with soil depth [*Sebag et al.*, 2016], with aging of bare fallow [*Barré et al.*, 2016], and with experimental soil warming [*Poeplau et al.*, 2019]. These gradients are somewhat analogues to soil maturation, and indicate that compounds richer with H (low ARQ) are preferably decomposed, enriching the remaining SOC with O. There are two possible explanations for the faster decomposition of compounds with low ARQ: 1) the oxidized compounds are not accessible for microbial decomposition or 2) the microbial community selects to consume reduced over oxidized compounds. The correlation of ARQ with soil moisture in the drying experiment might be related to accessibility, and the temperature effect on ARQ_{bs} provides some evidence for selectivity, but only to a limited degree.

According to the 'C quality theory' recalcitrant compounds require more enzymatic steps for decomposition [*Bosatta and Ågren*, 1999]. Each enzymatic step has its characteristic activation energy, thus a greater number of steps requires greater total activation energy. Temperature increases reduce the enzymatic activation energy and stimulate decomposition of compounds with high activation energy (i.e. less-decomposable compounds), faster than more labile compounds with lower activation energy. This greater sensitivity of recalcitrant compounds to temperature is reflected by higher values of the temperature coefficient Q_{10} [*Bosatta and Ågren*, 1999; *Fierer et al.*, 2005]. Correspondingly, the ARQ_{bs} increase with temperature presented in Figure 5a suggests substrates with higher ARQ require more energy to decompose. The positive effect of temperature on ARQ_{bs} is consistent with greater ARQ_{sa} measured at 30 cm depth in heated (+4^{o}C) over control soils during winter [*Hicks Pries et al.*, 2019]. Moreover, higher Q_{10} values were measured for the evergreen *Q. calliprinos* soils in comparison with the deciduous *Q. boissieri*, with corresponding higher ARQ_{bs} values under the *Q. calliprinos* (Fig. 6). However, the temperature effect on ARQ_{bs} was observed between 1^{o}C to 22^{o}C, while between 22^{o}C and 30^{o}C the ARQ_{bs} plateaued at ~0.8 (Fig. 6a). This may suggest that energy is not a limiting factor above 22^{o}C, and that a different factor, potentially physical protection, prevents decomposition of compounds with higher C_{ox}.

The drying-rewetting experiment implies that physically protected SOM may be indeed more oxidized, while more reduced compounds are dominating the decomposition flux (Fig. 8). The pulse of CO_2 released after soil wetting is thought to have two main C sources: microbial-C that is released to the soil to adjust cell osmolarity after the sudden wetting and C from SOM rendered accessible to microbes after disruption of soil structure [*Fierer and Schimel*, 2003]. The microbial-C is probably osmolites and short chain molecules that should decompose rapidly [*Fierer and Schimel*, 2003], while the released SOM-C may be more resistant to decomposition [*Degens and Sparling*, 1995]. Accordingly, the relative contribution of the SOM-C to respiration should have increased with time after rewetting. Thus, the observed gradual ARQ_{bs} increase

following the soil rewetting can be interpreted as a shift towards the ARQ value of the SOM rendered accessible. The ARQ_{bs} values measured on the 11th day (0.79-0.80) were higher than the basal 0.72-0.74 values, suggesting the newly accessible SOM was more oxidized than the original mix of SOM contributing to basal respiration. We speculate that similar, naturally occurring, rewetting events not captured in our periodic measurements might release pulses of respiration with increased contribution from compounds with high ARQ. However, the fraction of C respired by such event must be large for the overall ARQ_{sa} to achieve a 'balanced' value of ~1.

This apparent microbial preference for reduced compounds contradicts thermodynamic calculations predicting that oxidized compounds have lower free energy and therefore should be more favored substrates for decomposition [*LaRowe and Van Cappellen*, 2011]. However, the emerging perspective of SOM suggests that decomposability is not only a property of the organic matter itself (e.g. its energy content), but it is a combination of the preference of the decomposers, protection by minerals, O_2 saturation, and environmental drivers [*Keiluweit et al.*, 2017; *Kleber*, 2010; *Lehmann and Kleber*, 2015].

4.2 Low tree stem ARQ cannot be explained by substrate stoichiometry

The ARQ values we measured in tree stems were lower than usually expected, especially as transport is not a factor in a closed incubation. Normally, carbohydrates with ARQ = 1.0 are assumed to dominate respiration substrates for plants [*Hoch et al.*, 2003; *Masiello et al.*, 2008; *Plaxton and Podestá*, 2007]; however, we measured a mean ARQ_{ts} value of 0.39. This value is remarkably below the lipids-respiration ARQ of 0.73, which would be the lowest value expected from any respiration substrate in plants. Extensive lipid usage is not expected in the tree genera *Quercus* [*Hoch et al.*, 2003; *Sinnott*, 1918]. Furthermore, the mean value we measured is in accord with ARQ_{ts} values of 0.33-0.44 measured using the same method for the oak species *Quercus ilex*. These low values were also measured from *Quercus ilex* using stem chambers on intact trees [*Hilman et al.*, 2019].

Such low ARQ_{ts} values are difficult to explain. Damage during the tissue extraction from the stems might result in a burst of O_2 uptake. Observations of H_2O_2 production indicate that it increased the O_2 uptake temporarily, but this effect declined within two hours after epicormic shoots were wounded [*Tian et al.*, 2015]. Hence, a. wound response 24 h after harvesting is likely not important. The overall mean of ARQ measurement 24 h after harvesting (ARQ_{ts24}) was 0.68, lower than values expected even for 100% lipid substrates. We thus conclude that ARQ values are not an artifact of the sampling.

A recently published hypothesis explains the very low ARQ in tree stems as the result of dark fixation of CO_2 by the enzyme PEPC and incorporation of the fixed C into products such as organic acids like citrate and malate that can be exported to other tissues. In this case, the increase of ARQ_{ts} with time of incubation could result from inhibition of this process as those products accumulate [*Hilman et al.*, 2019]. In intact tree stems, malate can be transported in the xylem stream [*Schill et al.*, 1996] and contribute C to photosynthesis in leaves [*Hibberd and Quick*, 2002]. Alternatively, the fixation products might be exported via the phloem to the roots and be secreted to the soil as root exudates [*Hoffland et al.*, 2006; *Shane et al.*, 2004]. Most organic acid catabolism results in ARQ > 1.0 and therefore an increase in rhizosphere ARQ is predicted during their exudation if there is net export of fixed CO_2 from stems to roots.

Apparent evidence for the export of organic acids from stem to soil is found in the inverse relationship (R^2=0.41, P = 0.0618) between ARQ_{ts} at 20 cm and ARQ_{sa} in the underlying soil air observed for the deciduous *Q. boissieri* (Fig. 3). The lowest ARQ_{ts} values and highest ARQ_{sa}, which would in theory correspond with the greatest transport of organic acids, were measured during the foliated period for this species. During defoliation the ARQ_{ts} values of the *Q. boissieri* increased significantly, especially in the second winter that was wetter and colder (Fig. 1c). In contrast, while the evergreen *Q. calliprinos* exhibited almost the same seasonal changes in ARQ_{sa} as the deciduous *Q. boissieri*, its ARQ_{ts} values were rather uniform during the year and similar to the values measured in the foliated period of the *Q. boissieri*. This suggests that variability in ARQ_{sa} may not be related to ARQ_{ts}.

Comparing the seasonal patterns of the two species, low ARQ_{ts} values characterize photosynthetically active trees (Fig. 1c). This observation can support both the hypothesis of transport of CO_2 re-fixation products to photosynthetic sites and the hypothesis that products are transported below ground as root exudates, which is expected to occur when trees are active. However, it refutes the hypothesis that C re-fixation in the stem is primarily a pathway to reduce C losses when C is limited, as during winter dormancy. The relations in ARQ_{ts} values measured at stem heights of 130 and 20 cm indicate additional difference between tree species (Fig. 2). The ARQ values increase with height for *Q. boissieri* and decrease with height for *Q. calliprinos*. It can be speculated that the refixation products of the *Q. boissieri* are delivered to the soil, while in the *Q. calliprinos* the products are delivered to the canopy. Further elucidation of the potential for CO_2 fixation in trees stems requires measurements of PEPC activity and organic acid dynamics in tree stems.

4.3 Seasonality of Soil air ARQ and the potential of ARQ to partition soil respiration

The overall mean of ARQ_{sa} was 0.76 with values per campaign per species in the range of 0.60-0.92. The results are in agreement with the range of 0.23-1.14 measured in time-discrete soil tubes sampling [*A. Angert et al.*, 2012; *A Angert et al.*, 2015; *Hicks Pries et al.*, 2019], and higher than the values of 0.25-0.33 obtained also by tubes in soil depths of 10-60 cm in continuous measurement over one year [*Sanchez-Canete et al.*, 2018]. Our results are lower in comparison to ARQ equivalents of 0.90-1.06 measured using soil chambers [*Ishidoya et al.*, 2013; *Seibt et al.*, 2004], which might be explained by our tube sampling (measured at depths of 15 ±4) not accounting for respiration in the shallower soil horizons and litter layer that dominate the fluxes measured in soil chambers. Another possible reason for our low ARQ_{sa} results is over-correction of the soil atmosphere for CO_2 diffusional-enrichment in the soil. If advective gas exchange between the soil pore space and the atmosphere is dominant in our site, our diffusion correction will result in too low ARQ_{sa} values. Demonstration for that is soil gas transport model that predicts that the diffusion effect on soil pore $\delta^{13}CO_2$ increases with soil depth [*Egan et al.*, 2019]. Accordingly, we would expect similar effect of decrease of CO_2/O_2 with soil depth. However, we did not observe such trend in our results (data not shown). In addition, when gas diffusivity is low, depth play smaller role in the diffusion enrichment [*Egan et al.*, 2019]. Thus, we estimate that diffusional over-correction in the studied soils might happened only for summer and autumn results when soil was dry and diffusivity in the soils was high.

Our results suggest ARQ_{sa} is mainly driven by ARQ_{bs} and ARQ_{root}, with some effect of CO_2 dissolution in the soil water. The seasonal ARQ_{bs} measurements were almost always lower

than ARQ_{sa}, and when ARQ_{root} was measured its values exceeded ARQ_{sa}, implying ARQ_{sa} values are confined between those two end members (Fig. 1, 6). Hence, if CO_2 dissolution or other soil profile processes are known to have minor impact on ARQ_{sa} or can be quantified, the contributions of ARQ_{bs} and ARQ_{root} to ARQ_{sa} can be used to partition soil respiration to the heterotrophic (ARQ_{bs}) and autotrophic (ARQ_{root}) components (Fig. 7). In future studies it is recommended to include the root rhizosphere in the root incubation to better represent respiration derived from root exudates.

Mean winter ARQ_{sa} was 0.75 (measurements during the leafless period of the *Q. boissieri*) and mean summer ARQ_{sa} was 0.90 (August 2017, Fig. 1, 6), very similar to the 0.7-0.9 seasonal range observed at the Sierra-Nevada foothills [*Hicks Pries et al.*, 2019]. In that study it was hypothesized that the seasonal difference is due to shifting dominance between root respiration with more oxidized substrates during summer and bulk-soil respiration with more reduced substrates during winter. The results presented here suggest roots indeed respire more oxidized substrates with higher ARQ than bulk soil (Fig. 7a). However, ARQ_{bs} increased with temperature from 0.57-0.60 at 6^{o}C to 0.74-0.82 at 30^{o}C (Fig. 6), indicating a potential seasonality in ARQ_{bs} as appears from the temperature-corrected ARQ_{bs} values (Fig. 7b). In addition, ARQ_{root} also varied (0.73-0.96, Fig. 7a), demonstrating alteration in the respiratory substrates of both bulk soil and roots, indicating that the end-member values must be determined simultaneously with the bulk CO_2. Previous studies in Mediterranean oak-grass savannas showed that during dry season soil respiration can be dominated by roots [*Casals et al.*, 2011], or by bulk soil [*Tang and Baldocchi*, 2005].

The seasonal ARQ_{sa} values correlated positively with temperature and negatively with soil moisture, with strong auto-correlation between the two variables (Fig. 4). Similar relations were found by *Hicks Pries et al.* [2019], indicating the difficulty of disentangling the effects of temperature and soil moisture on ARQ in ecosystems where temperature and moisture are highly correlated. The backward selection technique we used attempts to resolve this issue, and indicates that on a yearly basis water-related parameters are the main factors controlling the seasonal ARQ_{sa} variability, while temperature has only a minor effect (Eq. 3). In contrast, we observed temperature control of ARQ_{bs} (Fig. 6) and ARQ_{sa} when we omitted late winter and spring from the analysis (the maximum growth period). Considering all observations, it appears that during the high growth period, temperature is a less important driver of ARQ_{sa} variability. A possible explanation is that root exudation and root respiration increase during the high growth period, and this dominates the temperature-related variability in decomposition of the bulk soil organic matter. This understanding matches the conclusion in *Hicks Pries et al.* [2019] that phenology drives the high ARQ_{sa} variability during February-June when soil had high volumetric water content in comparison to lower ARQ_{sa} variability when soil was dry (July-August).

Soil moisture variation can have a direct effect on ARQ_{sa} by dissolving respired CO_2 that otherwise would be released to the soil air. This process can explain the few ARQ_{sa} values that were equal to or lower than ARQ_{bs} during the second and wetter winter (Fig. 1, 6). When DIC-saturated water leaches to the groundwater there is net loss of CO_2, but if the water is taken up by the roots or evaporates, the dissolved respired CO_2 stays in the system. In the area of Odem forest, only 10-30% of annual precipitation (950 mm) leaches to groundwater [*Dafny et al.*, 2006], most of it during episodic intensive rain events during winter. Therefore we estimate the loss of respired CO_2 to groundwater is negligible in yearly scale. DIC uptake by roots is probably not substantial due to anatomical features [*Ubierna et al.*, 2009], and therefore it is most

probable that the dissolved CO_2 is released back to the pore space when the soil dries. Such degassing could explain the spike in ARQ_{sa} observed during the last campaign of the second winter (Fig 1,6).

4.4 Ecosystem CO_2 and O_2 balance

Photosynthesis and respiration are the key processes in the CO_2 and O_2 exchange between forests and the atmosphere. The O_2/CO_2 ratio in photosynthesis (photosynthetic ratio) is theoretically 1.0 when glucose is produced. Laboratory and field incubations of leaves and branches indeed observed photosynthetic ratio values close to 1.0 [*Gauthier et al.*, 2018; *Ishidoya et al.*, 2013], but [*Seibt et al.*, 2004] measured higher values of ~1.2. Higher photosynthetic ratio values can be related to NO_3^- assimilation, where electrons that are usually transferred to CO_2 are transferred to NO_3^- [*Bloom et al.*, 1989]. Assuming the oxidation ratio for biomass is nearly 1.0 [*Masiello et al.*, 2008], the photosynthetic ratio of 1.0 or 1.2 must be balanced by the same overall ratio for ecosystem respiration (i.e. respiration ARQ of 1 or 0.8, respectively). Ecosystem (canopy) measurements of nocturnal O_2 and CO_2 respiration fluxes measured over six years at the Harvard forest indicated an integrated oxidative ratio of 1.12, corresponding to an ecosystem ARQ of 0.89 [*M O Battle et al.*, 2019].

Calculation of the ecosystem-level ARQ from its components requires multiplying the ARQ of each component by its relative contribution to total ecosystem respiration. Unfortunately, we lack information about the respiratory fluxes in Odem forest, but studies in other Mediterranean forests indicate that 56-77% of total ecosystem respiration is from soil, 8-11% from stems, and 12-36% from foliage [*Guidolotti et al.*, 2013; *Maseyk et al.*, 2008; *Wieser et al.*, 2009]. Maintaining an ecosystem ARQ of 1.0 using our mean ARQ values for total soil respiration (0.76) and stem respiration (0.39), the foliage ARQ would have to be between 1.5 and 3.1. While those foliage ARQ values seem implausible, maintaining an ecosystem ARQ of 0.8 would require the foliage ARQ to be between more probably values of 1.0-1.4. ARQ values of 0.9-1.0 are characteristic values for tree branches and leaves [*Hanf et al.*, 2015; *Patterson et al.*, 2018] and assimilation of NO_3^- in barley leaves resulted in an ARQ of 1.51 [*Bloom et al.*, 1989]. These calculations used the arithmetic mean of the seasonal ARQ values rather than the flux-weighted mean, which could introduce bias. Additionally, as discussed above, ARQ_{ts} might be underestimates because of wound response, while ARQ_{sa} measured only at a depth of 15 cm in the soil may underestimate total soil respiration ARQ by missing contributions from respiration in the topmost soil horizons and surface litter. However, tree stem incubation ARQ measured after 24 hours, which was presumably after any wound response ceased, was 0.68, also lower than unity. In another site, monthly measurements of the soil surface ARQ using chamber over three years had a mean value of 0.9, also less than 1.0 (reported as OR of 1.11 ±0.01[*Ishidoya et al.*, 2013]). Given that a number of studies show that soil and stem ARQ values are below 1.0, the mean foliage ARQ must be above 1.0 for the overall ecosystem ARQ to be 1.0. Thus, leaves should on average respire more oxidized compounds than sugars, as is often presumed. Alternatively, the photosynthetic ratio could be greater than 1.0, as would result from significant NO_3^- assimilation and/or photosynthetic re-assimilation of internal C that produces O_2 without an uptake of atmospheric CO_2. The source for the internal C might be respired CO_2 from lower parts of the tree that is transported in the xylem stream to the leaves, as has been suggested in labeling experiments [*Stringer and Kimmerer*, 1993]. Solving this puzzle will require more and comprehensive CO_2 and O_2 measurements of different forest components.

5 Conclusions

We have presented here the first seasonal measurements of ARQ in several of the components of ecosystem respiration: tree stems, root-free bulk soil, and soil pore space air. Almost all the measured ARQ values were lower than would be expected compared to those expected from commonly assumed respiratory substrates. The lowest ARQ were observed in tree stem incubations, with values less than even if all respiratory substrates were lipids. The most plausible explanation is dark refixation of respired CO_2 [*Hilman et al.*, 2019], given that there was no transport affecting our tissue incubations. The ARQ observed for bulk (root-free) soil (ARQ_{bs}), were also less than 1.0, suggesting physical protection of oxidized compounds and preferential decomposition of reduced compounds. The ARQ_{bs} increase with temperature (Fig. 6) suggests according to the 'C quality theory' [*Bosatta and Ågren*, 1999] that compounds with higher ARQ require more energy to decompose. Eleven days after soil wetting ARQ_{bs} increased to higher values than basal values for soils maintaining constant soil moisture, possibly reflecting decomposition of protected SOM rendered more accessible after wetting. We were able to demonstrate an effect of Fe^{2+} oxidation in reducing ARQ_{bs} as hypothesized by [*A Angert et al.*, 2015; *Hicks Pries et al.*, 2019]. However, we found that under field conditions it is likely that this influence is important only when O_2 uptake rates are very low.

Variability in the ARQ over seasons and years indicated a number of the processes controlling ARQ. We found that the ARQ in soil pore space are (ARQ_{sa}) nearly always had values intermediate between bulk soil (ARQ_{bs}) and root (ARQ_{root}) respiration, suggesting that these two endmembers are the main drivers of ARQ_{sa} (Fig. 7) and can be used as a tool to partition soil respiration into soil and root/rhizosphere components. The seasonal variability in ARQ_{sa} was explained by variability in soil water parameters and not by temperature, although strong temperature control occurred during the low growth-period. Given the temperature control on ARQ_{bs}, we can conclude that ARQ_{bs} variability controls ARQ_{sa} variability during the low growth period. On three dates ARQ_{sa} was equal or lower than ARQ_{bs} (Fig. 7b), all of them during the second and rainier season. We estimate that this is the outcome of respired CO_2 dissolving in the soil water and decreased temporarily ARQ_{sa}.

On the ecosystem scale, ARQ_{sa} and ARQ_{ts} provide estimations for the gas exchange between two major components of ecosystem respiration: soil and tree stem respiration. While we observed an inverse correlation between ARQ_{ts} at 20 cm and ARQ_{sa} for one of the two tree species we studied, which support the hypothesis of C transport from tree stems to the soil via roots exudates, the overall ARQ values were below unity. Thus if the overall ecosystem ARQ must be close to 1.0, we hypothesize that the components we did not measure, including the shallow soil horizons, litter or the canopy, must have ARQ greater than 1.0; i.e. greater than expected.

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References

Angert, A., J. Muhr, R. Negron Juarez, W. Alegria Muñoz, G. Kraemer, J. Ramirez Santillan, E. Barkan, S. Mazeh, J. Q. Chambers, and S. E. Trumbore (2012), Internal respiration of Amazon tree stems greatly exceeds external CO_2 efflux, *Biogeosciences*, *9*(12), 4979-4991, doi:10.5194/bg-9-4979-2012.

Angert, A., and Y. Sherer (2011), Determining the relationship between tree-stem respiration and CO_2 efflux by δO_2/Ar measurements, *Rapid Commun Mass Spectrom*, *25*(12), 1752-1756, doi:10.1002/rcm.5042.

Angert, A., D. Yakir, M. Rodeghiero, Y. Preisler, E. Davidson, and T. Weiner (2015), Using O_2 to study the relationships between soil CO_2 efflux and soil respiration, *Biogeosciences*, *12*(7), 2089-2099.

Aon, M. A., D. E. Sarena, J. L. Burgos, and S. Cortassa (2001a), Interaction between gas exchange rates, physical and microbiological properties in soils recently subjected to agriculture, *Soil and Tillage Research*, *60*(3-4), 163-171, doi:10.1016/s0167-1987(01)00191-x.

Aon, M. A., D. E. Sarena, J. L. Burgos, and S. Cortassa (2001b), (Micro)biological, chemical and physical properties of soils subjected to conventional or no-till management: an assessment of their quality status, *Soil and Tillage Research*, *60*(3-4), 173-186, doi:10.1016/s0167-1987(01)00190-8.

Aubrey, D. P., and R. O. Teskey (2009), Root-derived CO_2 efflux via xylem stream rivals soil CO_2 efflux, *New Phytol*, *184*(1), 35-40, doi:10.1111/j.1469-8137.2009.02971.x.

Bais, H. P., T. L. Weir, L. G. Perry, S. Gilroy, and J. M. Vivanco (2006), The role of root exudates in rhizosphere interations with plants and other organisms, in *Annual Review of Plant Biology*, edited, pp. 233-266, Annual Reviews, Palo Alto, doi:doi:10.1146/annurev.arplant.57.032905.105159.

Barré, P., et al. (2016), The energetic and chemical signatures of persistent soil organic matter, *Biogeochemistry*, *130*(1-2), 1-12, doi:10.1007/s10533-016-0246-0.

Battle, M., M. Bender, P. P. Tans, J. White, J. Ellis, T. Conway, and R. Francey (2000), Global carbon sinks and their variability inferred from atmospheric O_2 and $\delta^{13}C$, *Science*, *287*(5462), 2467-2470.

Battle, M. O., et al. (2019), Atmospheric measurements of the terrestrial $O_2 : CO_2$ exchange ratio of a midlatitude forest, *Atmospheric Chemistry and Physics*, *19*(13), 8687-8701, doi:10.5194/acp-19-8687-2019.

Benavente, J., I. Vadillo, F. Carrasco, A. Soler, C. Liñán, and F. Moral (2010), Air Carbon Dioxide Contents in the Vadose Zone of a Mediterranean Karst, *Vadose Zone Journal*, *9*(1), 126-136, doi:10.2136/vzj2009.0027.

Berveiller, D., and C. Damesin (2007), Carbon assimilation by tree stems: potential involvement of phospho*enol*pyruvate carboxylase, *Trees*, *22*(2), 149-157, doi:10.1007/s00468-007-0193-4.

Berveiller, D., J. Vidal, J. Degrouard, F. Ambard-Bretteville, J. N. Pierre, D. Jaillard, and C. Damesin (2007), Tree stem phospho*enol*pyruvate carboxylase (PEPC): lack of biochemical and localization evidence for a C4-like photosynthesis system, *New Phytol*, *176*(4), 775-781, doi:10.1111/j.1469-8137.2007.02283.x.

Bloom, A. J., R. M. Caldwell, J. Finazzo, R. L. Warner, and J. Weissbart (1989), Oxygen and carbon dioxide fluxes from barley shoots depend on nitrate assimilation, *Plant Physiol*, *91*(1), 352-356, doi:10.1104/pp.91.1.352.

Bosatta, E., and G. I. Ågren (1999), Soil organic matter quality interpreted thermodynamically, *Soil Biology and Biochemistry*, *31*(13), 1889-1891, doi:10.1016/s0038-0717(99)00105-4.

Bowman, W. P., M. M. Barbour, M. H. Turnbull, D. T. Tissue, D. Whitehead, and K. L. Griffin (2005), Sap flow rates and sapwood density are critical factors in within- and between-tree variation in CO_2 efflux from stems of mature *Dacrydium cupressinum* trees, *New Phytol*, *167*(3), 815-828, doi:10.1111/j.1469-8137.2005.01478.x.

Burke, S. P., and S. A. Banwart (2002), A geochemical model for removal of iron(II)(aq) from mine water discharges, *Applied Geochemistry*, *17*(4), 431-443, doi:10.1016/s0883-2927(01)00092-0.

Casals, P., L. Lopez-Sangil, A. Carrara, C. Gimeno, and S. Nogués (2011), Autotrophic and heterotrophic contributions to short-term soil CO_2 efflux following simulated summer precipitation pulses in a Mediterranean dehesa, *Global Biogeochemical Cycles*, *25*(3), n/a-n/a, doi:10.1029/2010gb003973.

Chen, D., L. Zhou, X. Rao, Y. Lin, and S. Fu (2010), Effects of root diameter and root nitrogen concentration on in situ root respiration among different seasons and tree species, *Ecological Research*, *25*(5), 983-993, doi:10.1007/s11284-010-0722-2.

Cuezva, S., A. Fernandez-Cortes, D. Benavente, P. Serrano-Ortiz, A. S. Kowalski, and S. Sanchez-Moral (2011), Short-term CO_2(g) exchange between a shallow karstic cavity and the external atmosphere during summer: Role of the surface soil layer, *Atmospheric Environment*, *45*(7), 1418-1427, doi:10.1016/j.atmosenv.2010.12.023.

Dafny, E., A. Burg, and H. Gvirtzman (2006), Deduction of groundwater flow regime in a basaltic aquifer using geochemical and isotopic data: The Golan Heights, Israel case study, *Journal of Hydrology*, *330*(3-4), 506-524, doi:10.1016/j.jhydrol.2006.04.002.

De Vries, F. W. T. P., A. H. M. Brunsting, and H. H. Van Laar (1974), Products, requirements and efficiency of biosynthesis a quantitative approach, *Journal of Theoretical Biology*, *45*(2), 339-377, doi:10.1016/0022-5193(74)90119-2.

Degens, B., and G. Sparling (1995), Repeated wet-dry cycles do not accelerate the mineralization of organic C involved in the macro-aggregation of a sandy loam soil, *Plant and Soil*, *175*(2), 197-203.

Desrochers, A., S. M. Landhausser, and V. J. Lieffers (2002), Coarse and fine root respiration in aspen (Populus tremuloides), *Tree Physiol*, *22*(10), 725-732, doi:10.1093/treephys/22.10.725.

Dilly, O. (2001), Microbial respiratory quotient during basal metabolism and after glucose amendment in soils and litter, *Soil Biology and Biochemistry*, *33*(1), 117-127, doi:10.1016/s0038-0717(00)00123-1.

Dilly, O. (2003), Regulation of the respiratory quotient of soil microbiota by availability of nutrients, *FEMS Microbiol Ecol*, *43*(3), 375-381, doi:10.1111/j.1574-6941.2003.tb01078.x.

Dilly, O., and A. Zyakun (2008), Priming Effect and Respiratory Quotient in a Forest Soil Amended with Glucose, *Geomicrobiology Journal*, *25*(7-8), 425-431, doi:10.1080/01490450802403099.

Druschel, G. K., D. Emerson, R. Sutka, P. Suchecki, and G. W. Luther (2008), Low-oxygen and chemical kinetic constraints on the geochemical niche of neutrophilic iron(II) oxidizing microorganisms, *Geochimica et Cosmochimica Acta*, *72*(14), 3358-3370, doi:10.1016/j.gca.2008.04.035.

Egan, J. E., D. R. Bowling, and D. A. Risk (2019), Technical Note: Isotopic corrections for the radiocarbon composition of CO_2 in the soil gas environment must account for diffusion and diffusive mixing, *Biogeosciences*, *16*(16), 3197-3205, doi:10.5194/bg-16-3197-2019.

Emmerich, W. E. (2003), Carbon dioxide fluxes in a semiarid environment with high carbonate soils, *Agricultural and Forest Meteorology*, *116*(1-2), 91-102, doi:10.1016/s0168-1923(02)00231-9.

Fierer, N., J. M. Craine, K. McLauchlan, and J. P. Schimel (2005), Litter Quality and the Temperature Sensitivity of Decomposition, *Ecology*, *86*(2), 320-326, doi:10.1890/04-1254.

Fierer, N., and J. P. Schimel (2003), A Proposed Mechanism for the Pulse in Carbon Dioxide Production Commonly Observed Following the Rapid Rewetting of a Dry Soil, *Soil Science Society of America Journal*, *67*(3), 798-805, doi:10.2136/sssaj2003.0798.

Gauthier, P. P. G., M. O. Battle, K. L. Griffin, and M. L. Bender (2018), Measurement of Gross Photosynthesis, Respiration in the Light, and Mesophyll Conductance Using $H_2{}^{18}O$ Labeling, *Plant Physiol*, *177*(1), 62-74, doi:10.1104/pp.16.00741.

Gross, A., and A. Angert (2017), Use of ^{13}C-and phosphate ^{18}O-labeled substrate for studying phosphorus and carbon cycling in soils: a proof of concept, *Rapid Commun Mass Sp*, *31*(11), 969-977.

Guidolotti, G., A. Rey, E. D'Andrea, G. Matteucci, and P. De Angelis (2013), Effect of environmental variables and stand structure on ecosystem respiration components in a Mediterranean beech forest, *Tree Physiol*, *33*(9), 960-972, doi:10.1093/treephys/tpt065.

Hall, S. J., and W. L. Silver (2013), Iron oxidation stimulates organic matter decomposition in humid tropical forest soils, *Glob Chang Biol*, *19*(9), 2804-2813, doi:10.1111/gcb.12229.

Hanf, S., S. Fischer, H. Hartmann, R. Keiner, S. Trumbore, J. Popp, and T. Frosch (2015), Online investigation of respiratory quotients in Pinus sylvestris and Picea abies during drought and shading by means of cavity-enhanced Raman multi-gas spectrometry, *Analyst*, *140*(13), 4473-4481, doi:10.1039/c5an00402k.

Hawkins, H.-J., M. D. Cramer, and E. George (1999), Root respiratory quotient and nitrate uptake in hydroponically grown non-mycorrhizal and mycorrhizal wheat, *Mycorrhiza*, *9*(1), 57-60.

Hibberd, J. M., and W. P. Quick (2002), Characteristics of C4 photosynthesis in stems and petioles of C3 flowering plants, *Nature*, *415*(6870), 451-454, doi:10.1038/415451a.

Hicks Pries, C., A. Angert, C. Castanha, B. Hilman, and M. S. Torn (2019), Using Respiration Quotients to Track Changing Sources of Soil Respiration Seasonally and with Experimental Warming, *Biogeosciences Discuss.*, *2019*, 1-20, doi:10.5194/bg-2019-232.

Hilman, B., et al. (2019), Comparison of CO_2 and O_2 fluxes demonstrate retention of respired CO_2 in tree stems from a range of tree species, *Biogeosciences*, *16*(1), 177-191, doi:10.5194/bg-16-177-2019.

Hoch, G., A. Richter, and C. Korner (2003), Non-structural carbon compounds in temperate forest trees, *Plant, Cell and Environment*, *26*(7), 1067-1081, doi:10.1046/j.0016-8025.2003.01032.x.

Hoffland, E., R. Boogaard, J. Nelemans, and G. Findenegg (2006), Biosynthesis and root exudation of citric and malic acids in phosphate-starved rape plants, *New Phytologist*, *122*(4), 675-680, doi:10.1111/j.1469-8137.1992.tb00096.x.

Ishidoya, S., S. Murayama, C. Takamura, H. Kondo, N. Saigusa, D. Goto, S. Morimoto, N. Aoki, S. Aoki, and T. Nakazawa (2013), O_2: CO_2 exchange ratios observed in a cool temperate deciduous forest ecosystem of central Japan, *Tellus B: Chemical and Physical Meteorology*, *65*(1), 21120.

Kaplan, D., and M. Gutman (1996), Effect of Thinning and Grazing on Tree Development and the Visual Aspect of an Oak Forest on the Golan Heights, *Israel Journal of Plant Sciences*, *44*(4), 381-386, doi:10.1080/07929978.1996.10676659.

Keeling, R. F., and S. R. Shertz (1992), Seasonal and interannual variations in atmospheric oxygen and implications for the global carbon cycle, *Nature*, *358*(6389), 723-727, doi:10.1038/358723a0.

Keiluweit, M., K. Gee, A. Denney, and S. Fendorf (2018), Anoxic microsites in upland soils dominantly controlled by clay content, *Soil Biology and Biochemistry*, *118*, 42-50, doi:10.1016/j.soilbio.2017.12.002.

Keiluweit, M., T. Wanzek, M. Kleber, P. Nico, and S. Fendorf (2017), Anaerobic microsites have an unaccounted role in soil carbon stabilization, *Nature communications*, *8*(1), 1771.

Kleber, M. (2010), What is recalcitrant soil organic matter?, *Environmental Chemistry*, *7*(4), 320-332, doi:10.1071/en10006.

Lambers, H., F. S. Chapin, and T. L. Pons (2008), Respiration, in *Plant Physiological Ecology*, edited, pp. 101-150, Springer New York, New York, NY, doi:10.1007/978-0-387-78341-3_3.

LaRowe, D. E., and P. Van Cappellen (2011), Degradation of natural organic matter: a thermodynamic analysis, *Geochimica et Cosmochimica Acta*, *75*(8), 2030-2042.

Lehmann, J., and M. Kleber (2015), The contentious nature of soil organic matter, *Nature*, *528*(7580), 60-68, doi:10.1038/nature16069.

Liptzin, D., and W. L. Silver (2009), Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil, *Soil Biology and Biochemistry*, *41*(8), 1696-1702.

Ma, J., Z. Y. Wang, B. A. Stevenson, X. J. Zheng, and Y. Li (2013), An inorganic CO_2 diffusion and dissolution process explains negative CO_2 fluxes in saline/alkaline soils, *Sci Rep*, *3*, 2025, doi:10.1038/srep02025.

Maseyk, K., J. M. GrÜNzweig, E. Rotenberg, and D. A. N. Yakir (2008), Respiration acclimation contributes to high carbon-use efficiency in a seasonally dry pine forest, *Global Change Biology*, *14*(7), 1553-1567, doi:10.1111/j.1365-2486.2008.01604.x.

Masiello, C. A., M. E. Gallagher, J. T. Randerson, R. M. Deco, and O. A. Chadwick (2008), Evaluating two experimental approaches for measuring ecosystem carbon oxidation state and oxidative ratio, *Journal of Geophysical Research*, *113*(G3), doi:10.1029/2007jg000534.

Massman, W. J. (1998), A review of the molecular diffusivities of H_2O, CO_2, CH_4, CO, O_3, SO_2, NH_3, N_2O, NO, and NO_2 in air, O_2 and N_2 near STP, *Atmospheric Environment*, *32*(6), 1111-1127, doi:10.1016/s1352-2310(97)00391-9.

Miltner, A., F.-D. Kopinke, R. Kindler, D. Selesi, A. Hartmann, and M. Kästner (2005), Non-phototrophic CO_2 fixation by soil microorganisms, *Plant and Soil*, *269*(1-2), 193-203.

Patterson, A. E., R. Arkebauer, C. Quallo, M. A. Heskel, X. Li, N. Boelman, and K. L. Griffin (2018), Temperature response of respiration and respiratory quotients of 16 co-occurring temperate tree species, *Tree Physiol*, *38*(9), 1319-1332, doi:10.1093/treephys/tpx176.

Plaxton, W. C., and F. E. Podestá (2007), The Functional Organization and Control of Plant Respiration, *Critical Reviews in Plant Sciences*, *25*(2), 159-198, doi:10.1080/07352680600563876.

Poeplau, C., P. Barré, L. Cécillon, F. Baudin, and B. D. Sigurdsson (2019), Changes in the Rock-Eval signature of soil organic carbon upon extreme soil warming and chemical oxidation-A comparison, *Geoderma*, *337*, 181-190.

Pregitzer, K. S., M. J. Laskowski, A. J. Burton, V. C. Lessard, and D. R. Zak (1998), Variation in sugar maple root respiration with root diameter and soil depth, *Tree Physiol*, *18*(10), 665-670, doi:10.1093/treephys/18.10.665.

Pruyn, M. L., B. L. Gartner, and M. E. Harmon (2002a), Respiratory potential in sapwood of old versus young ponderosa pine trees in the Pacific Northwest, *Tree Physiol*, *22*(2-3), 105-116, doi:10.1093/treephys/22.2-3.105.

Pruyn, M. L., B. L. Gartner, and M. E. Harmon (2002b), Within-stem variation of respiration in *Pseudotsuga menziesii* (Douglas-fir) trees, *New Phytologist*, *154*(2), 359-372, doi:10.1046/j.1469-8137.2002.00380.x.

Rachmilevitch, S., H. Lambers, and B. Huang (2006), Root respiratory characteristics associated with plant adaptation to high soil temperature for geothermal and turf-type Agrostis species, *J Exp Bot*, *57*(3), 623-631, doi:10.1093/jxb/erj047.

Sanchez-Canete, E. P., G. A. Barron-Gafford, and J. Chorover (2018), A considerable fraction of soil-respired CO_2 is not emitted directly to the atmosphere, *Sci Rep*, *8*(1), 13518, doi:10.1038/s41598-018-29803-x.

Schill, V., W. Hartung, B. Orthen, and M. H. Weisenseel (1996), The xylem sap of maple (*Acer platanoides*) trees—sap obtained by a novel method shows changes with season and height, *Journal of Experimental Botany*, *47*(1), 123-133, doi:10.1093/jxb/47.1.123.

Sebag, D., et al. (2016), Dynamics of soil organic matter based on new Rock-Eval indices, *Geoderma*, *284*, 185-203, doi:10.1016/j.geoderma.2016.08.025.

Seibt, U., W. A. Brand, M. Heimann, J. Lloyd, J. P. Severinghaus, and L. Wingate (2004), Observations of O_2:CO_2 exchange ratios during ecosystem gas exchange, *Global Biogeochemical Cycles*, *18*(4), n/a-n/a, doi:10.1029/2004gb002242.

Severinghaus, J. P. (1995), Studies of the terrestrial O_2 and carbon cycles in sand dune gases and in biosphere 2, Columbia University.

Sexstone, A. J., N. P. Revsbech, T. B. Parkin, and J. M. Tiedje (1985), Direct measurement of oxygen profiles and denitrification rates in soil aggregates, *Soil science society of America journal*, *49*(3), 645-651.

Shane, M. W., M. D. Cramer, S. Funayama-Noguchi, G. R. Cawthray, A. H. Millar, D. A. Day, and H. Lambers (2004), Developmental physiology of cluster-root carboxylate synthesis and exudation in harsh hakea. Expression of phosphoenolpyruvate carboxylase and the alternative oxidase, *Plant Physiol*, *135*(1), 549-560, doi:10.1104/pp.103.035659.

Sinnott, E. W. (1918), Factors determining character and distribution of food reserve in woody plants, *Botanical Gazette*, *66*(2), 162-175.

Stephens, B. B., P. S. Bakwin, P. P. Tans, R. M. Teclaw, and D. D. Baumann (2007), Application of a Differential Fuel-Cell Analyzer for Measuring Atmospheric Oxygen Variations, *Journal of Atmospheric and Oceanic Technology*, *24*(1), 82-94, doi:10.1175/jtech1959.1.

Stringer, J. W., and T. W. Kimmerer (1993), Refixation of xylem sap CO_2 in *Populus deltoides*, *Physiologia Plantarum*, *89*(2), 243-251, doi:10.1111/j.1399-3054.1993.tb00150.x.

Tang, J., and D. D. Baldocchi (2005), Spatial–temporal variation in soil respiration in an oak–grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components, *Biogeochemistry*, *73*(1), 183-207, doi:10.1007/s10533-004-5889-6.

Teskey, R. O., A. Saveyn, K. Steppe, and M. A. McGuire (2008), Origin, fate and significance of CO_2 in tree stems, *New Phytol*, *177*(1), 17-32, doi:10.1111/j.1469-8137.2007.02286.x.

Tian, W. M., S. G. Yang, M. J. Shi, S. X. Zhang, and J. L. Wu (2015), Mechanical wounding-induced laticifer differentiation in rubber tree: An indicative role of dehydration, hydrogen peroxide, and jasmonates, *J Plant Physiol*, *182*, 95-103, doi:10.1016/j.jplph.2015.04.010.

Ubierna, N., A. S. Kumar, L. A. Cernusak, R. E. Pangle, P. J. Gag, and J. D. Marshall (2009), Storage and transpiration have negligible effects on $\delta^{13}C$ of stem CO_2 efflux in large conifer trees, *Tree Physiology*, *29*(12), 1563-1574.

Wieser, G., A. Gruber, M. Bahn, E. Catala, E. Carrillo, M. S. Jimenez, and D. Morales (2009), Respiratory fluxes in a Canary Islands pine forest, *Tree Physiol*, *29*(3), 457-466, doi:10.1093/treephys/tpp008.

Worrall, F., G. D. Clay, C. A. Masiello, and G. Mynheer (2013), Estimating the oxidative ratio of the global terrestrial biosphere carbon, *Biogeochemistry*, *115*(1-3), 23-32, doi:10.1007/s10533-013-9877-6.