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The Apparent Respiratory Quotient of Soils and Tree Stems and the Processes That Control It

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Key Points:

- The ratio of CO_2/O_2 fluxes in respiration (apparent respiration quotient) depends on the substrate stoichiometry and on biotic and abiotic non-respiratory processes
- Bulk-soil ARQ is governed by substrate stoichiometry in high respiration rates and by processes like Fe^{2+} oxidation during low rates
- ARQ in tree stems is lower than expected by carbohydrates respiration and can be explained by re-fixation of respired CO_2

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Abstract The CO_2/O_2 fluxes ratio (apparent respiration quotient [ARQ]) measured in soils and plants contains valuable information about the respiratory-substrate stoichiometry and biotic and abiotic non-respiratory processes. We investigated ARQ variability by measurements in soil pore space air, and in headspace air from incubations of bulk-soil and tree stem tissues (both fresh and 24-hr stored tissues) in 10 measurement campaigns over 15 months in a Mediterranean oak forest. Mean (range) ARQ values were: soil air, 0.76 (0.60–0.92); bulk soil, 0.75 (0.53–0.90); fresh stem tissues, 0.39 (0.19–0.70); and stored stem tissues, 0.68 (0.42–1.08). The variability in tree stems was assumed to be controlled by CO_2 re-fixation that lowered ARQ from 1.0, the value expected for carbohydrate respiration in plants. We estimate that the values of the stored tissues represent better stem metabolism since the fresh-tissue results contained a signal of wound-response O_2 uptake that further lowered ARQ. The mean bulk-soil ARQ (0.75) was considerably lower than expected by soil organic matter (SOM) stoichiometry (0.95). This lower value might represent the stoichiometry of the SOM sub-pool that supports respiration, and/or oxidative depolymerization that increases O_2 fluxes. Abiotic O_2 uptake was demonstrated to reduce bulk-soil ARQ down to 0.37 and consume Fe^{2+}, but estimated to have small effect under typical respiration rates. Soil-air ARQ was usually higher than bulk-soil ARQ and lower than root ARQ (which, when measured, ranged from 0.73 to 0.96), demonstrating the potential of ARQ to partition the autotrophic and heterotrophic sources of soil respiration. The limitations of this partitioning method are discussed.

Plain Language Summary Carbon dioxide is produced by the processes of both metabolic respiration by plants and microbial respiration and decomposition in soils. These 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 these processes, which are often collectively referred to as "respiration" is still incomplete. Here we investigated the use of the measured ratio CO_2 released to O_2 consumed, termed the apparent respiration quotient (ARQ), to investigate respiration in soils and tree stems. 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 cycling in terrestrial ecosystems. The expected substrates (carbohydrates) in tree stems and soils yield ARQ ≈ 1; however, we measured considerably lower values. The low ARQ values in the soil can be explained if microbes decompose compounds with low amounts of oxygen, which is surprising. No substrates can produce ARQ values as low as those we measured in stem core incubations, indicating other processes at work.

1. Introduction

New measurement methods can improve the understating and prediction of ecosystem processes. An emerging tool in biogeochemical studies is the coupled measurement of CO_2 and O_2 fluxes. In respiration the ratio CO_2 production/O_2 consumption is termed the "respiratory quotient" (RQ). The inverse term "oxidative ratio" (OR, 1/RQ) is also used, especially when describing ecosystem processes that include photosynthesis with O_2 production (where the fluxes direction is opposite to respiration). Both ratios depend primarily on the stoichiometry, of the respiratory substrate or the net synthesized biomass. The stoichiometry of an organic molecule determines the mean oxidation state (C_{ox}) of the C atoms in the molecule (LaRowe & Van Cappellen, 2011; Masiello et al., 2008). The more oxidized (higher C_{ox}) the molecule, the fewer moles of O_2 are consumed per mole of CO_2 released during complete oxidation and the RQ is higher (Table 1). For this reason RQ is often used to infer which

Table 1
Selected Compounds and Their Type, Formula, Mean Oxidation State of the C (C_{ox}), OR, RQ (ARQ), and the Gibbs Energy for Oxidation Half Reactions

Compound	Type	Formula	C_{ox} [a]	OR[b]	RQ (ARQ)[c]	Gibbs energy (kj mol C^{-1})[d]
Oxalic acid	Organic acid	$C_2H_2O_4$	3	0.25	4	−25.2
Glucose	Carbohydrate	$C_6H_{12}O_6$	0	1	1	60.3
Cellulose	Structural polysaccharide	$(C_6H_{10}O_5)_n$	0	1	1	60.3
Aspartic acid	Amino acid	$C_4H_7NO_4$	1	0.75 (NH_3)	1.33 (NH_3)	31.8
				1.25 (HNO_3)	0.80 (HNO_3)	
Lignin[e]	Structural polymer	$C_{100}H_{124}O_{43}$	−0.38	1.10	0.91	71.13
Oleic acid	Fatty acid (lipid)	$C_{18}H_{34}O_2$	−1.66	1.42	0.71	107.8
Methane	Inorganic gas	CH_4	−4	2	0.5	174.3

[a]The relation between molecular formula and C_{ox} described in Equation 5. [b]The oxidative ratio (OR) is often viewed as the ratio O_2 produced/CO_2 assimilated required for the biosynthesis of a compound. The relation between OR and C_{ox} depends on the N source in the ecosystem (see Section 2.5). [c]The respiratory quotient (RQ = 1/OR), the ratio CO_2 efflux/O_2 uptake in full aerobic oxidation of the compound. The value depends on the N-containing product. We use the term ARQ (apparent RQ) since in soils and tree stems additional processes can cause deviation from the theoretical RQ. [d]The Gibbs energy for oxidation half reactions at 25°C and 1 bar was calculated using LaRowe and Van Cappellen (2011) Equation 14: $G^o_{CoX} = 60.3 - 28.5 \times C_{oX}$. [e]Lignin formula is according to Baldock et al. (2004).

respiratory substrate is being used by plants (for example, Fischer et al., 2015). However, in soils and isolated plant organs biotic and abiotic non-respiratory processes can influence CO_2 and/or O_2 and lead to a ratio of CO_2/O_2 that differs from the substrate's RQ value. For this reason, we refer to the ratio CO_2 efflux/O_2 uptake measured in soils and tree stems as the "apparent RQ" (ARQ) (Angert et al., 2015; Angert & Sherer, 2011). In a recent study we suggested that ARQ measurements could be also used to separate autotrophic and heterotrophic respiration sources in soils due to different ARQ signatures (Hicks Pries et al., 2020). ARQ has therefore the potential to become a useful tracer, similar to $\delta^{13}C$ in the ability to identify respiratory substrates, processes, and to separate respiration sources (Gallagher et al., 2017). For meaningful use of ARQ as a biogeochemical tracer it is crucial to identify and separate the signals related to substrate stoichiometry and to non-respiratory processes. A first step toward this understanding is resolving the sources for ARQ variability.

1.1. The Processes That Can Affect ARQ Variability in Soils and in Tree Stems

The ARQ of soil heterotrophic respiration is usually approximated by root-free bulk-soil incubations (ARQ_{bs}). According to meta-analysis of the oxidative ratio (OR) in soil organic matter (SOM; Worrall et al., 2013) ARQ_{bs} values are expected to range between 0.77 and 1.11 with a median of 0.95. However, values of 0.27–0.94 measured previously from a variety of natural ecosystems and agricultural lands are mostly below these expected values (Angert et al., 2015; Aon et al., 2001a, 2001b; Dilly, 2001, 2003; Dilly & Zyakun, 2008; Severinghaus, 1995). The main processes that can drive deviation of ARQ_{bs} from the substrate stoichiometry include (Figure 1): (a) processes involving electron transfer that affect either O_2 uptake or CO_2 production without affecting both gases; (b) interactions involving the greater solubility of CO_2 in water and inorganic C cycling; (c) other processes that affect CO_2 more than O_2 or vice versa.

Enhanced O_2 uptake derived from abiotic oxidation of reduced species (e.g., 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+}, for example, are used as alternative electron acceptors. In that case, CO_2 is emitted 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 & 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.

Carbon dioxide is ~30 times more soluble than O_2, and reacts with water to form weak acids (HCO_3^- and H_2CO_3). Storage of respired CO_2 as dissolved inorganic carbon (DIC) in the soil solution can thus lower the measured ARQ_{bs}, especially in soils containing calcium carbonate with high pH (>7), where high concentrations of HCO_3^- and CO_3^{2-} significantly increase the DIC amount. However, if the water containing the DIC does not leach, the CO_2 is expected to degas back to the soil pore space when the soil is dried. Large ARQ fluctuations

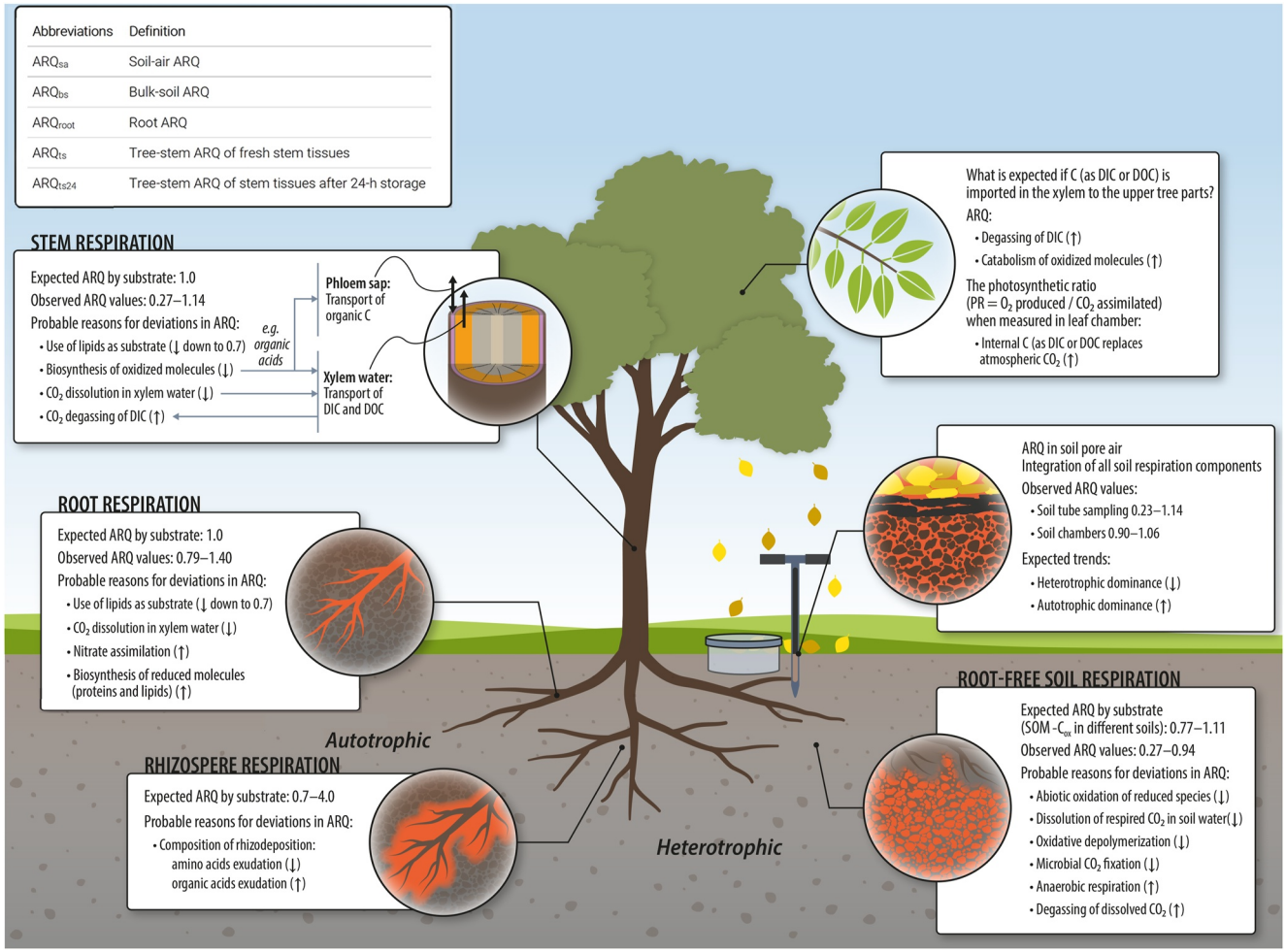

Figure 1. Observed apparent respiration quotient (ARQ, the measured CO_2 efflux/O_2 uptake) in different ecosystem components with plausible explanations for deviations from the expected value, and abbreviations. The expected ARQ is according to the putative-substrate stoichiometry (see Table 1): Carbohydrates for respiration in roots and stems; rhizodeposition composition for rhizosphere respiration, with values indicative of the end-members amino and organic acids; and soil organic matter composition for root-free soil respiration according to Worrall et al. (2013). The observed ARQ values are taken from the literature: Stem respiration (Angert et al., 2012; Angert & Sherer, 2011; Hilman et al., 2019); Root respiration (Hawkins et al., 1999; Hilman et al., 2021; Rachmilevitch et al., 2006; Shane et al., 2004); Root-free soil respiration (Angert et al., 2015; Aon et al., 2001a; 2001b; Dilly, 2001, 2003; Dilly & Zyakun, 2008; Severinghaus, 1995); Soil pore air, tube sampling (Angert et al., 2012, 2015; Hicks Pries et al., 2020; Sanchez-Canete et al., 2018), soil chambers (Ishidoya et al., 2013; Seibt et al., 2004). Included also are the expected effects on ARQ and the photosynthetic ratio (O_2 produced/CO_2 assimilated) if C is imported internally to the canopy.

due to precipitation and dissolution of carbonates are indeed observed in calcareous soils (Angert et al., 2015; Benavente et al., 2010; Cuezva et al., 2011; Emmerich, 2003; Gallagher & Breecker, 2020; Ma et al., 2013).

Other processes involving CO_2 and O_2 include root CO_2 uptake, SOM oxidation processes, and dark CO_2 fixation. DIC (or CO_2) uptake by roots is probably not substantial due to anatomical features (Ubierna et al., 2009). "Oxidative depolymerization" is the addition of oxygen to macromolecules that forms smaller and soluble molecules which can be readily digested by microbes (Kleber et al., 2015). If the oxygen-rich molecules escape respiration by leaching or binding to mineral surfaces, there is a net O_2 uptake and ARQ is lowered. Dark fixation of CO_2 by the microbial community is another process that can lower ARQ_{bs} (Akinyede et al., 2020; Miltner et al., 2005).

In plants, the primary respiration substrates are carbohydrates with ARQ = 1.0 (Hoch et al., 2003; Plaxton & Podestá, 2007). This value is expected for roots, but measurements of ARQ from roots (ARQ_{root}) often deviate from 1.0, ranging from 0.79 to 1.4 (Hawkins et al., 1999; Hilman et al., 2021; Rachmilevitch et al., 2006; Shane et al., 2004; Figure 1). ARQ_{root} values greater than 1.0 were explained by nitrate assimilation that consumes electrons otherwise transferred 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). Another process suggested to occur in roots is dissolution of a fraction of the root-respired CO_2 in the xylem water and its transport to aboveground tissues in the transpiration stream (Aubrey & Teskey, 2009; Grossiord et al., 2012). This CO_2 removal is expected to reduce ARQ_{root} during the daytime when transpiration is active. The ARQ associated with respiration in the rhizosphere depends on the composition of the root exudates that vary greatly in stoichiometry (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 (Table 1 and Figure 1).

Total soil respiration integrates both heterotrophic respiration in root-free soil and autotrophic respiration by roots. Measurements of soil air ARQ (ARQ_{sa}), representing total soil respiration, have been made by either soil chambers or soil pore space sampling using a tube (Figure 1 [Angert et al., 2012; Angert et al., 2015; Hicks Pries et al., 2020; Ishidoya et al., 2013; Sanchez-Canete et al., 2018; Seibt et al., 2004]). The large observed variability (0.23–1.14) can be attributed to two factors: variability in the relative weight of each of the soil respiration sources, and variability within each of the sources. Hicks Pries et al., 2020 found strong seasonality in ARQ_{sa} in western US forest conifer stand with summer versus winter values of 0.89 and 0.70, respectively. The seasonal variation, observed also in soil air $\delta^{13}CO_2$, was attributed to changes in respiratory substrates that switched between root-dominant respiration of more oxidized compounds during summer and bulk-soil respiration of more reduced compounds during winter (Table 1 and Figure 1).

Understanding ARQ measurements in tree stems is important for understanding whole ecosystem ARQ and tree-internal C cycles. Respiration in tree stems is also based on carbohydrates with expected ARQ (ARQ_{ts}) of 1.0. However, the mean ARQ_{ts} measured from stem chambers in tropical, temperate, and Mediterranean trees was 0.59 and values of 1 were rarely observed (Angert et al., 2012; Hilman et al., 2019; Figure 1). 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). However, CO_2 transport was found to have only a minor role in explaining low ARQ_{ts} in the investigated trees (Hilman et al., 2019). An alternative hypothesis for the low 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 (Berveiller & Damesin, 2007; Berveiller et al., 2007). The re-fixation produces oxidized organic acids that might end-up as root exudates with >1 ARQ (Hoffland et al., 1992; Lambers et al., 2008; Shane et al., 2004).

To explore the sources for ARQ variability, we conducted ARQ and bulk-soil respiration seasonal measurements in a Mediterranean oak forest with one evergreen and one deciduous species (soil pH < 7). Our hypotheses were (a) oxidation of the anoxia-product Fe^{2+} reduces ARQ_{bs}; (b) if soil redox condition is an important driver, ARQ_{bs} values will be higher in wet soils and lower in dry soils; (c) the overall respiration-weighted mean of ARQ_{bs}, which accounts for contrasting effects of redox condition, represents the respiratory substrate stoichiometry; (d) if ARQ_{sa} is the integration of ARQ_{bs} and ARQ_{root}, ARQ_{sa} values will be confined between the end members values; (e) if ARQ_{ts} represents CO_2 re-fixation and organic acids export to roots, its values will be anti-correlated with ARQ_{sa} and will be affected by leaf habit, with lower values in active versus dormant trees during winter. In addition we assessed the control of soil climate on ARQ and bulk-soil respiration variabilities.

2. Materials and Methods

2.1. Study Site

The study took place in Odem, an upland 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 temperatures 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 & 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 contains no carbonates, as determined by adding diluted H_3PO_4 (1 M) at 0°C and gradually warming to 80°C while tracking the CO_2 evolution. Soil pH is 6.6. Metal (Fe and Mn) content was determined using an ICP-MS (7500cx Agilent technologies).

2.2. Experimental Design

Samples from *Q. calliprinos* and *Q. boissieri* stems and underlying soils were collected in 10 campaigns between February 2017 and May 2018 at ~1.5 months intervals. We assessed soil climate variables from a nearby meteorological station. The tree stems were measured at two stem positions to account for possible vertical variations, and incubated at two time points to account for temporal metabolic effects. After initially performing ARQ_{bs} incubations at room temperature we realized that the different temperatures in the field may affect measured ARQ_{bs}. We therefore performed an additional experiment to test the temperature effect on ARQ_{bs} and examined how the corrected ARQ_{bs} relates to ARQ_{sa}. Three more experiments were conducted: (a) to evaluate the potential of ARQ to partition between soil respiration sources we measured ARQ_{root} and compared with ARQ_{bs} and ARQ_{sa}; (b) to test the ability of abiotic oxidation to reduce ARQ_{bs} we tracked changes in ARQ_{bs}, Fe^{2+}, and Mn^{2+} after recovery from anaerobic conditions; (c) to test ARQ_{bs} and Fe^{2+} changes in more realistic anaerobic conditions we performed a soil wetting-drying experiment.

2.2.1. Seasonal Measurements

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 × 2 samples × 3 trees × 10 campaigns). Every tree was sampled only once since sampling caused some disturbance to the soil and the stem (see below).

Surface soil from 0 to 10 cm depth 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 × 10 campaigns). Soil moisture was measured gravimetrically on ~3 g subsamples (available only for the last six 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 test tubes connected to ~3.6 mL glass flasks by Ultra-Torr fittings (Swagelok). The gas in the headspace had initial mean atmospheric values (20.95% O_2, 0.04% CO_2). Incubations were conducted usually 2 days after soil collection at room temperature.

For estimating ARQ_{ts} we performed stem tissue incubations, which were shown to produce equal values to stem-chamber ARQ in three species including the oak *Quercus ilex* (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), and since transport in the phloem is the pathway for C to flow from the stem to the roots. Cores were extracted using a 1.0 cm diameter cork borer, at 20 and 130 cm above the soil surface. A total of 60 samples were taken from each tree species (2 stem positions × 3 trees × 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 hr in the dark and at environmental temperatures. Metabolism in stem cores changes rapidly after harvesting; in a previous study ARQ_{ts} increased with time after harvesting from 0.4 to values closer to 1.0 while the O_2 uptake rate was maintained, indicating an relative increase in CO_2 efflux over time (Hilman et al., 2019). To observe potential temporal changes in ARQ_{ts}, the tissues were re-incubated 24 hr after harvesting (ARQ_{ts24}) for the same duration at room temperature. In the storage time stem tissues were wrapped with moist gauze cloth to avoid desiccation.

2.2.2. Comparison of Roots, Bulk Soil, and Soil Air ARQ and Temperature Sensitivity

To evaluate the potential for ARQ to partition the contributions of autotrophic and heterotrophic sources to total soil respiration, and to test the sensitivity of ARQ_{bs} to incubation temperature, we sampled additional trees. During January 2019 we measured ARQ_{sa}, ARQ_{bs}, and ARQ_{root} near three additional trees from each species. For ARQ_{root} we excavated fine roots (<2 mm), because of their highest specific respiration rates among root size classes (Chen et al., 2010; Desrochers et al., 2002; Pregitzer et al., 1998). 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°C to represent field conditions (field soil temperature was 6°C–8°C). Since we expected low respiration rates incubations lasted 24 hr. We also included in the comparison coarse roots of *Q. calliprinos* (<1 cm in diameter) collected in March 2018. Bulk soil incubations were conducted at temperatures of 6, 22, and 30°C and lasted 68–90 hr (2

samples × 3 trees). We also present ARQ_{bs} values for soils sampled in March and May 2018, when soil temperatures were 1°C and 22°C, respectively ($n = 1$).

2.2.3. Evaluation of the Effect of Abiotic O_2 Uptake on Bulk Soil ARQ

Two soil incubation experiments were undertaken to investigate the potential for abiotic O_2 uptake to affect ARQ_{bs}. In the first experiment we tested the response to temporary anaerobic conditions with un-screened soils (for maintaining their structure). Mason jars (1 L) with a small volume of soil (~150 mL, $n = 3$) and jars with a large soil volume (~550 ml, $n = 3$) were incubated for 13 days, to create high and low headspace O_2 concentrations, respectively. Headspace $[O_2]$ was measured by the end of the incubation, and soils were sampled for $[Fe^{2+}]$ and $[Mn^{2+}]$. The soils were then ventilated for 1.5 hr, before an overnight incubation. Air and soil samples were measured again at the end of this incubation. The soil moisture measured at the beginning of the experiment was 31% by weight.

In the second experiment we tracked ARQ_{bs} during a wetting-drying cycle, and measured $[Fe^{2+}]$ and soil moisture during the soil drying. The incubation set-up included Ultra-Torr Tee fittings connecting a test-tube with a drying-agent (magnesium perchlorate), a 3.6 ml flask, and a test-tube with soil. Soils were either 2-mm sieved ($n = 1$) or un-sieved ($n = 1$). Right before the experiment we conducted a control incubation to determine the basal ARQ_{bs} and respiration rate (CO_2 efflux). The latter was used to calculate the relative respiration rate (RR) during the experiment. After the preliminary incubation, soil was dried for 17 days using the drying agent, wetted, and dried again for 26 days. Flask incubations followed each other, with 1 hour ventilation period in between them. The first incubation lasted 17 days and encompassed the entire first drying period. Proceeding soil wetting, which was equivalent to a rainfall event of ~20 mm, the first three incubations lasted 1 day, and the next five incubations lasted 3–7 days. We repeated the soil wetting for the destructive Fe^{2+} and soil moisture measurements, done only on the sieved soil.

The soil $[Fe^{2+}]$ was measured by the Ferrozine method (Liptzin & 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, predominantly represents Mn^{2+} (Keiluweit et al., 2018).

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 & Angert, 2016]). The 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 a needle and injected into flow-through CO_2 (K33 ICB 30% CO_2 Sensor, CO_2 Meter, Inc) and O_2 (Fibox 3, PreSens-Precision Sensing) sensors, connected by plastic tubing. The O_2 sensor is a quenching-based optical fiber (optode) that reads the fluorescence from a sensing "spot.". We placed the "spot" in a 3 mm clear plastic aperture in an opaque lid of a custom-made 2-cm diameter flow-through cell, which was made from 4 mm thick aluminum base (to stabilize the temperature). From the outside of the aperture a connector for the optical fiber that reads the "spot" fluorescence was fixed.

2.4. ARQ and Respiratory Fluxes Estimations

To estimate the CO_2 efflux/O_2 uptake in total soil respiration (ARQ_{sa}) from the tube-sampled gas concentrations, the following equation that corrects for diffusion effects was applied (Angert et al., 2015):

$$ARQ = \frac{D_{CO2} \times ([CO_2]_s - [CO_2]_a)}{-D_{O2} \times ([O_2]_s - [O_2]_a)} = -0.76 \times \frac{\Delta CO_2}{\Delta O_2} \quad (1)$$

where D_{co2}, $[CO_2]_s$, and $[CO_2]_a$ are respectively the effective diffusivity of gaseous CO_2, and the CO_2 concentrations in the soil (measured value) and in the ambient air (assumed to be 0.04%). ΔCO_2 is therefore the difference in $[CO_2]$ between the soil and the atmosphere. The same definitions hold for O_2, where the concentration in the

ambient air assumed to be 20.95%. Since the O_2 flux is opposite in direction to the CO_2 flux we added negative sign for convenience. The effective diffusivity D depends on the structure of the pore spaces and on the diffusivity in air. It can be assumed that the structure is identical for CO_2 and O_2, therefore the ratio D_{co2}/D_{o2} depends only on the CO_2/O_2 diffusivity ratio in air, which is 0.76 (Massman, 1998). Temperature was assumed to impact the diffusivity of each species similarly.

Equation 1 is somewhat analogous to the Davidson equation (Davidson, 1995) that estimates the respired $\delta^{13}CO_2$ in soil from soil-air discrete samples. The equation corrects for the faster diffusion of $^{12}CO_2$ (in our case O_2) that enriches the soil air with the slower-diffusing $^{13}CO_2$ (in our case CO_2), and for difference in $\delta^{13}C$ and $[CO_2]$ between the soil and the air above the soil. The Davidson equation accounts for diffusion and diffusive mixing and thus yields the same respired $\delta^{13}CO_2$ regardless of soil depth (Bowling et al., 2015; Egan et al., 2019). Equation 1 is expected to similarly account for gas-mixing effects with depth (Angert et al., 2015). Indeed, ARQ_{sa} was similar when measured at depths of 10, 30, and 60 cm (0.3 [Sanchez-Canete et al., 2018]) and at 30 and 90 cm (0.8 [Hicks Pries et al., 2020]) Moreover, as ΔCO_2 and ΔO_2 are calculated with respect to atmospheric air, advective mixing of soil air with the above air is not expected to change the measured ARQ_{sa}. Nonetheless, since such mixing will violate the diffusional steady-state between different soil depths we avoided sampling in days with high wind speeds (>4 m s^{-1}).

ARQ_{bs}, ARQ_{ts}, ARQ_{ts24}, and ARQ_{root} were measured in closed-system incubations and calculated by the ratio between $[CO_2]$ and $[O_2]$ net changes in the incubation headspace, without the diffusion correction. Results of bulk-soil incubations from the different experiments are all referred as ARQ_{bs}, except from temperature-corrected ARQ_{bs} $\left(ARQ_{bs}^{corrected}\right)$ presented in Section 3.4. The reported ARQ_{bs} values were calculated after the measured $[CO_2]$ was further corrected for CO_2 dissolution. The correction was needed since some soil samples contained large volumes of water, which in combination with fairly high pH (6.6) is expected to cause a substantial amount of respired CO_2 to remain dissolved as DIC. DIC (mmol/L), the sum of dissolved CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}, was calculated using Equation 2 (Stumm & Morgan, 1996):

$$DIC = P_{CO2} \times k_h \times \left(1 + \frac{k_1}{10^{-pH}} + \frac{k_1 \times k_2}{\left(10^{-pH}\right)^2}\right) \quad (2)$$

where P_{CO2} is the partial pressure of gaseous CO_2 (= $[CO_2]/100$), k_h is Henry's constant that states the amount of dissolved CO_2 and H_2CO_3 in the water, and k_1 and k_2 are the first and second acidity constants, respectively. The constants are temperature dependent and calculated according to the incubation temperature (Harned & Davis, 1943; Harned & Scholes, 1941). To estimate the addition of DIC during incubation we assumed the initial DIC was in equilibrium with atmospheric $[CO_2]$ of 0.04%, and the final DIC in equilibrium with the final $[CO_2]$. The net change in the calculated DIC (ΔDIC) was converted to gaseous CO_2 equivalent and added to the measured $[CO_2]_{measured}$, yielding the corrected $[CO_2]_c$:

$$[CO_2]_c = [CO_2]_{measured} + 100 \times \frac{\Delta DIC \times W \times 22.4}{V_{HS}} \quad (3)$$

where W is the absolute amount of water in the sample (L) and V_{HS} is the headspace volume (mL). 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. The term 22.4 converts the units of the dissolved CO_2 (mmol) to mL gas.

The O_2 uptake and CO_2 efflux from the bulk-soil incubations were calculated using the equation (nanomole gas g^{-1} min^{-1}):

$$Flux = \frac{\Delta Gas \times V_{HS} \times BP}{t \times M \times I_t \times 8.314 \times 10^{-3}} \quad (4)$$

where ΔGas is net percent change in the gas concentration ($[O_2]$ or $[CO_2]$) during the incubation, 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°C, 24 hr) for dry weights.

2.5. Soil Organic Matter Stoichiometry

For estimating the expected ARQ_{bs} in the site we determined C_{ox} of the surface soil (0–10 cm depth, two species × 3 campaigns) using solid state ^{13}C cross polarization magic angle spinning NMR (^{13}C CP/MAS NMR), analyzed via molecular mixing modeling (MMM; Baldock et al., 2004; Hockaday et al., 2009). Analysis was carried out at Rice University. Spectra were recorded using a Bruker Avance 200 MHz solid-state NMR spectrometer (Bruker Corp., MA), equipped with 4 mm MAS probe and operated at a rotor spinning frequency of 12 kHz. A multiCP/MAS pulse sequence modified from Duan and Schmidt-Rohr (2017) was used in this study to obtain more quantitative biochemical compositions of the SOM. The MMM inputs are the sample's C/N ratio, the signal distribution across seven predefined ^{13}C NMR spectral regions associated with the main biochemical classes, and the molar ratios of C, N, H, and O assigned to each class. The soil total C and N contents were measured using catalytic combustion and subsequent chromatographic separation and detection of CO_2 and N_2 gases with a Costech ECS 4010 elemental analyzer. C_{ox} is calculated using the equation (LaRowe & Van Cappellen, 2011; Masiello et al., 2008):

$$C_{ox} = \frac{2d - b + 3c}{a} \quad (5)$$

where the coefficients are according to the MMM's resulting molecular formula $C_aH_bN_cO_d$. We calculated OR and ARQ (=1/OR) with different N sources and products; N_2: $OR = 1 - C_{ox}/4 + 3c/4a$, ammonia ($NH_3$): $OR = 1 - C_{ox}/4$, nitrate (HNO_3): $OR = 1 - C_{ox}/4 + 2c/a$.

2.6. Statistical Analysis

The relationships of the seasonal ARQ and bulk-soil CO_2 efflux with soil climate parameters were tested using a backward selection technique for multiple linear regression, including estimates of the interactions between each two factors. The tested parameters were: soil moisture (available for the last 6 out of 10 campaigns), the number of days passed since the last rain event, the accumulated rain in the 3 weeks prior to sampling, and soil temperature at 10 cm depth. Rainfall and temperature were measured in the nearby metrological station of El Rom (data courtesy of Meteo-Tech Ltd. Meteorological Services). When individual soil parameters were tested we applied a linear regression. Outliers were identified using residual plots. Effects of categorical variables were tested with one-way analysis of variance (ANOVA) and a *t*-test, after assuring homogeneity of variances using Bartlett's test. For unequal variances we used a Welch's test and nonparametric comparisons with Wilcoxon method. Significant differences were determined at $P < 0.05$. We used this analysis in the seasonal measurements to evaluate species, stem height, and incubation time (ARQ_{ts} and ARQ_{ts24}) effects, and in the temperature sensitivity experiment to evaluate species and temperature effects. To describe the correlation between ARQ_{ts} and ARQ_{sa} we used linear regression. All statistical analysis was done using JMP (JMP®, JMP Pro 13, SAS Institute Inc., Cary, NC, USA), except for the logarithmic fits we applied for the relation between ARQ_{bs} with temperature and CO_2 efflux that were done using the R function *nls* (R Core Team, 2019). We chose a logarithmic fit because it allows the expected stabilization of ARQ_{bs} on the substrate stoichiometry value in high respiration rates.

3. Results

3.1. Soil Properties

According to the measured stoichiometry of the SOM and assuming decomposed N ends up as N_2, the expected ARQ_{bs} in the site is 0.95 ± 0.01 (Table 2). The expected value varies with the N end product between end members of 0.90 for nitrate and 0.99 for ammonia. The soil beneath the two tree species did not differ in the stoichiometry and the expected ARQ. The metal concentrations ± SD (μg g^{-1}, $n = 6$) are: Fe 1.74 ± 0.47 and Mn 1.27 ± 0.07.

3.2. Bulk Soil Measurements

The dissolution correction increased the raw ARQ_{bs} values; from mean ± SE of 0.65 ± 0.02 to 0.72 ± 0.02 (range 0.51–0.88, Figure 2). The correction had some sensitivity to the parameters pH, W (soil water content), and V_{HS} (incubation headspace; Equations 2 and 3). For example, decreasing V_{HS} in 10%, increasing W in 10%, and

Table 2
Soil Stoichiometry and Calculated Ratios

Species	Date	^{13}C-NMR molar ratio relative to C				C_{ox}	Calculated ratios (range)[a]		Elemental-analysis content (%)	
		C	N	H	O		OR	ARQ[b]	C	N
Quercus calliprinos	October 2020	1.00	0.04	1.34	0.56	−0.089	1.055 (1.022–1.110)	0.95 (0.98–0.90)	13.7 ± 0.4	0.7 ± 0.0
	January 2021	1.00	0.05	1.32	0.56	−0.055	1.051 (1.014–1.114)	0.95 (0.99–0.90)	10.3 ± 0.2	0.6 ± 0.0
	April 2021	1.00	0.05	1.33	0.55	−0.078	1.059 (1.020–1.125)	0.94 (0.98–0.89)	8.2 ± 0.5	0.5 ± 0.1
Mean ± SD (N_2)						−0.074 ± 0.017	1.055 ± 0.004	0.95 ± 0.003	10.7 ± 2.8	0.6 ± 0.1
Quercus boissieri	October 2020	1.00	0.05	1.34	0.56	−0.074	1.054 (1.019–1.114)	0.95 (0.98–0.90)	14.5 ± 0.5	0.8 ± 0.1
	January 2021	1.00	0.04	1.32	0.59	−0.009	1.033 (1.002–1.085)	0.97 (1.00–0.92)	8.3 ± 0.1	0.4 ± 0.0
	April 2021	1.00	0.05	1.25	0.54	−0.028	1.043 (1.007–1.103)	0.96 (0.99–0.91)	5.4 ± 0.1	0.3 ± 0.0
Mean ± SD (*t* test between species)						−0.037 ± 0.033 ($P = 0.08$)	1.044 ± 0.01 ($P = 0.16$)	0.96 ± 0.01 ($P = 0.16$)	9.4 ± 4.65 ($P = 0.35$)	0.5 ± 0.3 ($P = 0.42$)
Overall mean (N_2)						−0.056 ± 0.031	1.049 ± 0.009	0.95 ± 0.01	10.1 ± 3.5	0.55 ± 0.2

[a]OR is calculated with N_2 as the N source for the ecosystem. In parenthesis the values when the N sources are ammonia – nitrate, respectively. [b]ARQ = 1/OR.

increasing pH from 6.6 to 6.8 resulted in an increase of the overall mean from 0.72 to 0.76. Varying the parameters in the same magnitude but in opposite direction yielded an overall mean of 0.69. The weighted mean of the corrected ARQ_{bs}, using CO_2 efflux rates for weighting, further increased the overall mean to 0.75. Species were found to differ significantly in their weighted means ($P = 0.03$, *t* test) where that of the *Q. calliprinos* was higher than *Q. boissieri* (0.77 vs. 0.72). The seasonal variability of ARQ_{bs} was not explained by the tested physical parameters in the backward selection technique. When temperature was tested individually it had a positive effect on ARQ_{bs} between 1–6 and 22°C, while between 22°C and 30°C ARQ_{bs} values were rather stable (Figure 3a). At the highest temperatures we observed a species effect with higher values in the *Q. calliprinos* (Figure 3a). The *Q. calliprinos* also had greater soil moisture than the *Q. boissieri* (Figure 3b).

A trend of higher ARQ_{bs} values with higher bulk-soil CO_2 efflux rates was observed for both the seasonal measurements and the temperature sensitivity test (Figure 4). Notably, ARQ_{bs} variability declined with increasing fluxes. A strong seasonal cycle was observed for the CO_2 efflux with maximal rates during spring (March-May) and minimal rates during the end of the summer (August-October; Figure 2d). The efflux rates of soil under the two tree species did not differ significantly ($P = 0.766$, *t* test). A reciprocal effect on CO_2 efflux rate (nmole CO_2 g^{-1} min^{-1}) was found between soil moisture (M, % dry soil) and soil temperature (T_{soil}, C°). The effect is described by the following equation:

$$CO_2 \ \text{efflux} = 20.96 \ \text{M} + 0.145 \ \text{T}_{\text{soil}} + (\text{T}_{\text{soil}} - 14.5) \times (\text{M} - 0.24) \times 0.73 - 4.63 \quad (6)$$

The actual CO_2 efflux versus the equation-predicted rate gives $R^2 = 0.77$ ($P < 0.01$). A correlation coefficient R^2 of 0.53 ($P < 0.01$) was calculated while assuming M is the only driving factor on the CO_2 efflux.

3.2.1. Relation Between ARQ_{bs} and Fe^{2+}

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% (anoxic) and 7.25% ± 0.07% (oxic), respectively ($n = 3$). As a result, $[Fe^{2+}]$ in the anoxic soils was higher than the oxic soils (0.89 ± 0.24 vs. 0.05 ± 0.01 mg g^{-1}, respectively). In the subsequent incubation performed after $[O_2]$ recovery to 20.95%, the rate of O_2 uptake was 2.9-fold faster in the previously anoxic soils compared to the oxic soils. The faster O_2 uptake was concurrent with $[Fe^{2+}]$ drop from 0.89 to 0.21 ± 0.04 mg g^{-1}, with ARQ_{bs} of 0.37 ± 0.01. The concentration of Mn^{2+} had stable value of 1.27 mg g^{-1}

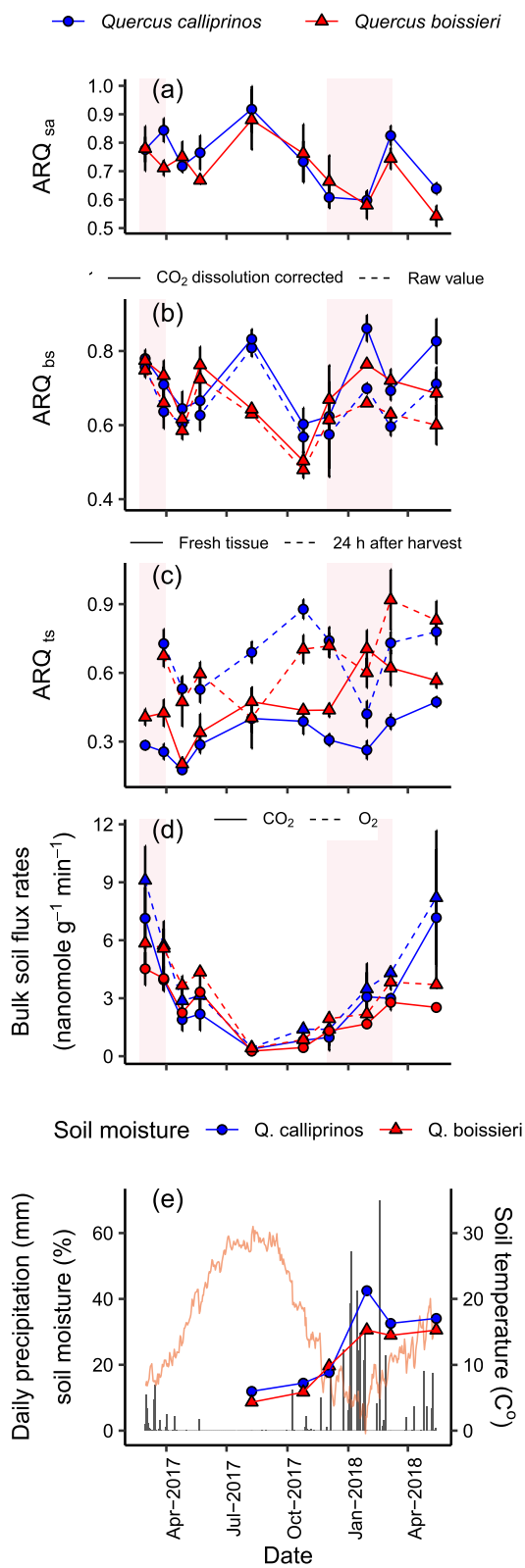
Quercus calliprinos
Quercus boissieri
(a)
ARQ sa
1.0
0.9
0.8
0.7
0.6
0.5
CO2 dissolution corrected
Raw value
(b)
ARQ bs
0.8
0.6
0.4
Fresh tissue
24 h after harvest
(c)
ARQ ts
0.9
0.6
0.3
(d)
CO2
O2
Bulk soil flux rates
(nanomole g−1 min−1)
12
9
6
3
0
Soil moisture
Q. calliprinos
Q. boissieri
(e)
Daily precipitation (mm)
soil moisture (%)
60
40
20
0
Soil temperature (C°)
30
20
10
0
Apr−2017
Jul−2017
Oct−2017
Jan−2018
Apr−2018
Date

Figure 2.

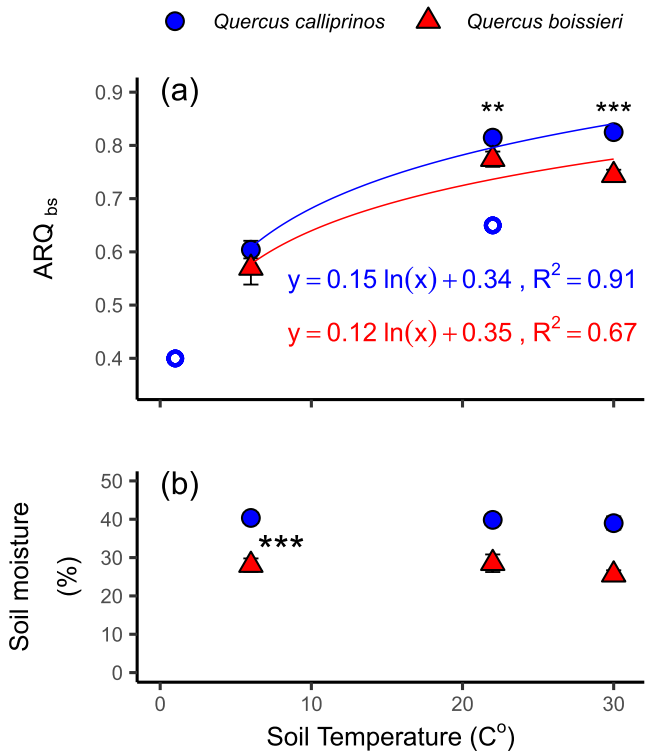

Figure 3. Results (mean ± SE) from bulk soil incubations at different temperatures. Closed symbols represent soils collected on January 2019 underneath three trees from each species with two soil samples per tree ($n = 6$). Empty symbols represent soils collected on March and May 2018 ($n = 1$). (a) ARQ_{bs} (ratio of CO_2 efflux/O_2 uptake) with logarithmic fit; (b) the gravimetric moisture of the soils. Asterisks indicate significance difference between species (**$P < 0.01$, ***$P < 0.001$) in t test or Welch test. For soil moisture, the significance test was made for all temperatures pooled together.

throughout the incubation. The ARQ_{bs} in the oxic soils following the $[O_2]$ recovery was 0.74 ± 0.02 while $[Fe^{2+}]$ maintained its initial 0.05 mg g^{-1} concentration.

The soil wetting-drying experiment induced variations in ARQ_{bs}, RR, and $[Fe^{2+}]$ (Figure 5). 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.

3.3. Soil Air Measurements

The overall ARQ_{sa} mean ± SE was 0.76 ± 0.02 (range 0.60–0.92, Figure 2a). The seasonal variability of ARQ_{sa} was explained by the water related parameters M, the number of days since the last rain event (D), and accumulated rain in the 3 weeks prior to sampling (R, mm) in the backward selection technique. A reciprocal effect was found between the last two factors. The statistical model is defined by the equation:

$$ARQ_{sa} = 0.47M + 0.02D + 0.004R + (D - 18) \times (R - 58.5) \times 3 \times 10^{-4} + 0.24 \quad (7)$$

With $P = 0.0002$ on F test and the correlation between the actual and predicted soil ARQ gives R^2 of 0.94. The effect of T_{soil} is small testing over all-year time scale, however, when omitting from the analysis data collected during late winter and spring and including only data from May 2017 to January 2018, ARQ_{sa} is found to be strongly dependent in temperature ($R^2 = 0.92$, $P < 0.0001$). The relation is given by the linear equation: $ARQ_{sa} = 0.01 \times T_{soil} + 0.6$. Concentrations of CO_2 and O_2 in the soils in single tube samplings ranged from 0.17%–2.25% to 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.

3.4. Comparison of ARQ in Bulk Soil, Roots, and Soil Air

In direct comparison ARQ_{sa} values were always lower than ARQ_{root} (range 0.73–0.96) and higher than ARQ_{bs} (Figure 6a). Assuming root and bulk-soil respiration are the only end members affecting the soil pore space air, their relative contributions could be estimated using a simple mixing model, where $ARQ_{sa} = X \times ARQ_{root} + (1 - X) \times ARQ_{bs}$ (Figure 6a). To compare the seasonal ARQ_{bs} measured in the laboratory to ARQ_{sa} measured in the field, we corrected ARQ_{bs} that was measured at room temperature to field temperature (Figure 6b). The correction was based on the incubation temperature (T) effect on ARQ_{bs} (Figure 3; both-species mean ARQ_{bs}):

$$ARQ_{bs} = 0.13 \times \ln(T) + 0.36 \quad (8)$$

For calculating the temperature-corrected ARQ_{bs} ($ARQ_{bs}^{corrected}$), the intercept term 0.36 was modified according to the actual room temperature that varied slightly between measurements and the measured seasonal ARQ_{bs}, resulting in Equation 9:

$$ARQ_{bs}^{corrected} = 0.13 \times \ln(T = field) + 0.36 - \left[0.13 \times \ln(T = room) + 0.36 - ARQ_{bs}\right] \quad (9)$$

Figure 2. Seasonal measurements of (a) mean ARQ_{sa} ± SE, the ratio of CO_2 efflux/O_2 uptake measured for soil air in depth of 15 ± 4 cm ($n = 3$); (b) ARQ measured from bulk soil incubation (ARQ_{bs}) where solid lines indicate the CO_2-disolution corrected values and dashed lines indicate the raw values ($n = 3$); (c) the mean ARQ measured for incubated stem tissues (ARQ_{ts}) extracted from 20 and 130 cm above the ground where solid lines indicate incubations conducted directly after the core was harvested and dashed lines indicate incubations conducted 24 hr after harvest ($n = 6$); (d) the CO_2 (solid lines) and O_2 (dashed lines) flux rates of the incubated bulk soils; and (e) daily precipitation (black bars) and soil temperature (orange 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.

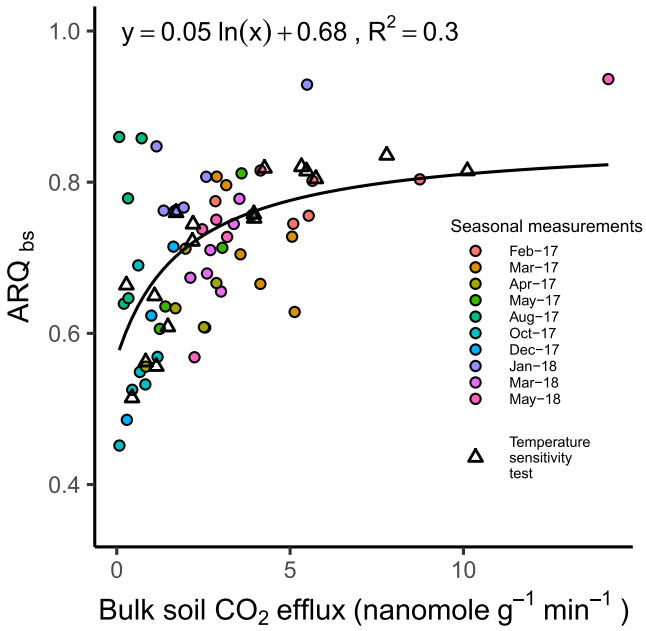

Figure 4. Scatter plot of ARQ (the ratio of CO_2 efflux/O_2 uptake) measured from bulk soil incubations (ARQ_{bs}) and the CO_2 efflux rate of the incubated bulk soils. Results are grouped by experiments: closed colored circles (month of sampling for the seasonal measurements) and empty triangles for the temperature sensitivity experiment (Figure 3).

Equation 9 can be further reduced:

$$ARQ_{bs}^{corrected} = ARQ_{bs} + 0.13 \times \ln\left(\frac{T = field}{T = room}\right) \quad (10)$$

3.5. Tree Stem Measurements

The strongest effect on ARQ_{ts} was time ($P < 0.0001$, *Welch* test); ARQ_{ts} values measured immediately after tissues extraction (overall mean ± SE of 0.39 ± 0.03, range 0.19–0.70) were much lower than ARQ_{ts24} measured 24 hr after the extraction (0.68 ± 0.04, range 0.42–1.08, Figure 2c). The sampling height of the stem tissues (20 and 130 cm) had no significant effect on ARQ_{ts} and ARQ_{ts24}, but we did observe significant species effect on ARQ_{ts} ($P = 0.0015$, *Welch* test) with mean values of 0.46 ± 0.03 for *Q. boissieri* and 0.32 ± 0.02 for *Q. calliprinos*. The species effect derives from a significant phenological effect during winter exfoliation, when the deciduous *Q. boissieri* had higher ARQ_{ts} values than the evergreen *Q. calliprinos* (0.51 ± 0.02 vs. 0.30 ± 0.02, $P < 0.0001$, *Welch* test). During the foliated period the higher *Q. boissieri* values were not significant (0.41 ± 0.03 vs. 0.34 ± 0.03, $P = 0.11$, *t* test). In contrast, the species did not differ in their ARQ_{ts24} values ($P = 0.66$, *t* test) also during winter exfoliation ($P = 0.07$, *Welch* test). The seasonal variability of the *Q. boissieri* was best explained by the accumulated rain in the 3 weeks prior to sampling, for ARQ_{ts} ($R^2 = 0.85$, $P < 0.001$), and for ARQ_{ts24} ($R^2 = 0.93$, $P < 0.001$). Soil moisture was also correlated with ARQ_{ts} ($R^2 = 0.71$, $P = 0.04$) and with ARQ_{ts24} ($R^2 = 0.8$, $P = 0.03$). For the *Q. calliprinos* no correlation was found. Inverse correlation with marginal significance was found between the *Q. boissieri* ARQ_{ts} at 20 cm above the ground and ARQ_{sa} ($R^2 = 0.41$, $P = 0.06$) after excluding 1 outlier point out of 10 (measured in April 2017 when ARQ_{ts} was minimal, Figure 3c).

4. Discussion

4.1. Bulk-Soil ARQ Is Lower Than Expected by SOM Stoichiometry

The overall weighted mean of the dissolution-corrected ARQ_{bs} is 0.75, well within the range of previous ARQ_{bs} assessments (Figure 1). The measured SOM-C_{ox} translates to OR of 1.049 ± 0.009 when N_2 is the N source (Table 2), almost the same as 1.055 ± 0.023, the median value in global soils meta-analysis study (Worrall et al., 2013). The OR value is equivalent to ARQ of 0.95, which is the expected ARQ_{bs} when the SOM is fully oxidized. If all the decomposed organic N ends up as nitrate ARQ_{bs} expected value is 0.90. The measured ARQ_{bs} value of 0.75 is thus appreciably below the expected values. Several potential processes can be ruled out as drivers for the low weighted mean ARQ_{bs}, including dissolution of respired CO_2 in the soil water (after the dissolution correction) and reversible effects such O_2 uptake by oxidizing Fe^{2+} or Mn^{2+} that should be canceled by anaerobic respiration that must occur at different time in the soil (e.g., to reduce Fe and Mn). However, other processes with potentially irreversible effects, including microbial re-fixation of respired CO_2 and oxidative depolymerization are both possible explanations. Actual respiratory substrate with different stoichiometry from the total SOM can also explain the low ARQ_{bs} values. Here we will discuss these three possible explanations:

1. Studies of CO_2 "dark" fixation showed that rates scaled with microbial biomass regardless of soil depth (Akinyede et al., 2020); under the assumption of correlation between microbial biomass and respiration, we speculate that a constant fraction of respired CO_2 is re-fixed regardless the season. If the re-fixation products, which are usually relatively oxidized, are mineralized shortly after synthesis, no net effect on ARQ is to be expected. However, if the re-fixed C persists in microbial biomass or SOM, there will be a net decrease in ARQ. Nonetheless, accounting for the highest observed re-fixation rates yields only slightly increase in the mean ARQ_{bs}, to 0.79 (5.6% from total CO_2 efflux [Akinyede et al., 2020])

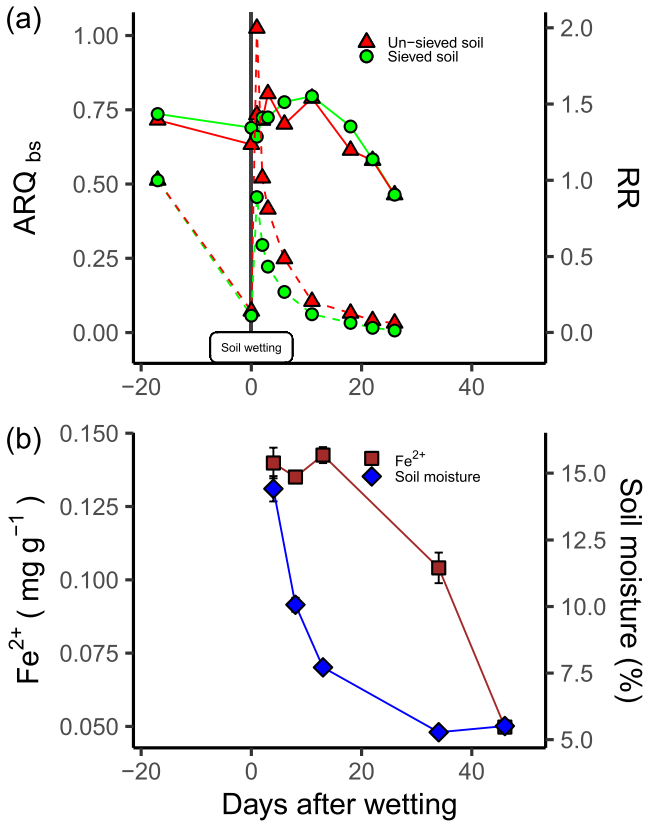

Figure 5. Results from the soil drying-rewetting experiment. The day of the rewetting is day 0. (a) ARQ_{bs} (ratio of CO_2 efflux/O_2 uptake) in solid lines and relative respiration rate (RR) in dashed lines for un-sieved and sieved (2 mm) soils. Each data point represents one measurement without replicates. (b) Mean ± SD of Fe^{2+} concentration (mg g^{-1}) and the gravimetric moisture of the sieved soil (%), measured in duplicate sub-samples of the sieved soil. Since the measurements are destructive, they were taken in an additional experiment following the experiment presented in panel (a).

2. Oxidative depolymerization breaks down macromolecules by introduction of oxygen-rich groups, which forms small molecules that microbes can uptake easily. The polymer oxidation step consumes O_2 and is expected to lower ARQ_{bs}. However, the mineralization of the highly oxygenated produced molecules is expected to have high ARQ_{bs}, with overall zero net effect on ARQ_{bs}. Net reduction of ARQ_{bs} might occur if the oxygen-rich molecules escape microbial digestion by leach or association with minerals, where an extended time period may pass until their mineralization (Kleber et al., 2015). To our knowledge there is no available method to estimate the actual in situ oxidative-depolymerization O_2 uptake, but methods for the potential activities of oxidizing enzymes are available. For example, the activities of phenol oxidases and peroxidases are reported to be in the range 0–50 μmol g^{-1} h^{-1} (Sinsabaugh, 2010), much higher than the O_2 uptake rates of 0–0.9 μmol g^{-1} h^{-1} measured here. However, despite the enzymatic activities are overwhelmingly enough to explain the current low ARQ_{bs}, marked differences are expected between standard enzymatic assays (undergone in optimal conditions and substrates) and field activities (Burns et al., 2013). Without direct comparison between ARQ_{bs} and oxidizing enzymes activity the true role of this process is uncertain
3. The difference between the measured and the expected ARQ_{bs} might suggest that the overall SOM is more oxidized than a smaller SOM pool that is more accessible to microbes and directly supports respiration. This suggestion contradicts the fact that oxidized compounds are energetically more labile than reduced compounds (Table 1). Protection of highly oxidized compounds on soil minerals can explain why the overall SOM is more oxidized than the sub-pool that supports respiration (Kleber et al., 2011). The suggested stoichiometry difference between the respiratory substrate and the total SOM is analogous to radiocarbon measurements, where bulk-soil respired CO_2 is much younger than the total SOM (Trumbore, 2000), suggesting most SOM has slow turnover rates with small contribution to respiration. Hence, rather than representing the total SOM stoichiometry, ARQ_{bs} might provide information about the stoichiometry of the actual labile pool utilized by microbes

To conclude, we estimate that the effect of CO_2 re-fixation is minor given previous measured rates, and that the lower than expected ARQ_{bs} is driven mainly by difference between the actual respiratory substrate and the total SOM stoichiometry, and by oxidative polymerization. Both processes lead to a gradual oxidation of the remaining, un-respired SOM. Hence, without new plant inputs the remainder of SOM will become more oxidized over time. This expectation is in line with an observed increase in O and a decrease in H contents in experiments where soils were subjected to SOM depletion via heating and bare fallow conditions (Barre et al., 2016; Poeplau et al., 2019).

4.2. Iron Redox Changes Affect ARQ_{bs} Only at Low Respiration Rates

ARQ_{bs} values were associated with respiration rates (approximated by CO_2 efflux), which varied by two orders of magnitude in our experiments (Figures 4 and 5a). At respiration rates above ~2.5 nmol CO_2 g^{-1} min^{-1} most ARQ_{bs} values were similar to the mean weighted seasonal value (0.7–0.8) and closer to the expected 0.95 value with relatively small variability, while in lower respiration rates larger ARQ_{bs} variability was observed (0.4–0.9; Figure 4). One possible explanation for this observation is greater sensitivity of the O_2 measurement for smaller O_2 fluxes. However, the low ARQ_{bs} values measured at low temperatures (Figure 3) and in the soil drying experiment (Figure 5a) seem like part of a trend and not just arbitrary analytical noise. Alternatively, as we hypothesized, the ARQ_{bs} and CO_2 efflux relation can be explained by a shift in the dominant processes. When respiration rates are low, slower processes (i.e., those normally contributing only in a minor way to larger O_2 fluxes), such

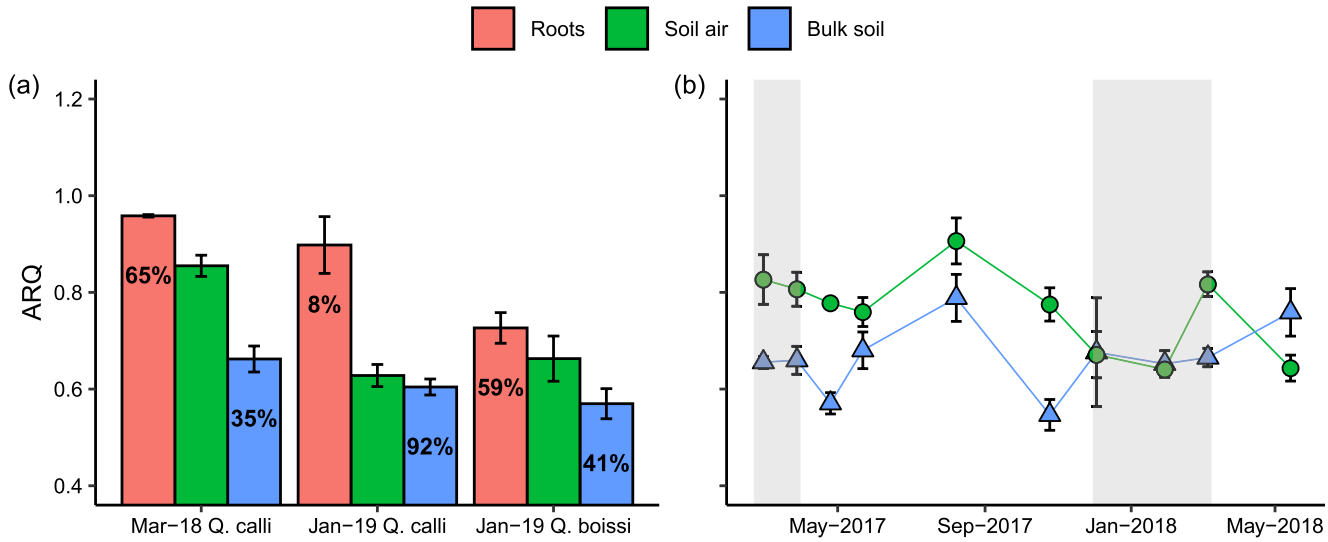

Figure 6. (a) A comparison of ARQ (ratio of CO_2 efflux/O_2 uptake) values (mean ± SE) measured from root incubations ($n = 2, 6, 6$), soil air ($n = 3$), and bulk-soil incubations at field temperature ($n = 3$). The x axis indicates the date of sampling and the tree species. The relative contributions (%) of roots and bulk-soil respiration to the total soil respiration are indicated in the bars; (b) The seasonal course of ARQ means of both tree species (±SE, $n = 6$), where the bulk soil values are temperature corrected according to Equation 10 (colors referred in the legend). Shaded periods indicate winter dormancy of the deciduous *Q. boissieri*.

as redox related changes in Fe, can have a measurable effect on ARQ_{bs}. As respiration rates increase the ARQ_{bs} value related to substrate-stoichiometry will drown out other effects.

The jar incubations demonstrate the maximal ability of abiotic oxidation of reduced species to affect ARQ_{bs} in the studied site. In soils recovered from anoxic conditions the O_2 uptake was 2.9-fold faster than in oxic soils, resulting in lower ARQ_{bs} (0.37 vs. 0.74). In a mass balance calculation, the observed oxidation of Fe^{2+} can explain one third of the O_2 uptake (Burke & Banwart, 2002), another third can be explained by the same microbial respiration as in the oxic soils, and the last third can be explained by oxidation of other reduced species and by faster oxidation of labile C that was protected by the anoxic conditions (Keiluweit et al., 2017). The soil drying-rewetting experiment indicates anoxic conditions have only a small influence under field conditions (Figure 5). The decrease in ARQ_{bs} values on the eleventh day after soil wetting seems to be the result of Fe^{2+} oxidation that occurred around the same time, but by this time point, respiration rates were already low. 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. In addition, the minimal $[O_2]$ measured in the soil air was 18.14%, therefore anoxia might present only in microsites and not in the whole soil profile in this upland forest. Moreover, the fast $[O_2]$ transition from ~1% to 20.95% in the jar incubations that resulted in the sharp ARQ_{bs} decrease is not expected in the field. We would instead expect a gradual $[O_2]$ increase with a smaller effect on ARQ_{bs}. From this we conclude that redox-related O_2 consumption is significant at the Odem forest only at low respiration rates.

4.3. Seasonality of Soil Air ARQ Indicates Soil Respiration at This Site Is Controlled by Bulk-Soil Respiration

The overall mean ARQ_{sa} was 0.76, within the range of previous studies (Figure 1). ARQ_{sa} was almost always higher than ARQ_{bs} and when ARQ_{root} was measured its values exceeded ARQ_{sa}, suggesting that ARQ_{sa} value might be the weighted mean of those two end members and that ARQ has a potential to be used for partitioning of soil respiration sources (Figure 6). However, ARQ is not always adequate for respiration partitioning. We observed ARQ_{sa} values that were equal to or lower than ARQ_{bs} at three dates during the second and wetter winter (Figures 2 and 6). We estimate that during these measurements substantial portion of the CO_2 in the soil profile dissolved and leached to deeper layers, resulting in large reductions in ARQ_{sa}. The use of ARQ for partitioning respiration sources is hence not recommended shortly after rain events. In comparison to other non-destructive methods like the bomb radiocarbon approach and canopy $\delta^{13}C$-labeling, the use of ARQ can be technically

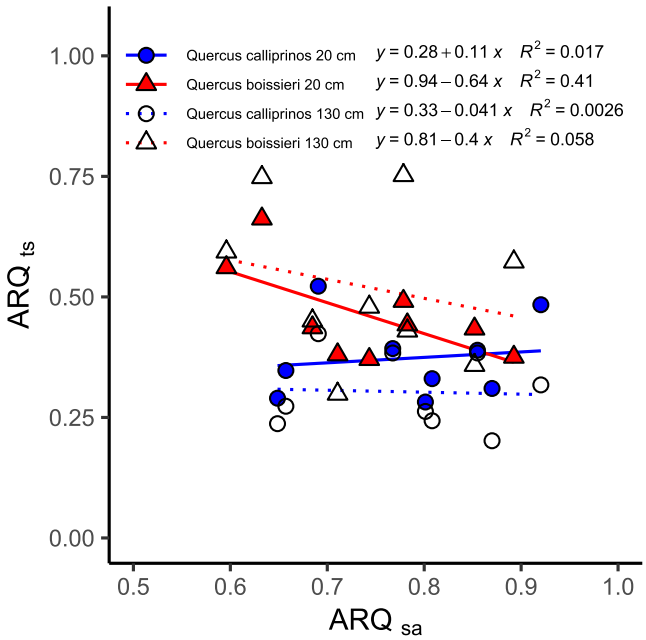

Figure 7. Scatter plot of stem apparent respiration quotient (ARQ) (ratio of CO_2 efflux/O_2 influx) measured from incubated stem cores containing phloem and cambium tissues (ARQ_{ts}) sampled 20 (closed symbols) and 130 cm (open symbols) above ground from the main trunks of the tree species *Quercus 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.

simpler and more cost-effective (Kuzyakov, 2006). There are a few considerations: different ARQ_{bs} signals from soils sampled from different depths, their temporal variability, and their relative contributions to the total respiration fluxes. Our results also indicate that the ARQ of the soil respiration components cannot be assumed a priori because of their large variability. Rather, to assess the end member values in mass balance calculation, separate measurements of bulk-soil and roots ARQ have to be conducted in parallel to each ARQ_{sa} measurement.

The backward selection technique indicates that on a yearly basis water-related parameters are the main factors controlling the seasonal ARQ_{sa} variability in this Mediterranean site, with negligible effect of T_{soil} (Figure 2a; Equation 7). With two orders of magnitude variability, the bulk-soil CO_2 efflux (at room temperature) was also mainly controlled by soil moisture (Figure 2d; Equation 6). We therefore estimate that bulk-soil respiration controls ARQ_{sa} variability. This is in line with other studies in Mediterranean climates that reported rather constant root respiration along the year (Tang & Baldocchi, 2005; Vose & Ryan, 2002). As discussed in the previous paragraph, water can also influence ARQ_{sa} via CO_2 dissolution in the soil profile. T_{soil} was an important factor with positive effect on ARQ_{sa} only outside the high growth period (May-January) when soil respiration rates were slow (Figure 2). A similar positive effect of T_{soil} on ARQ_{sa} was observed at 30 cm depth in heated soils (+4°C) during winter (Hicks Pries et al., 2020). This trend is in accordance with the effect of temperature on ARQ_{bs}; a positive effect when respiration rates are slow, and no effect when respiration rates are high (Figures 3 and 4).

4.4. Tree Stems ARQ Shows Evidence for Seasonal Variable Re-Fixation

The ARQ values measured in tree stem tissues were considerably lower than 1.0, the value expected from carbohydrate respiration (Table 1). Previously we have suggested that dissolution of respired CO_2 in the xylem water and re-fixation of respired CO_2 are the main processes that can explain low ARQ_{ts} values. The dissolution pathway can be excluded here since we incubated stem tissues that were detached from the vascular system. We surmise that ARQ_{ts}, with some particularly low values of <0.3, might be influenced by wound response since we incubated the tissues directly after cutting. Such damage might result in a burst of O_2 uptake that lasts few hours and lowers ARQ_{ts} (Tian et al., 2015). ARQ_{ts24} (measured 24 hr after tissues extraction) can be considered as less prone to artifacts, although metabolic change during the 24-hr period is possible (Hilman et al., 2019). The ARQ_{ts24} mean values of ∼0.7 could be explained by lipid respiration (Table 1). This is, however, not expected, especially in the tree genera *Quercus* (Hoch et al., 2003; Sinnott, 1918). ARQ_{ts} variability can be therefore perceived as variability in CO_2 re-fixation.

Our observations indicate significant phenological and seasonal effects on ARQ_{ts}. During the foliated period ARQ_{ts} was not statistically different among the two species. However during winter exfoliation ARQ_{ts} of the deciduous *Q. boissieri* was higher in ∼0.2 ARQ units than the evergreen *Q. calliprinos* (Figure 2c). In addition, the seasonal change of ARQ_{ts} and ARQ_{ts24} of the *Q. boissieri* were explained by water related factors suggesting low tree stem ARQ is associated with dry conditions. We hypothesized that if re-fixation of respired CO_2 by the enzyme PEPC is the process that lowers tree-stems ARQ, organic acids that are the enzyme's products might be exported via the phloem to the roots and be secreted to the soil as root exudates (Hoffland et al., 1992; Shane et al., 2004). In agreement, a recent study in a different Mediterranean forest in Israel reported that root exudation rates were highest in the dry season and were associated with high soil temperature and low soil moisture (Jakoby et al., 2020). However, we found only weak and marginal inverse relation between ARQ_{ts} and ARQ_{sa}, which was expected if the organic acids are quickly decompose in the soil (Figure 7). While one may conclude that export of organic acids from stems to soil is quantitatively unimportant, slow decomposition rates in the soil or root exudates secretion in shallower soil horizons than our sampling depth cannot ruled out.

5. Conclusions

Our observations provide several insights about the sources for ARQ variability. Total soil air ARQ variability is mainly driven by the relative weights of its two components, root respiration with high ARQ and bulk-soil respiration with low ARQ. The seasonal bulk-soil respiration (CO_2 efflux) varied by two orders of magnitude and therefore seems as the main driver of total soil air ARQ. At high respiration rates bulk-soil ARQ is rather invariable and notably lower than the SOM stoichiometry. Two processes can explain the low bulk-soil ARQ values: an SOM sub-pool that supports microbial respiration with a more reduced stoichiometry than the total SOM, and/or oxidative depolymerization. At low respiration rates bulk-soil ARQ is more variable due to greater effects of non-respiratory processes like oxidation of reduced Fe^{2+}. Variation in tree stems ARQ reflects variation in CO_2 re-fixation, with no evidence for substantial organic acids export from stems to rhizosphere. Overall this work demonstrates the ability of ARQ to provide valuable information about ecosystem processes.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The data are available in https://doi.org/10.6084/m9.figshare.12003654.v1.

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