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Characterizing soil organic carbon spatial and seasonal variability using Rock-Eval and CO2/O2 fluxes measurements

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Abstract. Soil organic matter (SOM) stores most of the terrestrial carbon, and changes in this storage can have a significant effect on the global carbon cycle. Various approaches have been used to understand the SOM transformations and stability. Here we tested the combination of two complementary approaches: 1) We estimated the long-term SOM stability, and the effect of decomposition on the stability and composition of the remaining fraction, by Rock-Eval pyrolysis. 2) We measured the respiratory CO2 and O2 fluxes, and their ratio (the Apparent Respiratory Quotient – ARQ) in soil incubations at different temperatures, to learn about the short-term processes. To study in detail the spatial and temporal variability, we examine soil samples from two sample sets: a regional set, and a local set that was used to study the seasonality and environmental variability within a site. The Rock-Eval analysis showed an effect of the slope aspect on SOM. The south-facing slope organic matter was more mature and stable. For the particulate organic matter fraction, we found an increase in O2 uptake rate with an increase in Hydrogen Index (HI), indicating that the respiration rates are higher when the reduced and easily degradable fraction is larger. The soil incubation experiments showed an increase in the ARQ values with temperature. This can be explained by higher respiration rates at high temperatures and the formation of anoxic microsites where electron acceptors alternative to O2 are used. This suggestion was supported by incubation of soil clods that at 23ºC resulted in ARQ values >1, implying anerobic conditions, while the addition of O2 to the headspace lowered the ARQ. Based on additional experiments, we further suggest that incubations at low temperatures can reflect the history of the soil and indicate past anaerobic conditions that resulted in soil rich in reduced chemical species, resulting in a lower ARQ. This line of reasoning can explain the lower ARQ measured for soil sampled in winter, since high moisture content limits oxygen diffusion and creates anaerobic microsites. Combining the measurements of ARQ with Rock Eval pyrolysis can provide a more complete understanding of the state of the organic matter in the soil.

1 Introduction

Soil is the largest terrestrial carbon reservoir, containing twice the carbon in the atmosphere and three times more carbon than vegetation. Therefore, even small change in soil carbon sequestration is meaningful to the global carbon cycle and climate change (Ramesh et al., 2019). The main source of CO_2 efflux from the soil is microbial respiration, which has increased globally over the recent decades, in a rate that is consistent with the increase in temperature (Bond-Lamberty et al., 2018; Bond-Lamberty and Thomson., 2010). Soil carbon is mainly stored as Soil Organic Matter (SOM), which is protected from microbial respiration through several mechanisms, such as physical occlusion, mineral protection of organic C, and

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anoxic microsites (zones of oxygen depletion in otherwise oxic soils). Within anoxic microsites, anaerobic respiration can be ~90% slower than aerobic respiration on a per-volume basis (Lacroix et al., 2022).

Angert et al. (2015) suggested using the ratio of CO2 efflux to O_2 uptake (Apparent Respiratory Quatient, ARQ) as a method for quantification and understanding of soil respiration. In the aerobic respiration process the substrate-organic compound is an electron donor, and O_2 is an electron acceptor, while in anaerobic respiration, other electron acceptors like Fe^{3+} or Mn^{3+} are used. The ARQ is dependent on the respiratory substrate stoichiometry – the more reduced the molecule, the more moles of O_2 are consumed per mole of CO_2 released during oxidation, and the ARQ is lower. Organic acid as a substrate will give an average ARQ of 1.4, sugars of 1, phenolics 0.95, lignin 0.88, proteins 0.77, lipids 0.73, and Methane 0.5 (Masiello et al., 2008). Deviation of ARQ from the substrate stoichiometry can be derived from processes like the dissolution of respired CO_2 in soil water, oxidative depolymerization (partial oxidation of organic matter can consume O_2 with no CO_2 emission), microbial CO_2 fixation, anaerobic respiration, and degassing of dissolved CO_2 (Hilman et al., 2022b). Hence, as will be detailed in the discussion, the ARQ measurements in soil can improve the understanding of soil microbiota substrate shifts, and processes like anaerobic respiration in microsites of well-aerated soils. For example, high respiration rates associated with higher temperatures and higher SOM content can create anoxic microsites, resulting in higher ARQ. Observing the temperature sensitivity (Q10) of respiration through both O_2 uptake rate and CO_2 efflux can provide valuable information regarding soil carbon interaction with global climate change. In addition, the ratio between oxygen consumption and CO_2 release (the inverse of ARQ) in soil respiration is an important parameter in estimates of global carbon sinks from atmospheric O_2 measurements (Keeling et al., 1996; Manning, 2001).

SOM cycling and stabilization in soils are affected by environmental factors such as temperature, soil texture, and mineralogy (Quideau et al., 2001). They are intimately associated with the SOM chemical structure (Kögel-Knabner, 2000). However, information on the SOM chemistry and properties in soils is still lacking. Very few techniques for characterizing and monitoring SOM dynamics are used routinely because of the common need for preliminary sample preparation or due to their complexity and cost (Albrecht et al., 2015). Rock-Eval pyrolysis emerges as a new tool for fast and easy carbon quantitation and characterization of soil organic matter thermal stability as well as supplying information on the composition of the organic matter, especially through the Hydrogen and Oxygen Index values (Disnar et al., 2003; Sebag et al., 2006; Seanger et al., 2013; Saenger et al., 2015). The Rock-Eval also allows assessing soil organic matter maturity through the calculation of the I index and the R index, which are based on the amounts of hydrocarbons released at different temperatures during the pyrolysis (Sebag et al., 2006; Albrecht et al., 2015). Studies in which Rock-Eval measurements are combined with CO_2 efflux measurements are sparse (Zhang et al., 2023; Soucémarianadin et al., 2018) , and we are unaware of studies combining Rock-Eval with O_2 measurements.

Here, we studied the SOM in soil samples, representing temporal (seasonal) and spatial (regional and local variability, see below) by both Rock-Eval analysis and soil incubations, conducted in different temperatures, in which the ARQ, the O_2 uptake rate, and the CO_2 efflux were measured. This enabled us to study soil organic carbon dynamics for both the most active pool, which is respired during a few days incubation, and the slower responding pools, as measured by the Rock-Eval, and to track the effect of environmental variability on the SOM.

2 Methods

2.1 Sites description

All the soil samples were taken from the Golan Heights in northern Israel, the sites were chosen due to the lack of soil carbonates, which may affect the CO_2 fluxes by carbonate mineral dissolution and precipitation processes. These sites' soils were previously studied (Dan et al., 1970-soil physical and chemical properties; Hilman et al., 2022b- ARQ in Odem Forest; Ben-Asher et al., 2017-soil creep efficiency in Mt. Baron). Two set of samples were taken: 1. a regional set was aimed to

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study how the soil parameters change with site environment and to describe spatial variability across sites, and 2. a local set was used to study in detail the seasonality and environmental variability within a site. The regional set was taken from 11 sites located in the north and center of the Golan Heights (Tables 1,2, Fig. 1), which are described in Dan et al. (1970) and in Hilman et al. (2022b) (Odem Forest). The mean temperature is 23ºC in summer and 7ºC in winter (Marom Golan meteorological station), and an annual precipitation is 600-1000mm. The main soil types are Red and Brown Mediterranean soils (FAO) (correlates with Rhodoxeralfs and Haploxeralfs in the USDA system). The vegetation includes species like *Crataegus aronia, Carlina hispanica, Hordeum bulbosum, and Echinops gaillardotii.* In Odem Forest the common species are *Quercus calliprinos and Quercus boissieri.*

The local soil samples set was taken from Mt. Baron, a volcanic cinder cone 1043 m a.s.l., which is located in the north of Golan Heights (33°15′N, 35°77′E, Fig. 1). Annual precipitation is 885mm (Ben-Asher et al., 2017), and the air temperatures is simlar to those in the regional set. Average soil temperature is 21.8°C in October, 4.6°C in February, and 21.2°C in June. The average relative humidity is 76% in October, 92% in February and 53% in June (Elrom meteorological station). The aridity index is 1.14 (Castagneri et al., 2020). The soil is Red Mediterranean Soil (FAO), which correlates with Typic Rhodoxeralfs in the USDA system. The vegetation includes annual and perennial grasses such as *Vicia tenuifolia Roth* (north slope), *Avena barbata, Daucus carota*, and trees like *Quercus boissieri* and *Crataegus aronia*. The average soil pH is 7.25 under tree canopies and 6.79 in open grassland. Dahlgren et al. (1997) found a similar increase in soil pH under Oak canopies in comparison to grassland and attributed it to greater cycling of base cations by the oak.

Table 1- The experiment description

	Site	Experiment
Regional set	11 sites at the Golan Heights	Spatial variability, 2 seasons, different moistures at 2 temperatures incubations
Local set	Mt. Baron	3 seasons, open grassland vs under tree canopies, northern vs. southern slopes, different elevation incubation at 4 temperatures + Rock-Eval
Clods	Mt. Baron	Artificial clods vs. sieved soil at 2 temperatures incubation with and without adding oxygen
POM and MAOM	Mt. Baron	Particular organic matter vs. mineral attached organic matter Rock-EVal

Table 2 – Location and general characteristics of the study sites.

Site	N	E	Classification	Texture	Elevation (m)	Precipitation (mm)	pH
Buqata	33°11'16.4"	35°46'50.7"	Typic Rhodoxeralfs	Clay	1090	900-1000	6.94
Dallawe	33°05'27.6"	35°46'19.5"	Typic Haploxeralfs	Sandy loam	890	700-800	6.9
Hushania northeast	33°00'12.0"	35°49'01.2"	Typic Haploxeralfs	Clay loam	779	600-700	6.84
Hushania southeast - upper	32°59'00.4"	35°49'43.1"	Aquic Chromoxererts	Clay	761	600-700	6.4

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Hushania southeast - lower	32°59'00.4"	35°49'43.1"	Aquic Chromoxererts	Clay	761	600-700	6.78
Juba	32°12'02.6"	35°44'08.8"	Typic Rhodoxeralfs	Clay	970	800-900	6.78
Mount Avital - lower	33°06'58.3"	35°47'10.2"	Typic Haploxeralfs	Loamy sand	1080	800-900	6.65
Mount Avital - upper	33°06'58.3"	35°47'10.2"	Typic Haploxeralfs	Clay loam	1116	800-900	6.6
Odem forest - Q. Boissieri	33°13'12.9"	35°44'54.1"	Lithic Rhodoxeralfs	Silt loam	1007	900-1000	6.42
Odem forest - Q. Calliprinos	33°13'12.9"	35°44'54.1"	Lithic Rhodoxeralfs	Silt loam	1007	900-1000	6.62
Tanuria	32°57'38.2"	35°47'50.8"	Aquic Chromoxererts	Sandy loam	620	600-700	6.86
Mount Baron	33°9'33.6"	35°46'41.0"	Typic Rhodoxeralfs	Clay loam	1043	885	7.02

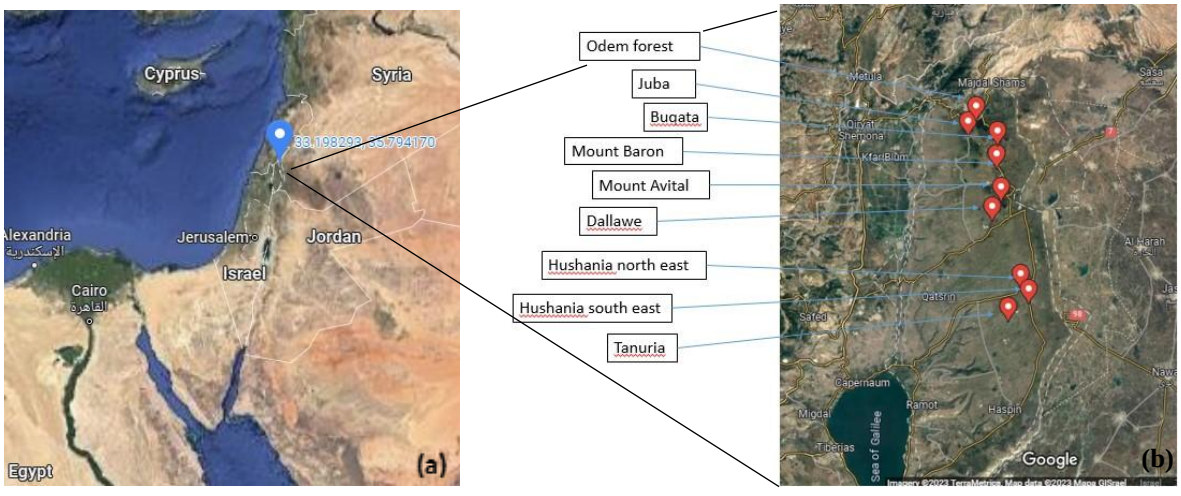

Fig. 1-(a) Golan Heights (b) Location of the study sites. The maps were provided by © Google Maps.

2.2 Sampling

Samplings for the Regional set took place in two campaigns in October 2020 and in January 2021. In each site, the sample was pooled from 2 sub-samples taken by a trowel from A-horizon at 0-10 cm depth in adjacent locations. The local set, detailed sampling at Mt. Baron, took place in October 2021, February 2022, and June 2022, which represent the beginning, middle, and end of the rainy season. At each campaign in Mt. Baron, the soil was sampled at 4 heights along the north and south slopes, under tree canopy, and in open grassland. Located in the northern hemisphere, the south-facing slope of the site has greater direct sunlight and drier soil. Each sample was pooled from 2 locations and taken by a trowel from A-horizon at 0-10 cm depth. The focus was on the surface layer since it is where most seasonal variability can be expected. On the southern slope, there are fewer samples under the tree canopy as there are fewer trees than in the northern slope. Litter from the open grassland and beneath the tree canopy of both slopes was sampled as well. Soil moisture content was measured gravimetrically (Majhi and Sarkar, 2019).

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2.3 ARQ

For measuring the ARQ, 3g of 2mm sieved soil was wetted with distilled water to different levels in the regional samples set (no wetting, 100 µl/g soil, 183 µl/g soil, field capacity (Dan et al., 1970)), or wetted to 60% of the water holding capacity (Namagembe et al., 2020) in the local samples set. The soil was incubated in a 6ml glass tube connected by Ultra-Torr tee fittings (Swagelok) to two 3.6ml glass flasks, equipped with with Louwer™ O-ring high-vacuum valves. The incubation was conducted in several temperatures between 5ºC to 43ºC. The length of incubations was adjusted to maintain aerobic conditions. At the cold incubation (5±1ºC) the first flask was closed after 6-7 days, and the second after 10-14 days, at room temperature incubation (23±1ºC) the first flask was closed after 2 days, and the second after 3 days, at 33ºC (only Mt. Baron samples) the first flask was closed after 3 hours and the second after 6 hours and at 43ºC (only Mt. Baron samples) the first flask was closed after 2.5 hours and the second after 5 hours.

To explore the effect of anaerobic microsites on ARQ we shaped artificial clods (1 cm diameter) with wetted soil from Mt. Baron. We expected that once anaerobic microsites would be formed in the clods, the O_2 influx to the soil would decrease (as alternative electron acceptors would be used), and ARQ would increase. To further test the effects of O_2 limitation we conducted an experiment in which we mitigated this limitation by enriching in oxygen the atmosphere of the incubation headspace. In this oxygen-enriched treatment, the incubation was set as usual with a glass tube connected by tee fittings to two glass flasks by Ultra-Torr. However, in these experiments one of the flasks was filled with pure O_2 instead of air before the experiment, and was opened to allow this O_2 to be mixed with the rest of the headspace at the beginning of each experiment. To trace the effect of the O_2 enrichment, these experiments were followed by second incubations with regular air, after ventilating the soil sample for 15 minutes. In these follow-up incubations, the tubes from 5ºC were transferred to incubation at 23ºC, and the tubes from 23ºC were moved to 5ºC. The ARQ was measured with a Hampadah system consisting of an infra-red gas analyzer (IRGA) for CO_2 measurement and a fuel-cell-based analyzer for measuring O_2 (Hilman and Angert, 2016).

2.4 Separation of particulate organic matter (POM) and mineral-associated organic matter (MAOM)

The organic matter of the local set of soils was separated to particulate organic matter (POM) and mineral-associated organic matter (MAOM) by a wet sieving method (Cambardella and Elliott, 1992; Garten et al., 2003). For this analysis, 10 grams of soil from Mt. Baron sieved to 2mm were dispersed by shaking overnight in 30 ml sodium hexametaphosphate (5 g L^{-1}) with 12 glass beads. The mixture was then sieved through a 0.053 mm sieve. The particulate organic matter (≥0.053 mm) was recovered by back-washing the sieve. The MAOM (<0.053 mm) was flocculated and collected using 1 g L^{-1} of $CaCl_2$ (Saenger et al., 2015). Both fractions were washed in distilled water and oven-dried to 60°C.

2.5 Rock-Eval

In the Rock-Eval, we analyzed soil samples from Mt. Baron and soil separated into POM and MAOM. In this analysis, a temperature-ramped pyrolysis stage is followed by a stage of temperature-ramped complete oxidation of the residual material. An FID detector measures hydrocarbons released during pyrolysis, while CO_2 and CO are detected by infrared absorbance during both stages. These measurements are used to calculate several parameters. The most important ones for the current study are detailed below and include the Hydrogen Index (HI, which relates to the hydrogen-to-carbon ratio), the Oxygen Index (OI, which relates to the oxygen-to-carbon ratio), and the total organic carbon (TOC) content (Albrecht et al., 2015). The parameters PC and RC represent the organic carbon content released in the pyrolysis stage and oxidation stage, respectively. In standard reporting of Rock-Eval data the TOC, PC, and RC are presented normalized to the dry soil weight and expressed as percent carbon. Here, we also normalized the PC and RC by TOC to assess the contribution of the different fractions of organic carbon to the TOC and to allow comparison between samples.

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Compared with organic-rich sedimentary rocks that have a single Gaussian peak in the FID above 300 °C, SOC usually has a more complex signal of overlapping peaks, with a saddle around 400 °C. The thermal stability of SOC is estimated from the Rock-Eval data, as detailed below, by the I-index, which is related to more labile SOC, and the R-index, which represents the more thermally stable SOC. The peak of the hydrocarbon released during the pyrolysis is divided into 4 components for different temperature ranges: A1 for 200–340 °C, A2 for 340–400 °C, A3 for 400–460 °C and A4 for 460< °C. The I-index and R-index are calculated according to the equations (Sebag et al., 2006; Albrecht et al., 2015):

$$\text{I-index} = \log((A1+A2)/A3) \quad (1)$$

$$\text{R-index} = (A3+A4)/100 \quad (2)$$

2.6 Statistical Analysis

The different sample locations and experimental implications were tested for homogeneity of variances using Bartlett's test. For the equal variances, we used one-way analysis of variance (ANOVA) and t-test for two groups in comparison and Tukey-Kramer when there were more groups in the comparison. For unequal variances, we used a Welch's test and nonparametric comparisons using Wilcoxon method for two treatments and Steel-Dwass for more groups than two. Significant differences were determined at $P < 0.05$. Robust Fit outliers were removed (K sigma 4, Huber).

All statistical analysis was done using the JMP software (JMP®, JMP Pro 17, SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Clods experiment

ARQ, O_2 uptake rate, and CO_2 efflux increased with temperature in both sieved soils and clods, while enriching the incubation headspace with oxygen lowered the ARQ (Tables 3,4). In incubation following the O_2 enrichment, the soils showed higher ARQ values regardless of temperature. At 23ºC , both the previously enriched and the non-previously-enriched incubations showed ARQ values greater than 1. O_2 addition raised the O_2 uptake rate in both incubation temperatures but raised the CO_2 efflux only at the 23ºC incubation (Table 4).

Table 3- ARQ, O_2 uptake rate, and CO_2 efflux in artificial clods and sieved soil incubations, cold 5ºC, and room temperature 22-24ºC. For each pair of clods and sieved soils at a given temperature, the 'a' and 'b' letters represent similarity or a significant difference in Wilcoxon test ($p < 0.05$) or *t*-test.

	Temperature	n	ARQ	Std. error		O_2 uptake rate (nmol 100 g^{-1} fresh soil s^{-1})	Std. error		CO_2 efflux (nmol 100 g^{-1} fresh soil s^{-1})	Std. error	
Artificial Clods	5ºC	8	0.74	0.003	a	1.85	0.02	b	1.33	0.07	b
Sieved soil	5ºC	10	0.75	0.015	a	2.19	0.11	a	1.63	0.06	a
Artificial Clods	23ºC	8	1.09	0.009	a	6.31	0.11	b	6.88	0.08	b
Sieved soil	23ºC	9	0.87	0.011	b	11.1	0.41	a	10.03	0.44	a

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Table 4- ARQ , O_2 uptake rate, and CO_2 efflux in artificial clods incubations, O_2 enriched vs. air. For each pair, different letters represent a significant difference in t-test ($p<0.05$).

Treatment	n	ARQ	Std Err		O2 uptake rate (nmol 100 g-1 fresh soil s-1)	Std Err		CO2 efflux (nmol 100 g-1 fresh soil s-1)	Std Err	
5ºC + O_2	6	0.58	0.014	b	2.35	0.04	a	1.35	0.04	a
5ºC air	6	0.83	0.011	a	1.51	0.04	b	1.25	0.04	a
23ºC + O_2	6	0.79	0.015	b	9.24	0.12	a	7.25	0.07	a
23ºC air	6	1.05	0.007	a	6.1	0.12	b	6.42	0.13	b
23ºC after 5ºC and O_2	6	1.12	0.006	a	4.81	0.1	a	5.4	0.11	a
23ºC after 5ºC air	5	1.11	0.004	b	4.89	0.11	a	5.4	0.12	a
5ºC after 23ºC and O_2	6	0.86	0.029	a	1.03	0.14	a	0.86	0.13	a
5ºC ater 23ºC air	4	0.77	0.011	b	1.36	0.17	a	1.05	0.02	a

3.2 Regional set

In the incubation of the regional set soils (spatial variability), the ARQ was higher in October than in January, with an average of 0.96±0.051 in October and 0.60±0.033 in January in the 5ºC incubation and 0.95±0.031 in October and 0.81±0.030 in January in the 23ºC incubation (Wilcoxon, $p<0.05$) (Fig. 2). No difference was found in the O_2 uptake rate between October and January in both the 5ºC incubation (Avg. of 1.4±0.9 nmol $100g^{-1}$ fresh soil s^{-1}) and 23ºC incubations (average of 5.4±4.5 nmol $100g^{-1}$ fresh soil s^{-1}). The wetted soils showed higher ARQ than the soils with no addition of water, with averages of 0.76±0.031 and 0.58±0.065 in the 5ºC incubation and 0.90±0.019 and 0.73±0.033 in 23ºC respectively (t-test, $p<0.05$). The water amount had no effect. Wetted soils showed higher values of O_2 uptake rate compared with no addition of water at 23ºC (averages of 5.7±0.4 and 2.3±0.9 nmol $100g^{-1}$ fresh soil s^{-1}) (t-test, $p<0.05$), but not in the 5ºC incubations (average of 1.4±0.4 nmol $100g^{-1}$ fresh soil s^{-1}). No correlation was found between ARQ and O_2 uptake rate, pH, soil moisture, bedrock type, bedrock age, soil texture, elevation, precipitation, cation exchange capacity, and specific surface area nor between O_2 uptake rate and these parameters.

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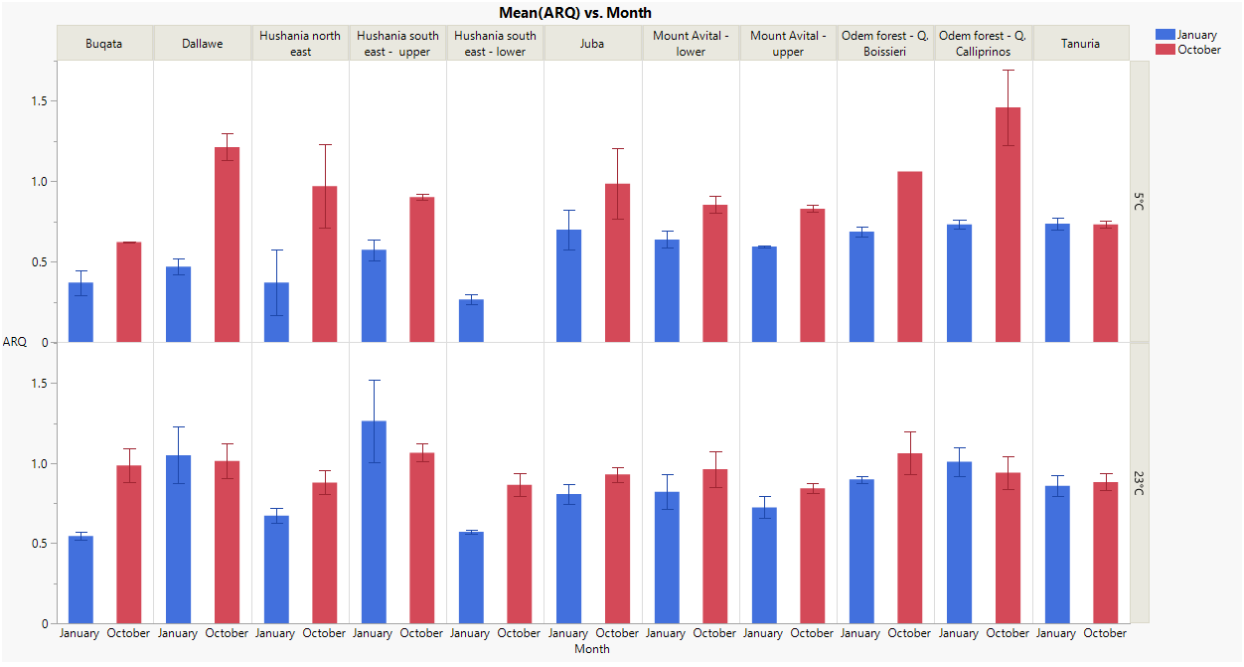

Fig. 2 - Golan incubations – 5ºC and 23ºC ARQ by month

3.3 Local set

The soil moisture content in Mt. Baron was higher on the northern slope in comparison to the southern slope in February, with values of 32.9% ± 0.7% and 28.7±0.7%, respectively. There were no differences between north and south in June and October, with averages of 8.1%±1.1% and 8.7%±2.4%.

The ARQ in Mt. Baron soil 5ºC incubations was the highest in October, lower in June, and the lowest in February, with values of 0.81±0.021, 0.70±0.020, and 0.45±0.020, respectively (Fig. 3). In the 23ºC incubations October and June had higher ARQ than February with averages of 0.90±0.022, 0.89±0.023 and 0.83±0.020 respectively ($p<0.05$ (Steel Dwass)) (In 5 samples out of 27 in October, all of the oxygen was consumed during incubation, there was no effect on the ARQ, probably due to the decreased respiration at this phase). The O_2 uptake rate in the 5ºC incubation showed the highest values in October, lower in June, and the lowest in February, with averages of 2.6±0.1, 1.3±0.1, and 0.6±0.1 nmol $100g^{-1}$ fresh soil s^{-1}, respectively (fig. 4). In the 23ºC incubations October and June had higher O_2 uptake than February with averages of 14.1±1.2, 12.1±1.2 and 3.3±1.1 nmol $100g^{-1}$ fresh soil s^{-1} respectively (Steel Dwass, $p<0.05$). In the incubations at 33ºC and 43ºC, there was no difference between months in ARQ (averages of 1.00±0.213 and 1.05±0.173, respectively. The 33ºC incubation was performed only in June and February). In the incubations at 33ºC the O_2 uptake rate was higher in June than in February, with averages of 32.2±1.9 vs. 7.9±1.9 (Wilcoxon, $p<0.05$), and at 43ºC October and June had higher rates than February with averages of 36±3.7, 35.4±2.7, and 13.7±2.6 nmol $100g^{-1}$ fresh soil s^{-1} respectively (Steel Dwass, $p<0.05$).

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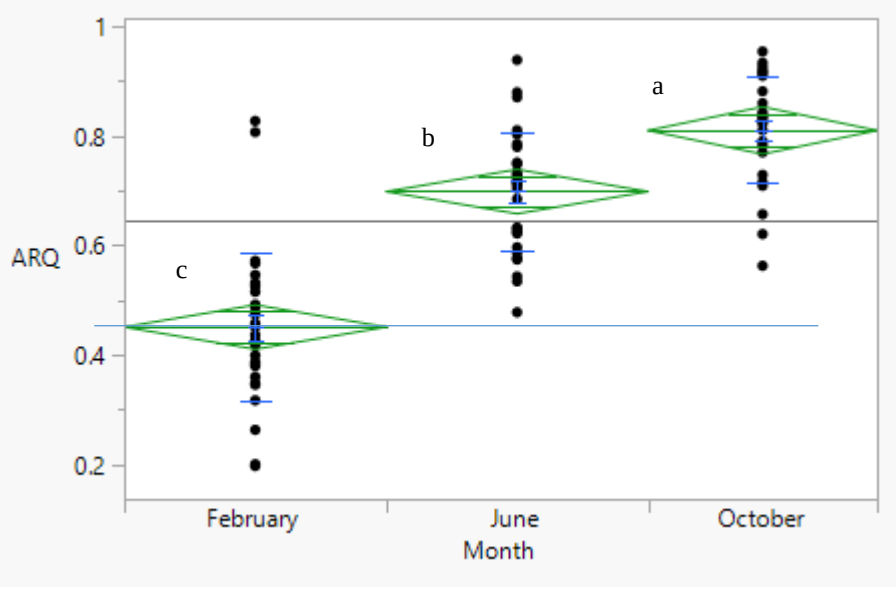

Fig. 3– ARQ by month in Mt. Baron soil incubations at 5ºC. The line across the middle of each diamond represents the treatment's mean. The top and bottom of each diamond represent the 95% confidence interval. The error bar represents the standard error. The horizontal line is the grand mean. Different letters represent a significant difference in the Tukey Kramer test ($p<0.05$).

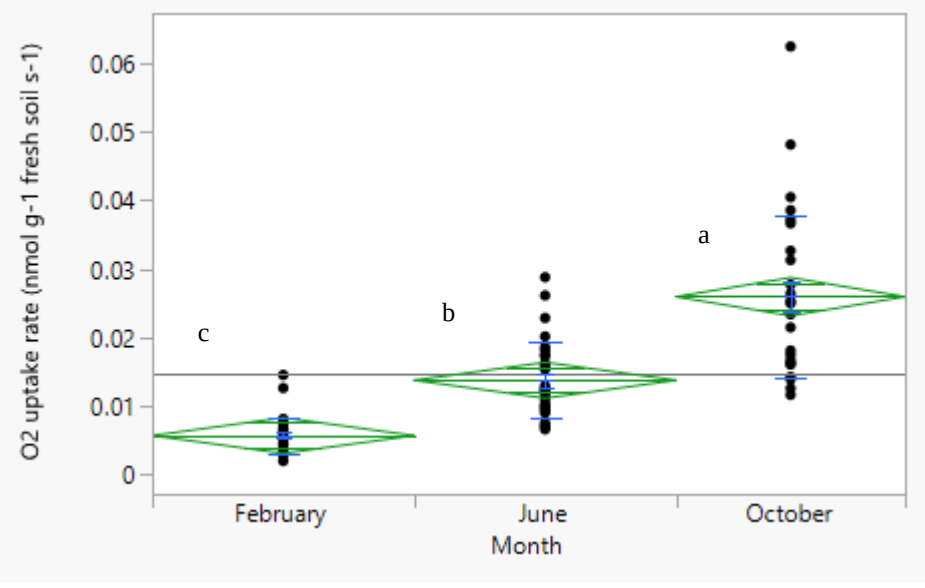

Fig. 4– O_2 uptake rate by month in Mt. Baron soil incubations at 5ºC. The line across the middle of each diamond represents the treatment's mean. The top and bottom of each diamond represent the 95% confidence interval. The error bar represents the standard error. The horizontal line is the grand mean. Different letters represent a significant difference in Steel Dwass test ($p<0.05$).

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The rates of O_2 uptake and CO_2 efflux at Mt. Baron soils rose exponentially with temperature, and the calculated Q10 values are between 2 to 3 (Table 5). The ARQ values also increased with temperature (Fig. S1).

Table 5 - Q10 values of O_2 uptake rate and CO_2 efflux in Mt. Baron soil incubations ± standard error.

Month	Q10 O_2	Q10 CO_2
October	2.1±0.10	2.2±0.09
February	2.3±0.03	2.8±0.03
June	2.4±0.12	2.6±0.12

ARQ under tree canopy was lower than in open grassland in the 5ºC incubation, equal in 23ºC, and higher at 33ºC, and 43ºC (Fig. 5). The O_2 uptake rate in the 5ºC incubation was higher in grassland than under canopy with averages of 1.7±0.2 and 1.2±0.2 nmol $100g^{-1}$ fresh soil s^{-1} (Wilcoxon, $p<0.05$). In the warmer incubation, there were no differences in O_2 uptake rate between soils under canopy and grasslands (averages of 9.5±2.6, 20.2±4.1, and 26.8±4.1 for 22-24ºC, 33ºC, and 43ºC). There were no differences in ARQ and O_2 uptake rate between the north and south slopes, but the differences between soils under tree canopies and grasslands in the 5ºC incubation were more pronounced in the southern slope. There was no correlation between ARQ and elevation nor between O_2 uptake rate and elevation.

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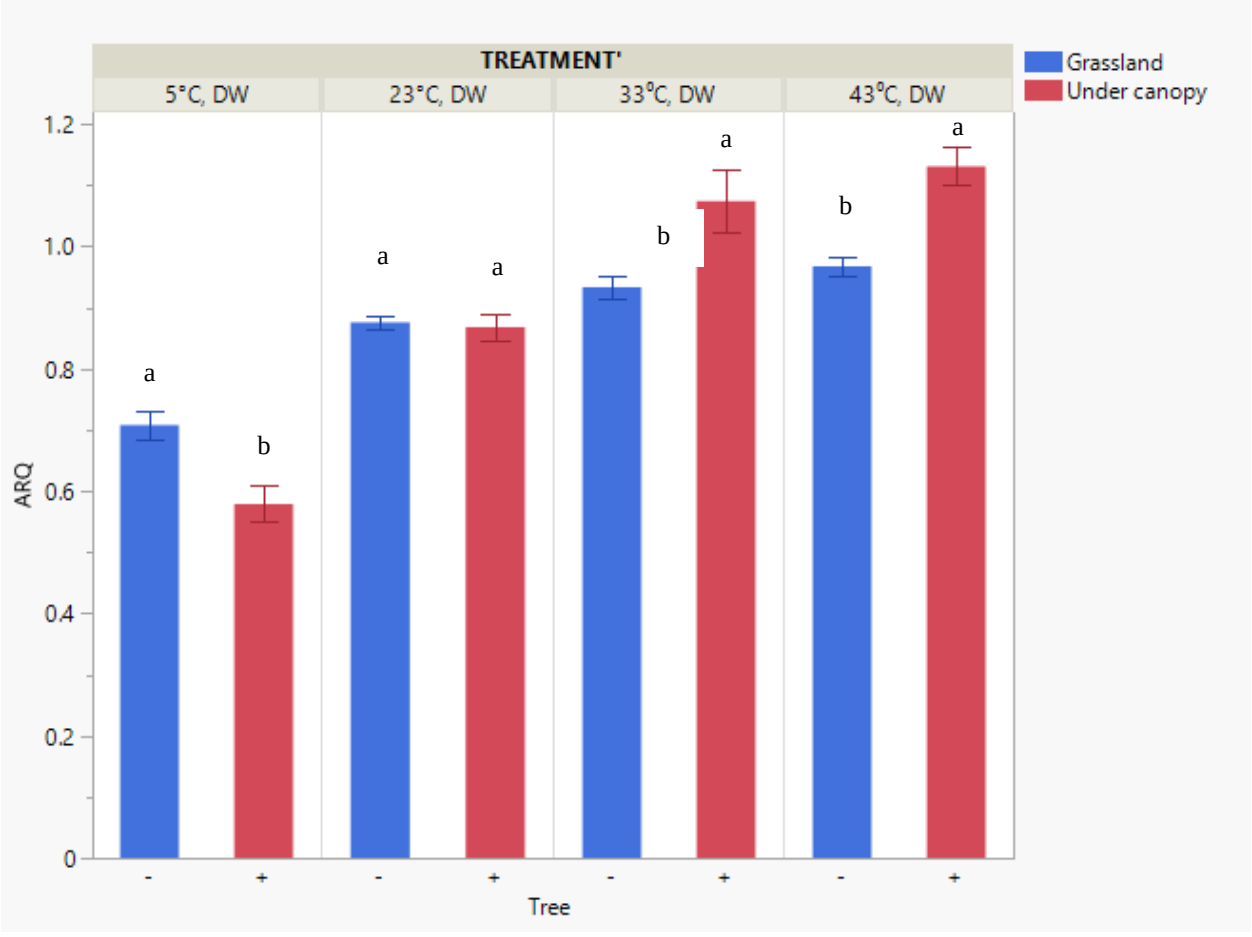

Fig. 5 – Average ARQ in Mt. Baron soil incubations at different temperatures, under canopy (+) vs. grassland (-). Different letters represent a significant difference in Wilcoxon test or in t-test (5ºC treatment) (p<0.05) for each (+, -) pair.

In the Rock-Eval analysis of north versus south slopes, the north slope samples showed higher TOC and HI values than the south slope, while RC/TOC, R index in open grassland, and I index under trees, were lower in the north than in the south slope (table 6). The HI was higher in Oaks litter than in grass, with values of 414.5±23.05 and 307.5±23.05, respectively. The OI of Oaks litter was lower than grass with values of 183.5±11.24 and 247.5±11.24.

Table 6 – North versus south slopes differences in Rock-Eval parameters in Mt. Baron soil

	North	South	Prob>F
TOC (%)	4.1	3.2	0.0395
HI	165	131	0.0371

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RC/TOC	0.77	0.79	0.0123
R index in open grassland	0.63	0.68	0.0448
I index under tree	0.01	0.06	0.0416

At the POM fraction, I index, HI and TOC are higher at the north slope while R index and RC/TOC are lower at this slope (Table 7).

Table 7 – North/south slopes differences in Rock-Eval parameters at the POM fraction in Mt. Baron soil.

	North	South	Prob>F
R index	0.39	0.51	0.0131
I index	0.44	0.25	0.0115
HI	402	294	0.0066
TOC	8.8	5.3	0.0053
RC/TOC	0.60	0.69	0.0042

Averaging the samples from all locations, throughout the year, the TOC is lower in February, the I index is lower in October, and the R index is higher in October. The HI is higher in October than in February, and the OI is higher in February than in October. The RC is higher in June than in February while the RC/TOC is higher in February than in October. The ARQ, O2 uptake rate, and CO2 efflux are higher in October, lower in February, and lowest in June (Table 8). In this section we chose to refer to the ARQ and respiration rate at the 5°C incubation which preserve soil state in the field, all incubation results are presented at the incubation section.

Table 8 - Seasonal changes in Rock-Eval and 5ºC incubation parameters, averaged over all sampling locations in Mt. Baron soil. S1 and RC were also normalized by TOC. Different letters ("a", "b") represent a significant difference in Tukey Kramer test or in Steel Dwass test (p<0.05).

	October	February	June	Prob>F
TOC (%)	a	b	a	
	4.03	2.90	4.10	0.0143
R index	a	b	b	
	0.70	0.66	0.64	0.0024
I index	b	a	a	
	-0.01	0.06	0.07	0.0069

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HI	a	b	ab	
	173.0	121.2	155.1	0.0319
OI	b	a	ab	
	196.5	232.7	212.8	0.0059
RC (%)	ab	b	a	
	3.02	2.29	3.15	0.0110
RC/TOC	b	a	ab	
	0.76	0.80	0.78	0.0036
ARQ 5ºC	a	c	b	
	0.81	0.45	0.70	<0.0001
O2 uptake rate 5ºC (nmol 100 g^{-1} fresh soil s^{-1})	a	c	b	
	2.59	0.57	1.38	<0.0001
CO2 efflux 5ºC (nmol 100 g^{-1} fresh soil s^{-1})	a	c	b	
	2.19	0.31	1.02	<0.0001

We found a correlation between HI and TOC (R^2=0.77, Fig. S3), and between PC and the 5ºC incubation rates, both O2 uptake (R^2=0.53, Fig. S5) and CO_2 efflux (R^2=0.58, Fig. S6). We also found a correlation between the ratio RC/TOC and OI (R^2=0.57, Fig. S7). An inverse correlation was found between I index and R index (R^2=0.99, Fig. S1), HI and OI (with more moderate gradient in the south) (R^2=0.78 in the north and R^2=0.57 in the south, Fig. S4). The TOC, I index and HI are higher at the POM fraction while R index, OI, Tmax and TpkS2 are higher at the MAOM fraction (Table 9).

Table 9 – POM and MAOM Rock-Eval parameters in Mt. Baron soil

	MAOM	POM	Prob>F
TOC (%)	2.60	7.07	<0.0001
R index	0.58	0.45	<0.0001
I index	0.16	0.35	<0.0001
HI	137.1	347.9	<0.0001
OI	251.4	155.1	<0.0001
Tmax (°C)	393.8	337.2	<0.0001
TpkS2 (°C)	434.4	375.8	<0.0001

In the MAOM fraction, the HI and PC/TOC have a positive correlation to elevation (the HI to elevation R^2=0.67, Fig. S8, the PC/TOC to elevation R^2=0.76, Fig. S9) while there is an inverse correlation between RC/TOC and elevation (R^2=0.74, Fig. S10).

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In the POM fraction, there is a positive correlation between ARQ 5ºC and I index (R^2=0.46, Fig. S12), PC/TOC (R^2=0.46, Fig. S15) and HI (R^2=0.51, Fig. S17), between O_2 uptake rate 5ºC and I index (R^2=0.49, Fig. S13), and between O_2 uptake rate 5ºC and HI (R^2=0.55, Fig. S18). Inverse correlation is between ARQ 5ºC and R index (R^2=0.43, Fig. S11), OI (R^2=0.39, Fig. S14), and RC/TOC (R^2=0.51, Fig. S16).

4 Discussion

4.1 Respiration rates and ARQ response to temperature and past conditions

The Q10 values of O_2 uptake rate and CO_2 efflux are around 2-3 (table 5) and are similar to the values reported in the literature (Hilman et al., 2022a; Meyer et al., 2018; Curiel et al., 2004; Chen et al., 2010). The Q10 values for O_2 fluxes were lower than for CO_2, suggesting that some of the processes involving O_2 are different from the processes involving CO_2, e.g., Fe oxidation or oxidative depolymerization.

In the incubations of sieved soils, the ARQ values showed an increase with temperature (Fig. S1), which is similar to what we found in lab incubations in a previous study in the same region (Hilman et al., 2022b). A possible reason for that is that higher respiration rates, associated with the higher temperatures, cause anoxia in SOM-rich microsites and that anaerobic respiration at these microsites resulted in high ARQ. This is since in anaerobic respiration, O_2, as an electron acceptor, is replaced by alternative electron acceptors like Fe^{3+} and NO_3 (Hicks Pries et al., 2020). A possible similar effect of anoxic microsites on ARQ and Fe^{2+} concentrations was shown for the wetting and drying of soil aggregates (Figure 5, Hilman et al., 2022b).

An alternative explanation for shifts in ARQ with temperature could be changes in the respiratory substrate. For example, ARQ values above 1 could result from the decomposition of organic acids. However, we did not find references showing higher organic acids decomposition at higher temperatures. Similarly, low ARQ values of around 0.6 could be explained by lipids as the respiratory substrate. Again, there is no evidence that lipid utilization will be more common in lower temperatures, and the higher energy barrier for lipid utilization (LaRowe and Van Cappellen, 2011) makes this suggestion unlikely. Few studies (Angert et al., 2015, Dilly, 2001) reported ARQ values above 1 following sucrose addition to soil. Such an increase in ARQ can be explained by an increase in the anaerobic microsites volume due to a substrate-induced increased respiration rate. However, a recent study (Smart et al., 2024) suggested that such a temporary increase in ARQ to values well above 1 following sucrose amendment is the result of microbial biosynthesis of biomass and extracellular compounds. However, the model presented in that study cannot explain the ARQ values below 1 we often measured, and our experiments did not include substrate amendment.

We are left then with the anoxic microsite hypothesis as the most likely explanation for the ARQ dependence on temperature. Based on this hypothesis, the coldest incubations, at 5ºC, which are associated with lower respiration rates, create less anoxic microsites during the non-saturated experimental conditions. Thus, we suggest here that 5ºC incubations provide better information on the SOM currently in the soil. Moreover, we suggest that incubation at 5ºC can provide information on the soil's past state. For example, if the soil was subjected to anaerobic conditions and gained reduced chemical species like Fe(II) and reduced organic matter, the ARQ in subsequent 5ºC incubation will be low due to their oxidation.

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To test directly if higher ARQ values are related to anoxic microsites, we set up the experiments with oxygen-enriched headspace, and with the artificial clods. We expected the oxygen enrichment would decrease the anoxic volume, and thus decrease the ARQ, and that the clods would have the opposite effect. Indeed, we found ARQ values higher than 1.0 for the 23ºC incubation of artificial clods, which might be due to anoxic microsites, created by respiration rates that are higher than the slow oxygen diffusion in the clods.

Also, in agreement with our hypothesis about the relationship between anoxic microsite and ARQ we found that in contrast to clods incubations with regular air, in sieved soil, and in incubations of clods in oxygen-enriched hedspace, the ARQ values were below 1. We suggest that in the 5ºC temperature incubations, the oxygen consumption rate was too slow to form such anoxic microsites (Tables 3, 4). In a similar way, enriching the headspace with O_2 also limited the formation of those microsites, as indicated by ARQ values below 1. However, it should be noted that the lower ARQ at high O_2 can also be explained by an increase in oxidase activity and increased depolymerization of lignin and other aromatic substances, which consume oxygen without releasing CO_2 (Li et al., 2022; Quideau et al., 2001).

In an additional experiment, the clods that were incubated previously at 23ºC were incubated again immediately after the first incubation at a 5ºC temperature. This experiment was aimed at testing the effects of past conditions. The ARQ values at the second incubation of formerly non-O_2-enriched samples were lower than the ARQ of samples formerly enriched with O_2. As suggested above, the second incubation, in this case, might reflect soil history and be related to the formation of anaerobic conditions in microsites in the first (non-O2-enriched) incubation, and oxidation of these reduced chemical species during the second incubation.

The same logic can be used to explain the lower ARQ measured in the winter in both the regional sample set (spatial variability) and the local set (Mt. Baron temporal variability) (Figs 2-3, S2). High moisture content in the winter limits oxygen diffusion and creates anoxic microsites that form reduced chemical species. In agreement, a previous study found lower ARQ during winter for soil-air in-situ measurements (Hicks Pries et al., 2020; Hilman et al., 2022b) which is a different approach than the lab incubation approach used here. During the incubation in better-aerated conditions (sieved soil) in the lab, reduced species oxidation lowers the ARQ measured at the low temperatures (which does not promote the creation of anoxic sites again). In October, which is at the end of the dry season, the soils are sampled after being dry for several months. As a result, the soils are well aerated and oxidized, and since no reduced species are present the ARQ in the incubations is higher. McLatchey and Reddy (1998) found that the soil microbial biomass gradually decreased as soils became more reduced. This, in addition to the low temperatures effect on the microbial community in the winter, can explain the O_2 uptake rate values, which are the lowest in January, increase in June, and highest in October (Fig. 4). Another possible explanation for the high October ARQ is the buildup of litter that was not decomposed during the dry season because of the lack of available moisture. This, together with the partial breakdown of organic polymers by sun radiation and the high summer temperatures (Austin and Vivanco, 2006; Schechter et al., 2023), creates a large pool of readily decomposable SOM.

4.2 Differences between soil under trees and open grassland

Previous research suggested that oak trees create enhanced fertility islands with nutrient enrichment beneath the canopy (Dahlgren et al., 2003; Dahlgren et al., 1997; Perakis et al., 2007). Lacroix et al., (2022) suggested that oxygen demand, rather than supply, can regulate anoxic microsite formation. Accordingly, the increased fertility under oak canopies might induce higher activity and increased oxygen consumption that forms anoxic microsites. This can explain the high ARQ values found under tree canopies at high-temperature incubations (Fig 5). Indeed, Uroz et al. (2010) found indications for such anoxic conditions under oaks by detecting sequences related to anaerobic ammonium-oxidizing bacteria. Anaerobic conditions in microsites at the past, which generated reduced chemical species, can explain the lower ARQ values under tree canopy at the low-temperature incubation. Anoxic microsites may slow SOM turnover (Lacroix et al., 2022), which can explain the lower O_2 uptake rates found under tree canopies at low-temperature incubation. This formation process of anoxic microsites might be acting more on the southern slope due to higher temperatures, which promotes faster respiration. The

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anoxic microsites can preferently inhibit microbial oxidation of lipids and waxes (Keiluweit et al.,2016). The presence of lipids and easily degradable components such as alkyl-C can explain the higher I index under the trees in the south slope (Table 6) (Albrecht, 2015). This correlates well with the low ARQ values measured beneath the tree canopy at the 5ºC incubation since lipid oxidation is associated with low ARQ. In addition, for oak leaf litter the HI was higher, and OI was lower than in the grass litter, suggesting more lipid-like compounds with lower ARQ under the oaks. Expanding our hypothesis of what happens under oak canopies and observing the ARQ and its dependence on oxygen demand is interesting in the context of climate change.

Global climate change can have mixed effects as more intense rain events can increase the size of anaerobic domains in soils, while prolonged droughts may lower it (Keiluweit et al.,2016). It can be expected that rising temperatures will lead to higher oxygen consumption and increased soil anoxic microsite formation, especially in fertile landscapes (as we see under oak canopies), which in turn can increase the stability of soil organic carbon. This scenario is expected with higher probability in regions like the Indian subcontinent, which combine high soil nutrient content (total exchangeable bases) (Huston and Wolverton, 2009), with increased flood risk under the warming climate (Ali et al., 2019) and relatively high annual temperatures which are predicted to rise in 2.5-5°C by the end of the century (Kumar et al., 2006).

4.3 Differences between the south and the north slopes

We found higher soil moisture in the north slope during winter, as well as higher TOC, HI values, and lower RC/TOC ratio (Table 6), probably resulting from higher primary production of organic matter at this wetter slope. The intensive growth of *Vicia tenuifolia Roth* at the grassland on the north slope supplies large amounts of fresh organic matter and is probably related to the lower R index at the north slope's grassland. The POM of the north is more labile according to the R and I index. In addition, the HI, and the RC/TOC values of the POM (Table 7) are in agreement with the HI and RC/TOC of the bulk soil samples, which showed less degraded organic matter at the north slope (Table 6).

4.4 Seasonal variations

The TOC is higher in June and October, while in February, after the beginning of the rainy season, TOC levels are lower, probably as a result of intensive decomposition. (Table 8). The decrease in HI, and the increase in OI and RC/TOC also agree with this suggestion of strong SOM degradation from October to February, which leaves behind SOM with lower H/C ratio and higher O/C ratio (Alburquerque et al., 2009) (H-C bonds are more energetic than O-C bond) and a higher fraction of carbon that cannot be release by pyrolysis. Indeed, while sampling in February, we found much less litter on the ground than in the other samplings. Between June and October, the changes in the I and R indices imply a strong reduction in the labile organic matter, and the organic matter that is left in October is more stable. Although the TOC in June and in October is similar, and so are the soil temperature and soil moisture, the respiration rate in the 5ºC incubation is higher in October. Kukumägi et al. (2014) found increased biomass below ground at misting experiments and hypothesized higher humidity increases heterotrophic respiration, it might be that the higher relative humidity in the period before sampling in October led to a better state of the microbial community and to higher respiration rates than June.

4.5 Relationship between soil indices

The correlations between the Rock-Eval indices (Figs. S3-S8) may indicate the following: The HI to TOC correlation shows that the SOM becomes less reduced during decomposition, as was found previously (Barré et al., 2016). This is also indicated by an increase in OI. Less organic matter is associated with more degraded SOM, probably due to the

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decomposition of the more liable fraction. We also found that the more decomposable organic matter is during pyrolysis (PC), the more respiration there is in 5ºC incubation (Figs. S5-S6). This observation shows a link between thermal stability and biological decomposition. In agreement, we also found that the more oxygenated the SOM (higher OI) is, the less it is decomposable during pyrolysis (higher RC/TOC).

In addition, we found in these surface soils less mineral-associated organic matter than particulate organic matter, yet the POM fraction was separated only by size (as in Garten et al. (2003), and not by density, as in John et al. (2005)), and is far from being pure organic matter as indicated by its low TOC (Table 9). According to all the Rock-Eval parameters, the MAOM is more degraded and more thermally stable. In the MAOM fraction, the HI and PC/TOC increased with elevation, and the RC/TOC decreased with elevation (Figs. S8-S10). It could be due to erosion and accumulation of fine particles with more degraded soil organic matter down the slope.

According to the correlations between R index, I index, HI, OI, PC/TOC, or RC/TOC, and ARQ at the POM fraction, the ARQ is lower when the SOM is more degraded and more oxidized soil (Figs. S12-S13, S15-S18). This is opposed to the expectation that the ARQ would be lower for substrates that are more reduced due to higher oxygen consumption per mole of CO_2 released. This can be explained by the fact that only a small fraction of the SOM is available for bacterial consumption. Siewert et al (2012) found a correlation between thermal mass losses at temperature interval from 260 to 320°C and soil respiration in 1 day incubation, similarly, we found a positive correlation between O_2 uptake rate at 5°C and I index in the POM fraction (Fig. S14) (again thermal versus biological indicators for SOM stability) and an increase in O_2 uptake rate with an increase in HI (Fig. S18), indicating that the respiration rates are higher when the reduced and easily degradable fraction is larger.

5 Conclusions

Combining the measurements of CO_2 and O_2 fluxes during soil incubations with Rock Eval pyrolysis can provide a wider and more complete understanding of the state of the organic matter in the soil. Rock-Eval measurements characterize the total organic matter in the soil, while the incubations show the small but important, fraction of organic matter that is immediately biologically available. Based on our results, we suggest that incubation at low temperatures can reflect the history of the soil and indicate past anaerobic conditions that resulted in soil rich in reduced chemical species expressed in a lower ARQ.

Vegetation type was found to affect the ARQ in soil and the respiration rate. Slope facing was found to be important as the south-facing slope organic matter was more mature and stable. Seasonality affected ARQ, respiration rate, and the Rock-Eval parameters, with a large difference between fall and winter in all parameters. The current detailed study of spatial (regional and within a single site) and temporal (seasonal) variations in respiration and Rock-Eval parameters should be used to better plan and understand border studies that are done in lower spatial resolution and are often based on single-time sampling.

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