

1 Subsurface CO₂ dynamics in a temperate karst system reveal 2 complex seasonal and spatial variations

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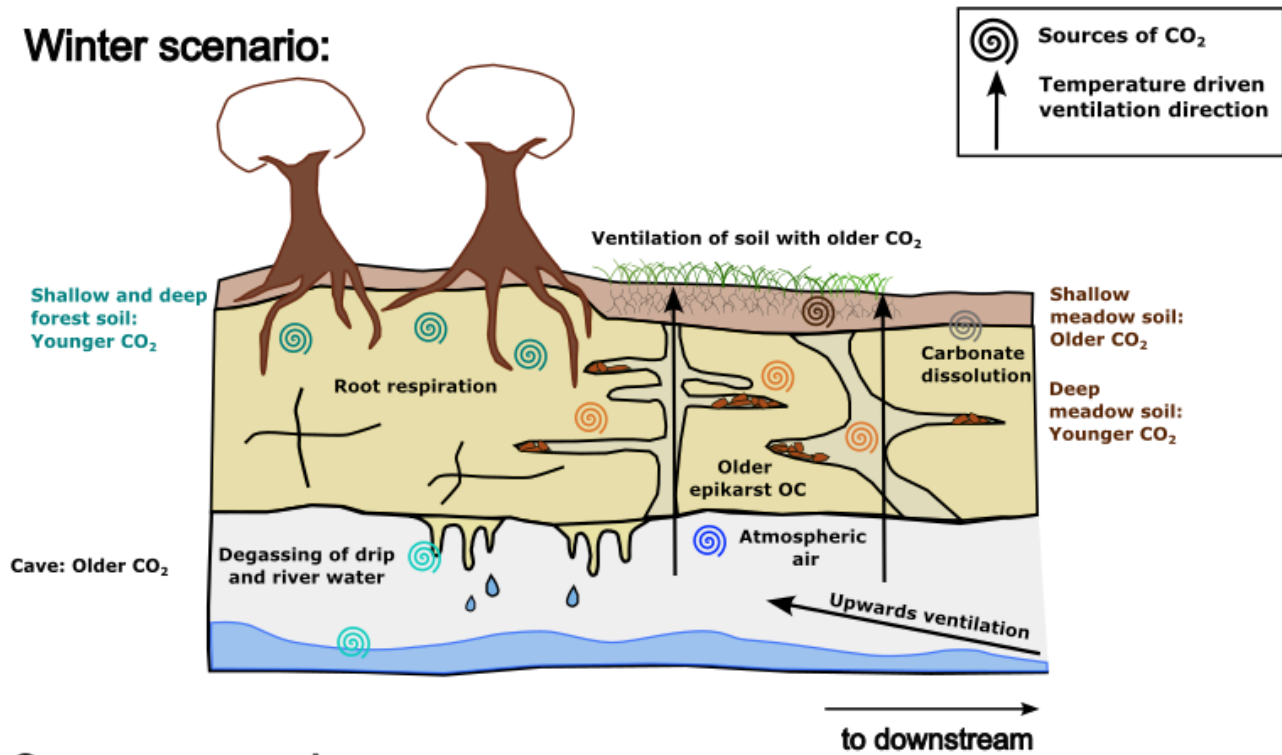
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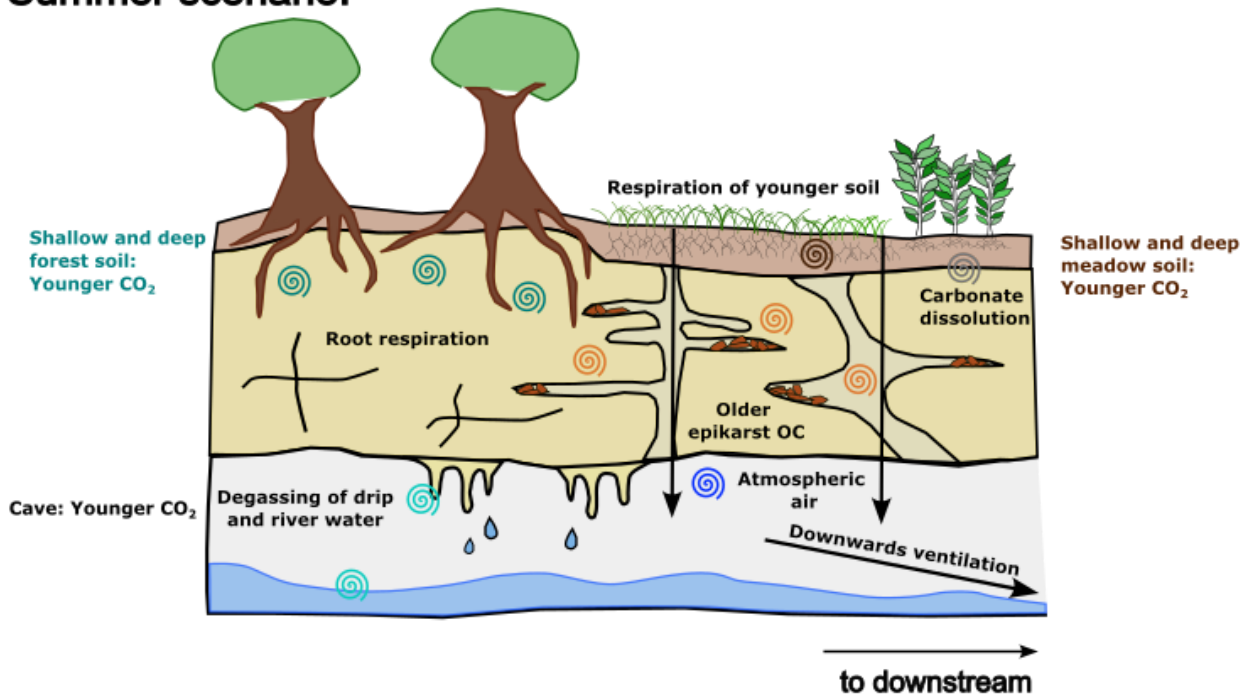
12 **Abstract.** Understanding the carbon cycle of the terrestrial Critical Zone, extending from the tree canopy to the aquifer, is
13 crucial for accurate quantification of its total carbon storage and for modelling terrestrial carbon stock responses to climate
14 change. Caves and their catchments offer a natural framework to sample and analyse carbon in unsaturated zone reservoirs
15 across various spatial and temporal scales. In this study, we analyse the concentration, stable carbon isotopic ratio ($\delta^{13}\text{C}$), and
16 radiocarbon (^{14}C) compositions of CO₂ from the atmosphere, boreholes (0.5 to 5 m depth), and cave sampled every two
17 months over two years at Milandre cave in northern Switzerland. High concentrations of up to 35,000 ppmV CO₂ are
18 measured in the boreholes. The $\delta^{13}\text{C}$ values of CO₂ in the boreholes reflect the $\delta^{13}\text{C}$ of C3 plants ($\sim -26\text{‰}$) which dominate
19 the catchment ecosystem. Shallow meadow boreholes host older CO₂ in winter and modern CO₂ in summer, while forest
20 ecosystems consistently export modern CO₂ ($F^{14}\text{C} = \sim 1$) to the unsaturated zone. Cave CO₂ concentrations exceed
21 atmospheric levels and are diluted by temperature-driven seasonal ventilation. Keeling plot intercepts indicate that the cave
22 CO₂, which mixes with atmospheric CO₂, is younger in summer ($F^{14}\text{C} = 0.94$) and older in winter ($F^{14}\text{C} = 0.88$), with a $\delta^{13}\text{C}$
23 consistent with the C3 plant dominated catchment. Mixing models utilising drip water dissolved inorganic carbon ^{14}C
24 suggest that varying carbonate dissolution and degassing dynamics do not explain the $F^{14}\text{C}$ variation and concurrent $\delta^{13}\text{C}$
25 stability of the mixing endmember. Rather, contributions from aged carbon reservoirs in the deeper unsaturated zone are
26 likely. This study provides valuable insights into CO₂ source dynamics and cycling within the karstic Critical Zone,
27 highlighting the impact of seasonal variations and ecological factors on downward carbon export from terrestrial ecosystems.

28

Winter scenario:



Summer scenario:



30 1 Introduction

29
31 Within the context of current and future climate change, investigations into the response of Critical Zone carbon pools to
32 rising temperatures and changing hydroclimatic conditions are crucial (Brantley et al., 2007). Recent studies have
33 specifically highlighted the unsaturated zone as a potentially important but poorly constrained reservoir of gaseous CO₂
34 (Mattey et al., 2016; Noronha et al., 2015; Keller, 2019; Stewart et al., 2022). Estimates suggest that between 2 to 53 PgC
35 could be present in the form of CO₂ in the unsaturated zone globally (Baldini et al., 2018). This carbon is particularly
36 vulnerable to changes in the water table level, whereby rises may easily result in the rapid release of CO₂ into the atmosphere
37 (Baldini et al., 2018). Despite its importance, comprehensive assessments integrating spatial and temporal variability of
38 shallow subsurface CO₂ remain scarce.

39
40 Understanding unsaturated zone CO₂ dynamics is complicated by the different sources of carbon contributing to the
41 subsurface reservoir. The CO₂ present in the unsaturated zone is often referred to as ground air and was first defined as CO₂
42 produced by microbial oxidation of organic material which was transported from the surface (Atkinson, 1977). However,
43 ground air can also refer more generally to high CO₂ concentrations in the subsurface, without linking its presence to a
44 particular source. Early evidence for the presence of a ground air reservoir was the observation of high CO₂ concentrations
45 found in deep cave passages with poor connection to the catchment surface, suggesting an endogenous source of CO₂
46 (McDonough et al., 2016). The age profile of this high concentration CO₂ reservoir, however, varies by site, suggesting
47 significant variability in its source and processing. CO₂ reservoirs of a lower F¹⁴C, corresponding to an older age, have been
48 reported (Breecker et al., 2012; Noronha et al., 2015; Mattey et al., 2016; Bergel et al., 2017), as well as modern subsurface
49 CO₂ pools, likely derived from ecosystem respiration of recently fixed carbon (Campeau et al., 2019; Tune et al., 2020).
50 Consequently, the primary sources of subsurface CO₂ generally considered are 1) the outside atmosphere (Kukuljan et al.,
51 2021), 2) catchment soil and vegetation respiration (Breecker et al., 2012; Li et al., 2024), 3) microbial respiration in the
52 unsaturated zone (Mattey et al., 2016; Ravn et al., 2020), 4) carbon dissolved from the carbonate bedrock (Milanolo &
53 Gabrovšek, 2015), and 6) volcanic and metamorphic hydrothermal input (Chiodini et al., 2008; Girault et al., 2018).

54
55 Carbon isotope analysis of CO₂ can be used to differentiate the contributing sources to the subsurface gas mixture.
56 Specifically, the $\delta^{13}\text{C}$ of CO₂ in the unsaturated zone can provide information about the influence of biological fractionation
57 by overlying catchment vegetation and microbial respiration, and the influence of carbonate dissolution and subsequent
58 degassing (Breecker et al., 2017; McDonough et al., 2016). In addition, due to the contrast between the ¹⁴C content of
59 biospheric carbon, which ranges from bomb peak enriched to >10,000 years old, and carbonate rock which is typically
60 radiocarbon dead, ¹⁴C measurements of subsurface CO₂ can provide information for both source apportionment and carbon
61 turnover rates in the terrestrial subsurface (Noronha et al., 2015).

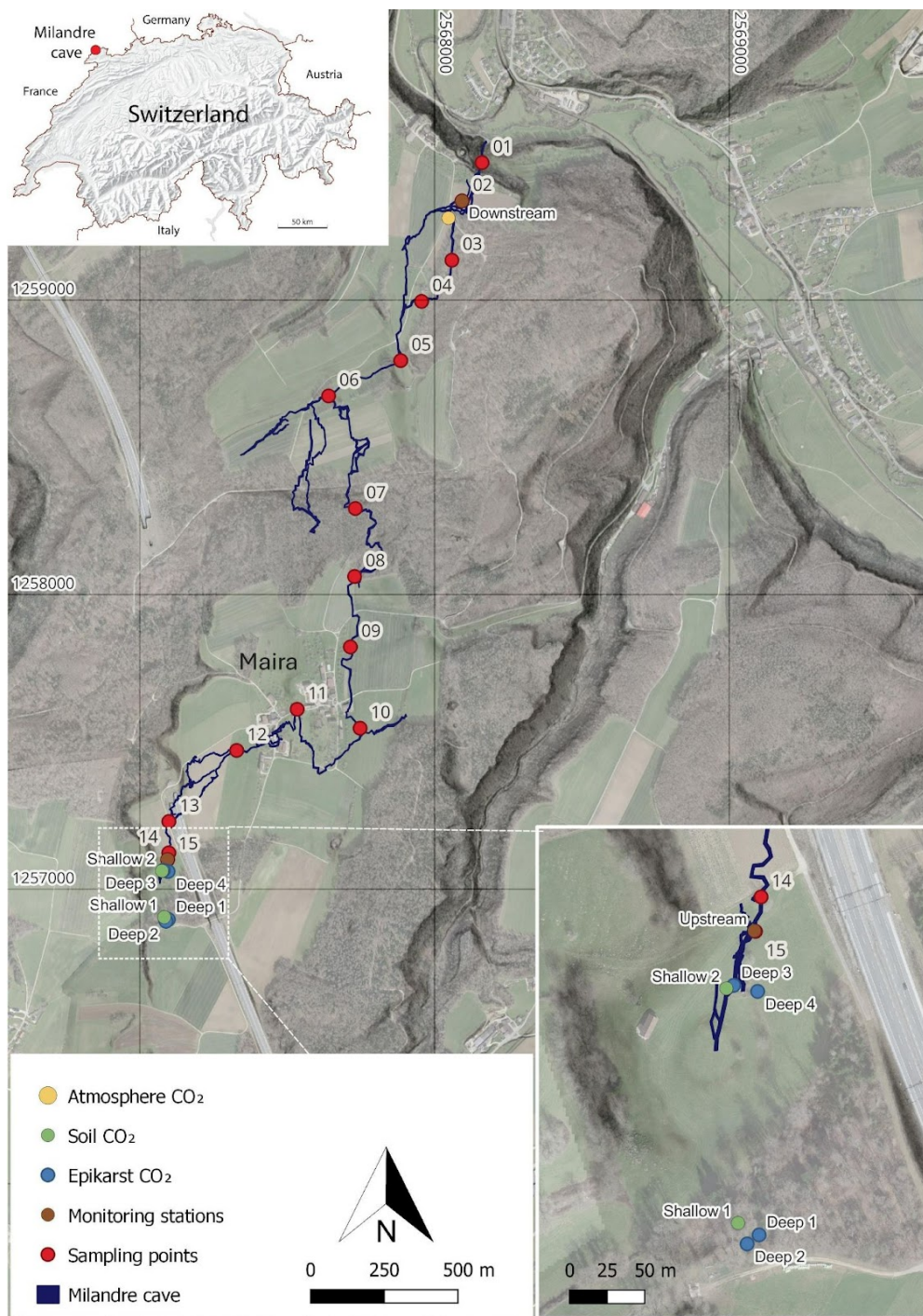
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Though there has been extensive work investigating unsaturated zone CO₂ reservoirs, questions remain about the concentration, composition and sources of ground air on temporal and spatial scales. This study seeks to address this gap by presenting a detailed assessment of CO₂ concentrations and isotopic compositions over 2 years within the Milandre cave karst system in Switzerland. We aim to 1) Determine the relationships between pCO₂, δ¹³CO₂, and ¹⁴CO₂ in Milandre cave and its catchment; 2) Assess the effect of seasonality on CO₂ concentration and isotopic characteristics and 3) Gain insights into the provenance of CO₂ and how these evolve over time.

2 Site Overview

Milandre cave (47.4852 ° N, 7.0161 ° E, 373m a.s.l.) is located in the municipality of Boncourt in the Jura canton, NW Switzerland (Fig. 1). The Milandre karst structure formed within the Jura mountains, a sub-alpine mountain range which lies laterally in a northwest-southeast direction. The cave is located within the external Plateau unit of the Jura mountains which consists of thin sub horizontal Mesozoic limestone units that have experienced deformation which produced imbricates and strike-slip faults (Sommaruga, 2011). More specifically, the Milandre karst system formed within the Late Jurassic (Middle Oxfordian) St-Ursanne Formation which overlies Early to Middle Oxfordian marls (Jeannin, 1998). The unsaturated zone ranges between 40 and 80 m depth. The active karst level developed in the epiphreatic zone, with the underneath phreatic zone extending for ~ 20 m to reach the low permeable Lower Oxfordian marls (Liesberg Mb) (Perrin et al., 2003). The cave is formed over 2.7 km, with 10.5 km of galleries forming along N-S oriented fractures. The Milandre river flows for 4.5 km in a northerly direction in the lower part of the cave and exits via the perennial Saivu spring where it joins the Allaine River, and the Bame temporary spring. Clays and silts, deposited during hydraulic backflooding, are particularly present in the downstream part of Milandre cave where they contribute to the sediment load during mild flood events. In contrast, during medium to intense flow, allochthonous sediment transport is often associated with higher microbial concentrations (Vuilleumier et al., 2021). Quantifying the contribution of microbial respiration to the cave carbon cycle was beyond the scope of this study and should be addressed by future research. The Milandre river drains a catchment area of approximately 13 km² (Perrin et al., 2003). There is a series of dolines in the catchment area which form depressions ~10 to 20 m deep in the landscape. Notably, one doline is located in the meadow partially covering the upstream section of Milandre cave (Fig. 1).

89



90 Figure 1. Map of the main passages of the Milandre cave network. The location of the atmospheric sampling (yellow), the soil
 91 (green) and unsaturated zone boreholes (blue), and the cross-trip sampling (red) are annotated. Cave survey modified from Gigon
 92 & Wenger (1986). Base map from ©swisstopo.

93 Over the past 40 years, land use in the catchment area has been dominated by farmland (37 %), forest (36 %), and meadows
94 (12 %) as estimated by aerial photo analysis (Jeannin et al., 2016). The crops grown primarily include maize and tobacco,
95 two C4 plants. Long-term land use analysis shows that farmland has decreased slightly in this area and special infrastructures
96 have increased due to the construction of a motorway that overlies part of the cave. The soils of the area are influenced by
97 the ecosystem that is overlying it. Forest soils are shallow leptosols (< 10 cm deep) that are rich in fragments of the
98 carbonate bedrock. The shallow forest soils transition into deeper (up to ~ 80 cm) organic rich histosols in the meadows,
99 particularly in the dolines. The roots from the forest vegetation likely extend into the epikarst and unsaturated zone, however
100 no roots are visible within the cave itself.

101

102 The Jura region experiences a marine west coast, warm summer climate (Cfb classification) (Kottek et al., 2006). Daily
103 temperature measurements from the Fahy MeteoSwiss weather station located ~ 9.5 km SW from Milandre cave show a
104 mean temperature of 9.4 °C (1991 to 2020). Temperature seasonality is strong, with monthly mean temperatures fluctuating
105 between a minimum of -1.2 °C in January and a maximum of 18.2 °C in July from 1991 to 2020 (MeteoSwiss, 2024). In
106 contrast, temperatures within the cave remain almost constant year-round, and vary between 10.3 and 11.0 °C (Affolter et al.,
107 2020). The temperature difference between the outside and in-cave temperature drives dynamic ventilation on seasonal
108 scales (Garagnon et al., 2022). Regional meteoric precipitation shows a mean of 1,046 mm year⁻¹. Monthly precipitation data
109 indicate that precipitation in this area is well dispersed throughout the year (MeteoSwiss, 2024). Monitoring of tritium (³H)
110 in stalagmite drip water was used to estimate the residence time of seepage water at ~ 5.5 to 6.6 years (Affolter et al., 2020).

111 3 Materials and methods

112 3.1 Gas sampling set up

113 All gas samples were taken every two months from December 2021 to January 2024. The samples were collected in 5 L
114 sampling bags (Cali-5-Bond, Calibrated Instruments, USA) using a handheld pump. The gas was dried through a glass tube
115 filled with granular magnesium perchlorate (~ 83 % purity, Supelco, Germany) to reduce the effects of humidity which may
116 affect the isotopic composition of the sample. The sampling set up and procedure were identical for all gas samples. The CO₂
117 concentration was monitored during line flushing using a nondispersive infrared (NDIR) CO₂ sensor (SCD30, Sensirion,
118 Switzerland) to ensure accurate sampling. The magnesium perchlorate was exchanged before each sampling day. Prior to
119 analysis, the samples were stored away from direct sunlight in cool indoor temperatures for a maximum of six weeks. The
120 same bags were reused for each sampling location and were flushed with N₂ gas for at least 48 h before sampling to reduce
121 cross contamination risks.

122

Atmospheric samples were collected at a defined sampling site above the cave (Fig. 1). Before sampling, the line was manually flushed for 1 min. To reduce the risk of breath contamination, the sampling set up was attached to 3 m long Teflon tubes, and samples were always taken against air flow direction.

126

The unsaturated zone air was sampled from six boreholes of varying depths between 0.5 and 5 m (Table 1). Spatially, the boreholes cover a large portion of the cave's upstream hydrological catchment, and are overlain by contrasting vegetation cover (mixed-deciduous forest or a grass meadow) (Fig. 1) (Table 1). The gas was sampled in two lines from depths of 0.5 to 0.85 m (Shallow 1), three lines from 0.6 to 1.5 m (Shallow 2), and in single lines from a depth of 5 m (Deep 1, Deep 2, Deep 3, Deep 4). Due to the nature of the installation, it was not possible to assign a specific depth to the multi-line boreholes. All boreholes were drilled in 2013 and are equipped with aluminium tubes compacted by layers of gravel, bentonite and sand. The sampling line was flushed for 1 min once attached to the borehole, and then samples were taken into the Cali-5-Bond bags. Due to the nature of the borehole installation, we assume that the samples were not taken in steady state conditions, where the production rate of CO₂ would equal the sampling rate.

ID	Well names	SISKA ID	Easting (CH1903+/LV95) (Swiss grid)	Northing (CH1903+/ LV95) (Swiss grid)	Depth (m)	Number of sampling lines	Classification	Ecosystem cover
SG-04	Shallow 1	CO2-13	2°567'083	1°256'904	0.5 to 0.85	2	Soil	Forest
SG-07	Shallow 2	CO2-10	2°567'074	1°257'068	0.6 to 1.5	3	Soil	Meadow
SG-05	Deep 1	EPI-2	2°567'097	1°256'896	5	1	Unsaturated Zone	Forest
SG-10	Deep 2	EPI-4	2°567'089	1°256'890	5	1	Unsaturated Zone	Forest
SG-08	Deep 3	EPI-7	2°567'080	1°257'063	5	1	Unsaturated Zone	Meadow
SG-09	Deep 4	EPI-5	2°567'096	1°257'059	5	1	Unsaturated Zone	Meadow

136

Table 1 Summary of the boreholes sampled in Milandre cave catchment noting sample depth, number of sampling lines, classification in soil/ unsaturated zone, and ecosystem cover type. The ID's are the names given to the boreholes for this study, and are concurrent with the supplementary data provided. SISKA (Swiss Institute for Speleology and Karst Studies) IDs refer to the original names of the boreholes at the time of installation.

Cave air was sampled in two locations within Milandre cave proximal to both entrances, Downstream (topographically lower) and Upstream (topographically higher), with the same procedure as for the atmospheric samples (Fig. 1). To gain

insight into possible spatial variability within the cave, a through trip across the main passage of the cave was carried out in September 2021, and 14 samples were collected (Fig. 1). The trip was taken against the direction of airflow to reduce the risk of breath contamination, from the downstream to upstream entrance.

3.2 Continuous O₂ and CO₂ concentration monitoring

Soil gas O₂ and CO₂ concentrations were measured continuously nearby Shallow 2 at 40 cm depth and recorded at 10 min intervals between April 2023 and June 2024 using a SCD-41 CO₂ sensor (± 50 ppmV + 5 % of reading) (Sensirion, Switzerland) and SGX-40X O₂ sensor (Amphenol SGX, Sensortech, Switzerland). Calibration was cross-checked with an independent, handheld multi-gas monitor (XAM 5600, Dräger, Germany) before installation.

3.3 CO₂ concentration and $\delta^{13}\text{C}$ analysis

The concentration of CO₂ and the stable carbon isotopic composition ($\delta^{13}\text{C}$) in all gas samples was measured using a cavity ringdown spectrometer (CRDS) G2131-I Isotopic CO₂ instrument (Picarro, USA) at ETH Zurich (CO₂ concentration = 0.2 ppm, $\delta^{13}\text{C} = < 0.1$ ‰ precision). Standard gases with known CO₂ concentrations, 399.6 ppmV and 2,000 ppmV CO₂ in synthetic air, were measured in addition to standard gases of known $\delta^{13}\text{CO}_2$ values, -27.8 ‰ and -2.8 ‰ VPDB, for offline calibration. As there was no equivalent standard gas available for high CO₂ concentrations as in some samples, linearity of the concentration data produced by the CRDS is assumed. All standards and samples were measured by directly attaching the sample bags to the inlet of the CRDS that was previously fitted with a magnesium perchlorate dryer, and measuring gas concentration and isotopic composition for 2 min after reaching steady state. We used the mean and standard deviation of this measurement interval for evaluation. $\delta^{13}\text{C}$ refers to the ratio between the two stable carbon isotopes with respect to the Vienna Pee Dee Belemnite (VPDB) standard:

$$\delta^{13}\text{C} (\text{‰}) = \left(\frac{\frac{^{13}\text{C}}{^{12}\text{C}}_{\text{sample}}}{\frac{^{13}\text{C}}{^{12}\text{C}}_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

3.4 Radiocarbon analysis

The CO₂ from each gas sample was converted into approximately 1 mg of graphite using an Automatic Graphitization Equipment (AGE, IonPlus, Switzerland) (Wacker et al., 2010a) coupled with a custom-made carbon inlet system. In this process, each sampling bag was successively attached to the inlet system which dried the sample through a fine-grained magnesium perchlorate water trap via a vacuum line. The volume of air required to sample was calculated from the CO₂ concentration of the sample, allowing the appropriate amount of CO₂ to be trapped within a stainless-steel coil that was

174 cooled by submersion in a liquid N₂ bath. After trapping, the CO₂ was sublimated by submerging the coil in a room
 175 temperature water bath and then adsorbed onto a zeolite trap using helium as a carrier gas between the coil and the trap. The
 176 CO₂ adsorbed onto the trap was then thermally desorbed and filled into the AGE reactor, where it was reduced to graphite
 177 with hydrogen over an iron catalyst (Wacker et al., 2010a). A modern standard of Oxalic Acid II gas (F¹⁴C = 1.3407, Oxa II,
 178 NIST SRM 4990C, HEKAL AMS Lab, Hungary) and a radiocarbon fossil reference CO₂ gas (F¹⁴C = 0, CO₂ ≥ 99.7 %
 179 Vol.abs, Carbagas, Switzerland) were also graphitized and used to calibrate the measurements. The resulting graphite was
 180 pressed into targets for radiocarbon analysis through AMS analysis with a MIni Carbon DAting System (MICADAS,
 181 Ionplus, Switzerland) (Synal et al., 2007; Szidat, 2020). The resultant radiocarbon data were corrected using the BATS (4.3)
 182 software (Wacker et al., 2010b). In this study the ¹⁴C content of samples will be discussed in F¹⁴C notation, which represents
 183 Fraction Modern (defined as the 1950 atmosphere) according to Reimer et al., (2004):

184

$$F^{14}C = \frac{\frac{{}^{14}C}{{}^{12}C}_{sample}}{\frac{{}^{14}C}{{}^{12}C}_{standard}} \quad (2)$$

186 3.5 Dissolved inorganic carbon analysis

187 Drip water dissolved inorganic carbon (DIC) ¹⁴C was measured to constrain the DIC degassing endmember. Samples were
 188 measured over one year from December 2021 and December 2022 at 9 drip sites focused on the actively dripping galleries
 189 nearby Upstream and Downstream (Fig. 1). Using a 5 mL syringe, 1 mL of drip water was collected directly from the soda
 190 straw stalactites on the cave roof to reduce fractionation effects associated with degassing. The water was injected directly
 191 into pre-cleaned Exetainer® (Round Bottom, Borosilicate, 938W, Labco Limited, UK) vials which had been flushed with
 192 helium gas and pre-spiked with 150 µL of 85 % H₃PO₄ (Suprapur®, 85 %, Merck KGaA, Germany). The ¹⁴C content of the
 193 DIC was measured by sampling the vial headspace using the Carbonate Handling System (CHS, Ionplus, Switzerland) and
 194 transferring the gaseous sample to the AMS using a gas handling system. Here, the CO₂ was introduced to the gas ion source
 195 through the CHS at a flow of 50 mL min⁻¹. The ¹⁴C content for each sample was measured for ~ 60 cycles. Standard material
 196 with a carbonate matrix of similar composition to the DIC samples were used as standards (IAEA C1 (F¹⁴C = 0) and C2
 197 (F¹⁴C = 0.411), sodium bicarbonate (NaHCO₃, Sigma Aldrich, USA) and potassium bicarbonate (KHCO₃, Sigma Aldrich,
 198 USA) (both F¹⁴C = 0)).

199

200 3.6 Data analysis and statistics

201

202 All data and statistical analyses and all graphs were generated using the Python 3 programming language (Van Rossum &
 203 Drake, 2009).

204

Relationships between CO_2 concentration, $\delta^{13}\text{C}$, $F^{14}\text{C}$ for each sample type and the Mean Monthly Temperature (MMT) and Mean Monthly Precipitation (MMP) from Fahy weather station were explored using Spearman's rank correlation coefficient analysis (Kokoska & Zwillinger, 2000). A significantly correlated relationship between two variables is defined by $p < 0.05$, with the correlation coefficient denoted by “rho”, and number of samples involved in the analysis as “n”.

4 Results

4.1 Atmospheric CO_2

The atmospheric samples had a CO_2 concentration ranging from ~ 380 ppmV (August 2023) to ~ 485 ppmV (December 2022) with a mean of ~ 440 ppmV. The $\delta^{13}\text{C}$ ranged from -12.5 ‰ (February 2023) to -7.6 ‰ (August 2023) and had a mean of -10.1 ‰ ($n = 17$). The CO_2 sampled was typically modern, though had some fluctuation from $F^{14}\text{C}$ 0.98 (December 2022) to 1.02 (June 2022) and a mean of $F^{14}\text{C}$ 1.0 (Fig. 2).

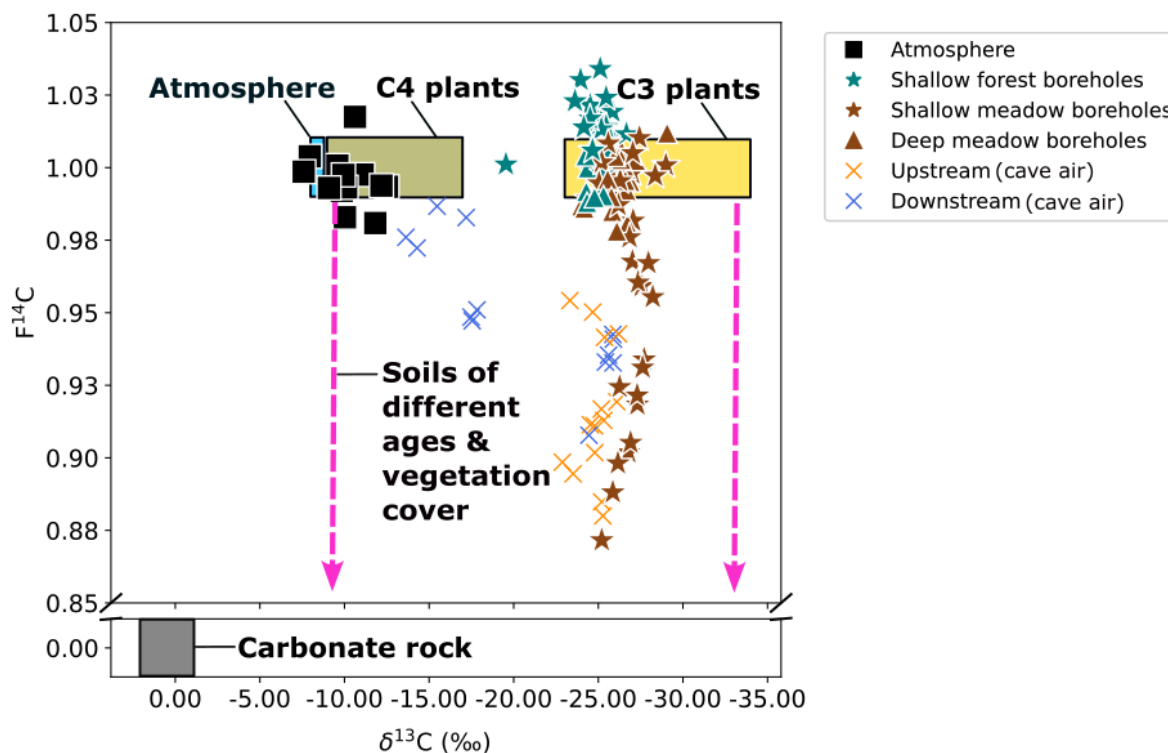


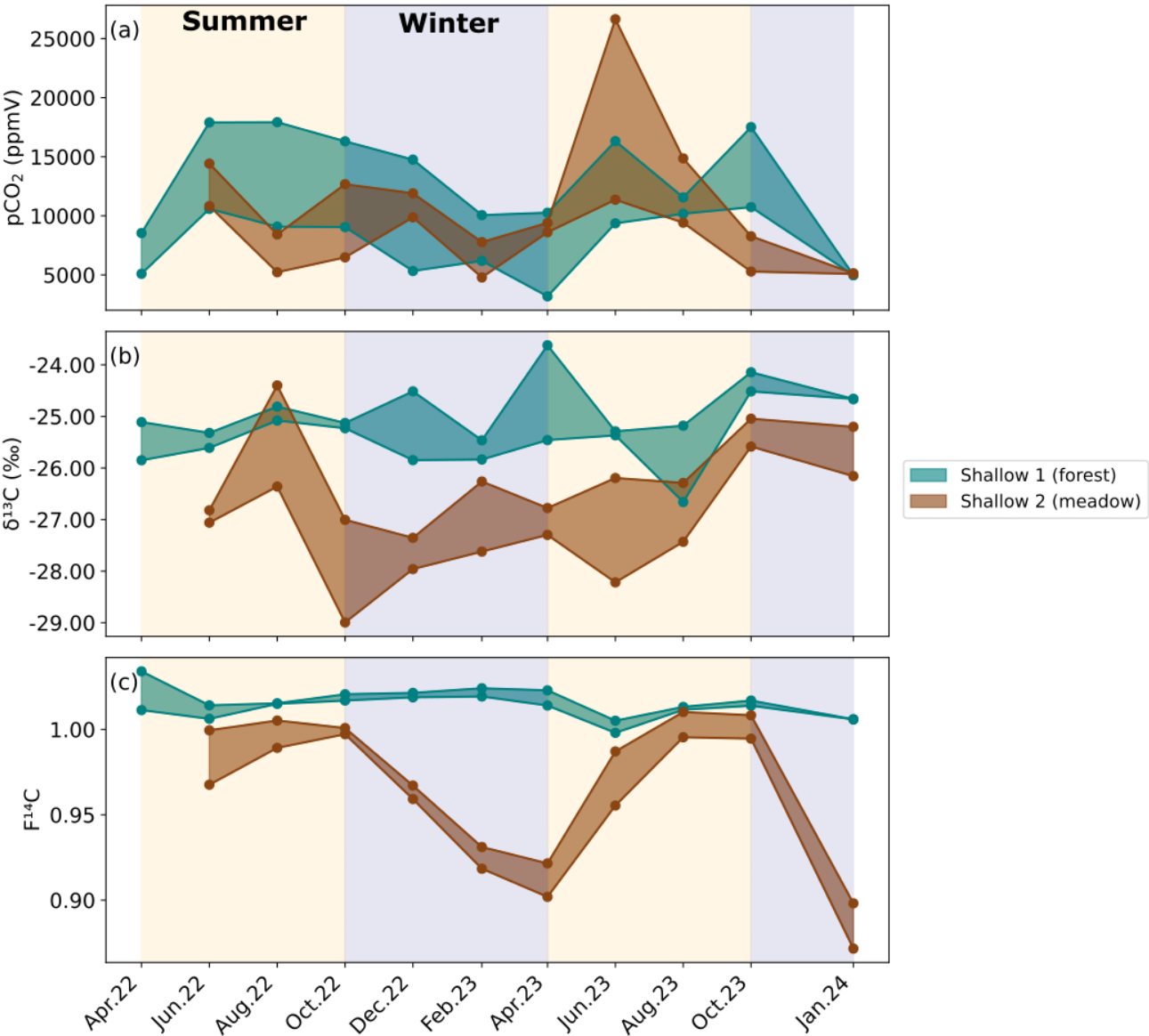
Figure 2. $\delta^{13}\text{C}$ and $F^{14}\text{C}$ of all gas samples over the monitoring period. The theoretical carbon reservoirs present in the system are shown with the atmosphere (blue), C3 plants (yellow), C4 plants (green), soils of various ages and compositions (pink arrows), and carbonate rock (grey).

221 4.2 Soil Zone CO₂

222 Soil boreholes have higher CO₂ concentrations and lower $\delta^{13}\text{C}$ compared to the atmosphere (Fig. 2). Concentrations in
223 boreholes that sample the soil zone (Shallow 1 and Shallow 2) (Fig. 3a) vary significantly depending on sampling depth,
224 cover type, and season. The Shallow 1 boreholes (forest) show seasonal high pCO₂ in the summer months from June to
225 October, which steadily declines during winter until it begins to rise again after April. In these boreholes CO₂ concentrations
226 vary from a maximum of $\sim 18,000$ ppmV in August 2022 to a minimum in January 2024 of $\sim 1,200$ ppmV, with a mean of
227 $\sim 10,700$ ppmV ($n = 21$). The CO₂ concentrations in Shallow 2 (meadow) boreholes range from $\sim 27,000$ ppmV in June 2023
228 to $\sim 4,800$ ppmV in February 2023 with a mean of $\sim 9,900$ ppmV ($n = 30$).

229

230 The $\delta^{13}\text{C}$ varies by 7.0 ‰ in shallow forest boreholes over the sampling period ranging from a minimum of -26.7 ‰ in
231 August 2023 to a maximum of -19.5 ‰ in January 2024 (Fig. 3b). There is ~ 4.0 ‰ isotopic variation in the shallow
232 meadow boreholes with a minimum of -29.0 ‰ in October 2022 and a maximum of -24.4 ‰ in August 2022. The pCO₂ and
233 $\delta^{13}\text{C}$ are negatively correlated in both shallow forest and shallow meadow boreholes (forest: $\rho = -0.47$, $p = 0.02$, $n = 25$.
234 meadow: $\rho = -0.49$, $p = 0.01$, $n = 30$), and $\delta^{13}\text{C}$ is on average lower in shallow meadow than shallow forest boreholes.



236

237 Figure 3. Maximum and minimum a) CO₂ concentration, b) δ¹³C and c) F¹⁴C of the three soil borehole samples from Shallow 1 in
238 the forest (green), and the two sampling lines in Shallow 2 in the meadow (brown). Summer (yellow) and winter (blue) seasons are
239 highlighted by the coloured background. Concentrations are shown as ranges between maxima and minima as they aggregate over
240 three sampling lines in the soil per location (Table 1).

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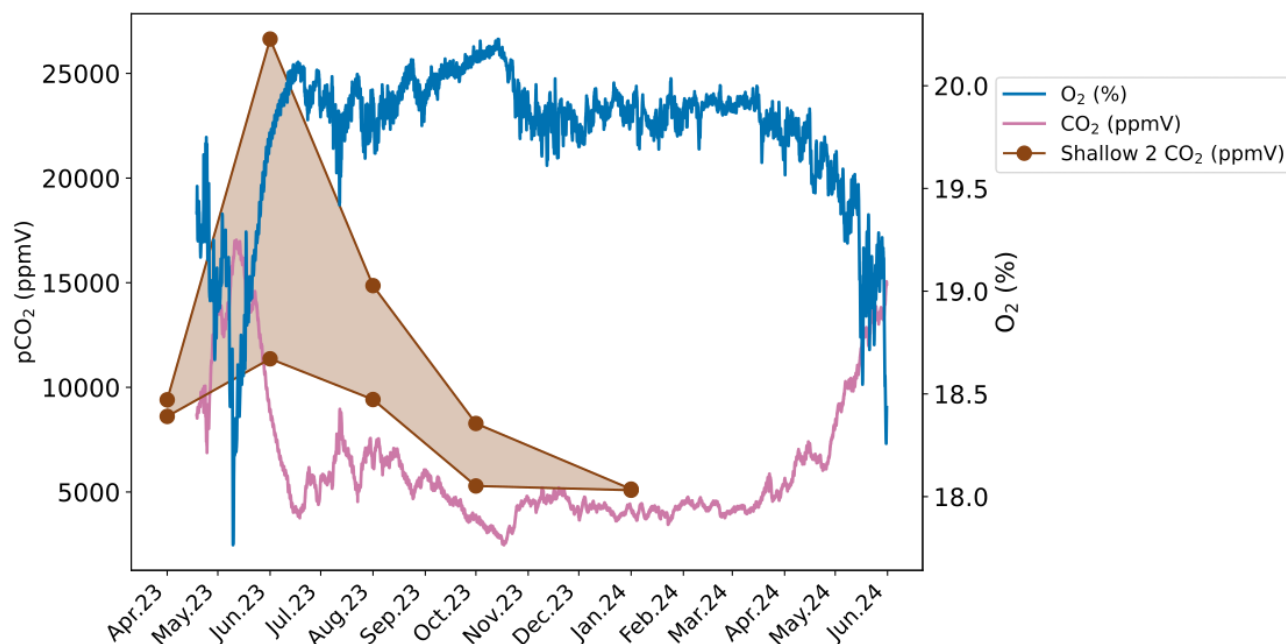
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243 $F^{14}C$ in Shallow 1 boreholes (forest) shows little annual variability and fluctuates slightly around a modern value (max =
244 1.03 in April 2022, min = 1.0 in June 2023) (Fig. 3c). Conversely, the shallow 2 (meadow) boreholes show distinct annual
245 variation in $F^{14}C$, which steadily decreases from modern (1.0) in August 2022 and 2023 to a low in April 2023 and a
246 minimum in January 2024 (0.87). The samples from the shallow forest boreholes have a positive correlation between $F^{14}C$
247 and $\delta^{13}C$ ($\rho = 0.48$, $p = 0.02$, $n = 25$). In the shallow meadow borehole samples, we observe a positive correlation between
248 $F^{14}C$ and MMT ($\rho = 0.67$, $p = 0.001$, $n = 30$). All other tested parameters show no significant correlations (see Appendix
249 A).

250

251 The continuously measured O_2 and CO_2 concentrations nearby Shallow 2 in the meadow show an inverse relationship (Fig.
252 4). The concentrations are relatively stable throughout most of the year with higher O_2 and lower CO_2 concentrations. This
253 pattern is disturbed by a sharp decrease in O_2 and an increase in CO_2 observed around May/June of both 2023 and 2024.
254 These different conditions last around one month before returning to the typical concentrations. The O_2 concentration ranges
255 from a maximum of 20.2 % in August 2023 to a minimum of 17.8 % during a brief interval in May 2023. Conversely, the
256 CO_2 concentration peaks at $\sim 17,000$ ppmV in May 2023 and is the lowest at $\sim 2,500$ ppmV in August 2023. The Shallow 2
257 boreholes also show peaks in CO_2 concentration in May to June 2023 (Fig. 4).

258



259

260 Figure 4. Continuously measured CO_2 (pink) and O_2 (blue) concentrations from the soil zone nearby the Shallow 2
261 boreholes. CO_2 concentrations from Shallow 2 are also plotted (brown).

262

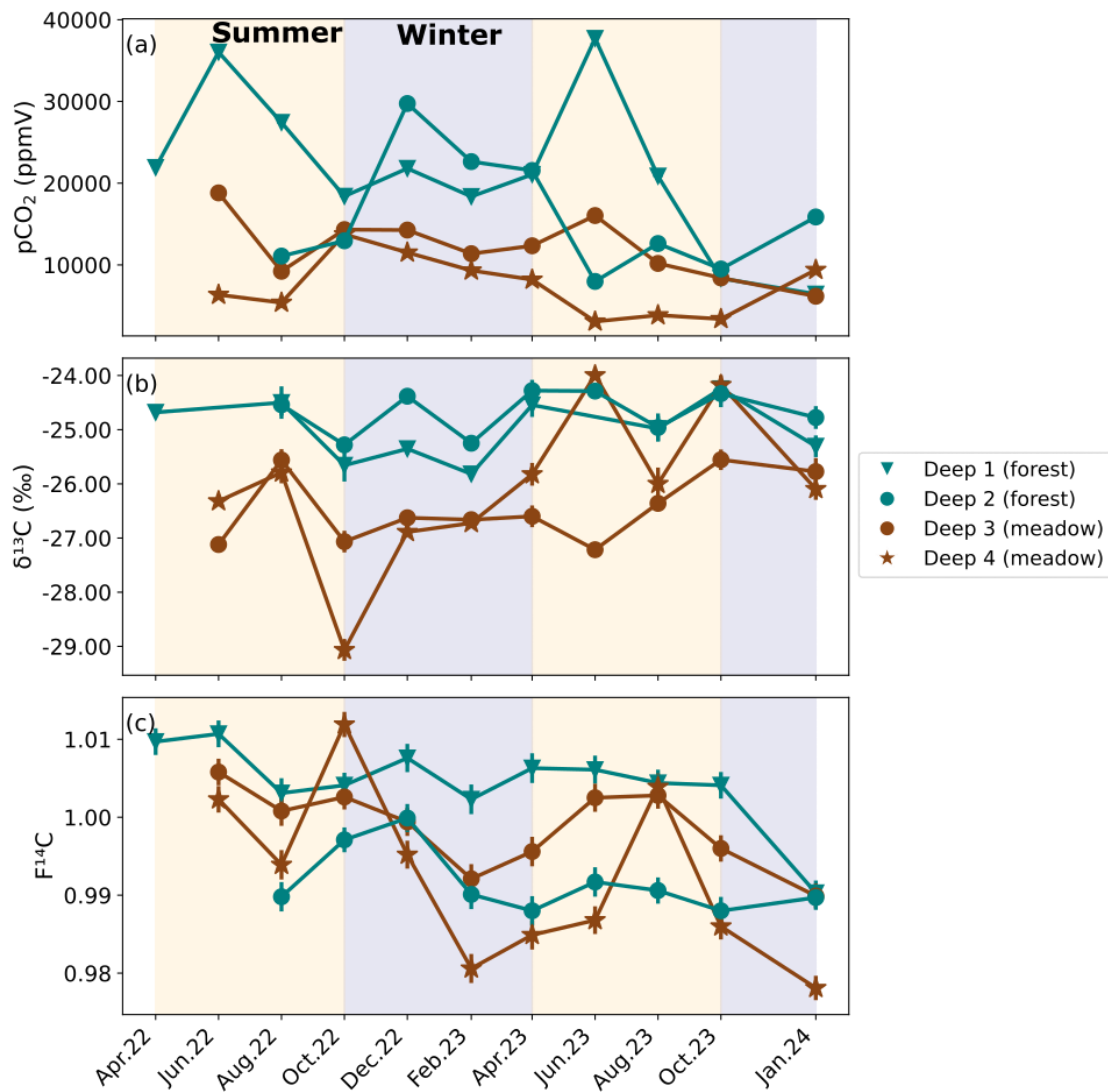
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264 4.3 Unsaturated Zone CO₂

265 Unsaturated zone CO₂ concentrations varied widely during the sampling period but are generally very high (>10,000 ppmV).
266 On average, the forest boreholes Deep 1 and Deep 2 have higher concentrations than the meadow boreholes Deep 3 and
267 Deep 4. Concentrations are generally highest in the Deep 1 borehole (forest) (Fig. 5a) with a maximum of ~ 37,000 ppmV in
268 June 2023 and a similarly high concentration peak in June 2022. The lowest values (~ 21,000 ppmV) at this borehole are
269 recorded in the winter months. Deep 2 (forest) shows an opposite trend with its highest CO₂ concentrations in autumn,
270 winter, and spring (max = ~ 29,000 ppmV in December 2022). The lowest CO₂ concentrations were measured during the
271 summer months (min = ~ 7,900 ppmV in June 2023). There is comparatively less variability in the Deep 3 and Deep 4
272 boreholes (both in the meadow). In Deep 3, concentrations fluctuate from ~ 6,100 to ~ 19,000 ppmV with no obvious
273 seasonality. Deep 4 has higher concentrations over the winter period and lower during summer, peaking at ~ 14,000 ppmV in
274 October 2022 and dropping to an overall minimum of ~ 3,000 ppmV in June 2023.

275

276 The $\delta^{13}\text{C}$ of the majority of unsaturated zone CO₂ samples is very stable around ~ -26 ‰ (Fig. 5b). Similar to the shallow
277 boreholes, samples from deep meadow boreholes (Deep 3 and 4) have on average slightly lower $\delta^{13}\text{C}$ values. In the meadow
278 boreholes Deep 3 and Deep 4, the $\delta^{13}\text{C}$ and pCO₂ are negatively correlated ($\rho = -0.66$, $p = 0.001$, $n = 21$) (see Appendix A).
279



280

281 **Figure 5. a) CO_2 concentration, b) $\delta^{13}C$ and c) $F^{14}C$ of unsaturated zone borehole samples from forest boreholes Deep 1 and Deep 2**
 282 **(green), and meadow boreholes Deep 3 and Deep 4 (brown). Summer (yellow) and winter (blue) seasons are highlighted by the**
 283 **coloured background.**

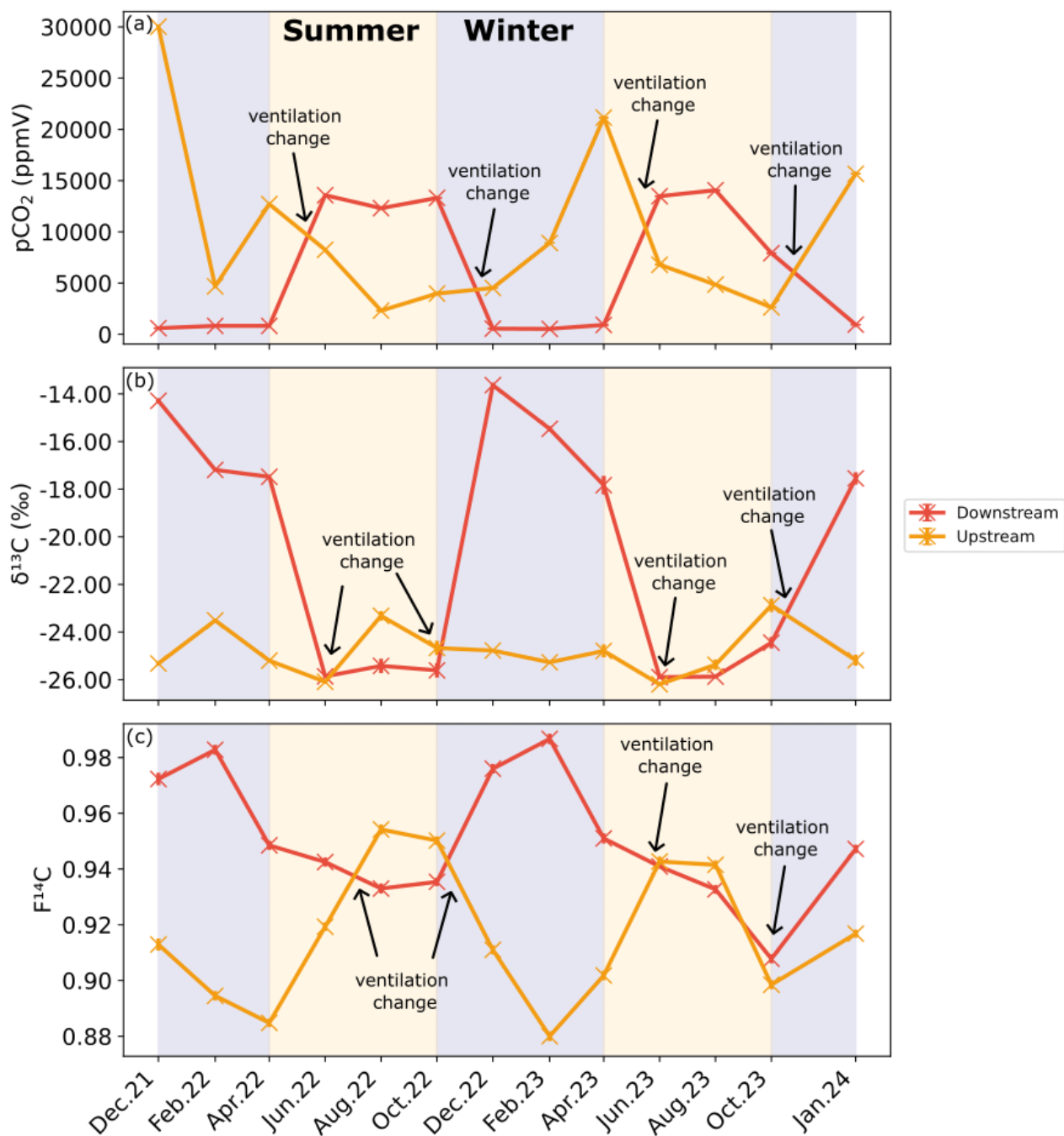
284 The $F^{14}C$ of unsaturated zone CO_2 samples is relatively stable throughout the sampling period, ranging from 1.01 (Deep 1,
 285 June 2022) to 0.98 (Deep 4, October 2022) (Fig. 5c). A possible seasonal pattern can be observed in the meadow boreholes
 286 (Deep 3 and Deep 4) with lower $F^{14}C$ values in the winter and spring months and higher $F^{14}C$ during summer, similar to the
 287 observation from the shallow meadow boreholes. No clear seasonal pattern is observed for Deep 1 and Deep 2. The $F^{14}C$
 288 values of unsaturated zone CO_2 samples are negatively correlated with their $\delta^{13}C$ value ($\rho = -0.54$, $p = 0.01$, $n = 21$) and
 289 positively correlated with MMT ($\rho = 0.53$, $p = 0.01$, $n = 21$). All other tested parameters show no significant correlations.

290

291 4.4 Cave CO₂

292 The CO₂ concentrations at the Downstream and Upstream sampling points in the cave are inversely correlated (Fig. 6a).
293 Downstream shows a higher pCO₂ from June to October in 2022 and 2023, with a maximum of ~ 14,000 ppmV in August
294 2023. The lowest concentrations are seen between December and April, reaching near atmospheric concentrations with a
295 minimum of ~ 420 ppmV in December 2021. Conversely, the highest CO₂ concentrations at the Upstream site were
296 measured during the winter months, with a maximum of ~ 30,000 ppmV in December 2021. The lowest CO₂ concentrations
297 at Upstream were observed during the summer months with a minimum of ~ 2,300 ppmV in August 2022. Overall,
298 concentrations at the Upstream site reach higher maxima and do not approach atmospheric values at their minimum, as at the
299 Downstream site. The isotopic composition of CO₂ is inversely related to its concentration, with higher CO₂ concentrations
300 coinciding with lower F¹⁴C and δ¹³C values. Seasonal trends differ between sites, at the Downstream site δ¹³C and F¹⁴C
301 values are higher in winter and lower in summer, whereas at the Upstream site, δ¹³C and F¹⁴C values show the opposite
302 pattern (Fig. 6b and c). The δ¹³C in Downstream ranged between -25.9 ‰ in June 2023 and -13.6 ‰ in December 2021, and
303 the F¹⁴C between 0.91 in October 2023 and 0.98 in February 2023. At Upstream, the opposite relation with CO₂
304 concentration is well expressed for F¹⁴C, but not as clear for δ¹³C, with minimal variability over the entire study period. The
305 δ¹³C in Upstream fluctuates slightly around a mean of -24.8 ‰.

306

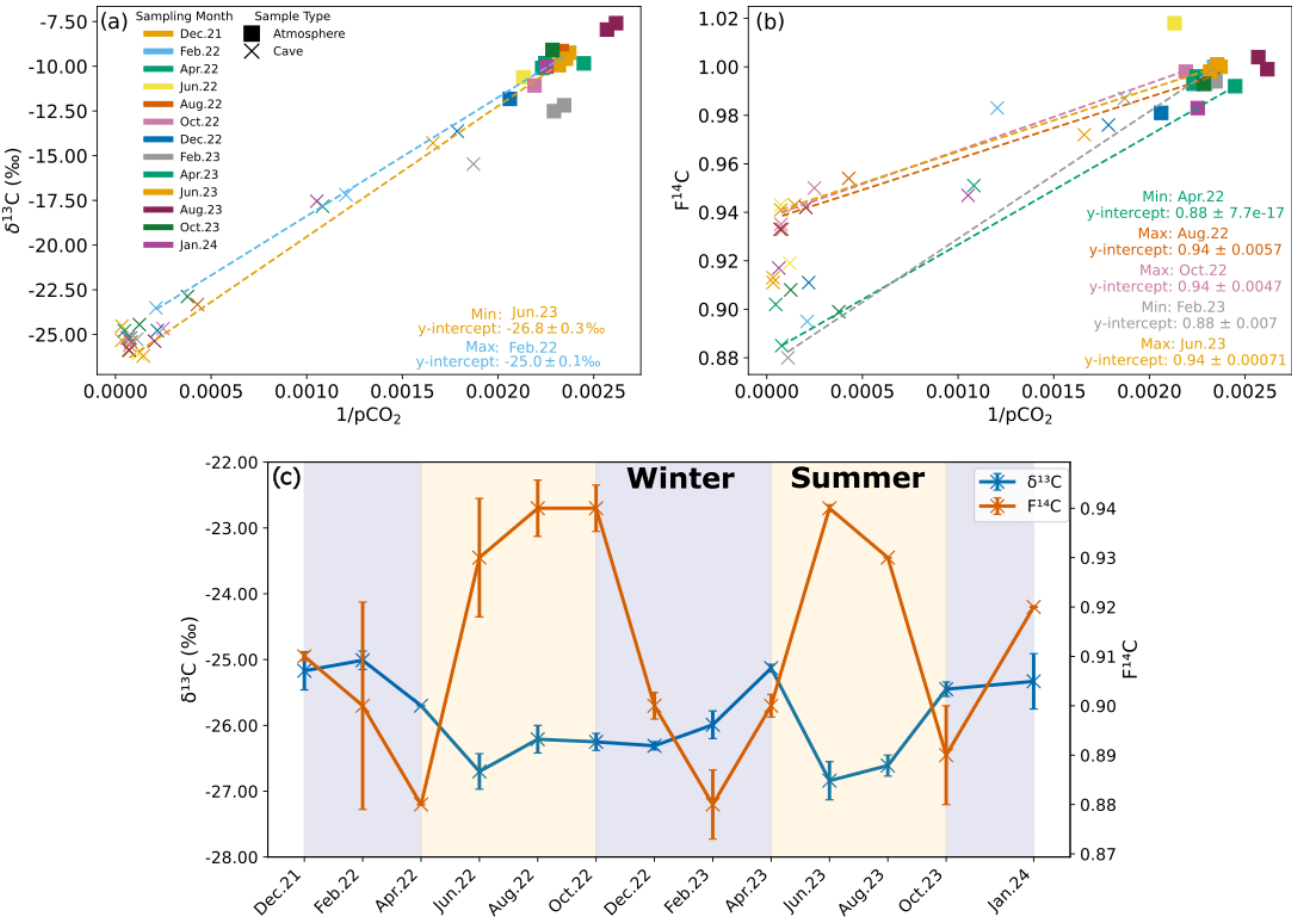


312 The $F^{14}C$ of CO_2 in Upstream is overall lower than that of Downstream, increasing over late spring 2022 and into summer,
313 peaking during August 2022 at 0.95. Decreasing $F^{14}C$ is observed during winter and into spring with lows of ~ 0.88 in
314 February 2023. At the Downstream site, the pCO_2 and $\delta^{13}C$ ($\rho = -0.9$, $p = 0.0001$, $n = 14$), pCO_2 and $F^{14}C$ ($\rho = -0.83$, $p =$
315 0 , $n = 14$), and $\delta^{13}C$ and MMT ($\rho = -0.79$, $p = 0.0001$, $n = 14$) are negatively correlated. Moreover, the pCO_2 and MMT
316 ($\rho = 0.53$, $p = 0.05$, $n = 14$), and $\delta^{13}C$ and $F^{14}C$ ($\rho = 0.74$, $p = 0$, $n = 14$) are positively correlated. At the Upstream site,
317 pCO_2 and MMP are positively correlated ($\rho = 0.55$, $p = 0.04$, $n = 14$), and pCO_2 and MMT are negatively correlated ($\rho =$
318 -0.54 , $p = 0.05$, $n = 14$) (See Appendix A). All other tested parameters show no significant correlations.

319

320 To constrain the isotopic composition of the end members contributing CO_2 to the cave air mixture, we use the Keeling plot
321 approach (Fig. 7a & b). This approach assumes that cave air is a mixture of two main sources, the atmosphere (with known
322 concentration and isotopic values), and a second source of a priori unknown composition. The y-intercepts of the Keeling
323 plots represent the isotopic composition of the contributing end member which mixes with atmospheric air inside the cave
324 (Keeling, 1961; Pataki et al., 2003) (Fig. 7a & b). We find that over time, the isotopic composition of the endmember varies
325 for both $\delta^{13}C$ and $F^{14}C$ (Fig. 7c). The endmember $\delta^{13}C$ value varies by ~ 2.0 ‰ from -26.8 ‰ in June 2023 to -25.0 ‰ in
326 February 2022. The variation in the $F^{14}C$ is larger, ranging from 0.88 in February 2023 to 0.94 in June 2022, August 2022,
327 October 2022, and June 2023 (ca. ~ 0.06 $F^{14}C$). Maxima in the $F^{14}C$ value derived for the endmember through the Keeling
328 plot generally correspond to decreases in $\delta^{13}C$, with two $F^{14}C$ maxima occurring in June to October 2022 and in June to
329 August 2023.

330



333 **Figure 7. Keeling plots for $\delta^{13}\text{C}$ (a) and $F^{14}\text{C}$ (b) of cave (Upstream and Downstream sites) and atmospheric samples from all**
334 **monitored months. The dashed regression lines denote the months with the maximum and minimum y-intercepts for $\delta^{13}\text{C}$ and**
335 **$F^{14}\text{C}$. c) Keeling plot y-intercepts for $\delta^{13}\text{C}$ (blue) and $F^{14}\text{C}$ (orange) over time. Summer (yellow) and winter (blue) seasons are**
336 **highlighted by the coloured background.**

338 Samples taken along the main passage of the cave during the through trip show increasing $p\text{CO}_2$ from the lower entrance
339 (location 1, corresponding to Downstream monitoring site, $\sim 6,900$ ppmV) to a plateau of $\sim 14,000$ ppmV from sample point
340 4 to 9 towards the centre of the cave (Fig. 8a). The $p\text{CO}_2$ decreases sharply after sample point 9 until point 11 ($\sim 6,600$
341 ppmV). The $p\text{CO}_2$ increases briefly at sample point 12 and then decreases again upon approach to the cave exit (Upstream).
342 The $\delta^{13}\text{C}$ values remain essentially constant at -25.5‰ through the initial segments of the cave, beginning to increase
343 slightly past sample point 9 (Fig. 8b). The $\delta^{13}\text{C}$ value is -25.8‰ at sample point 9 and progressively increases as $p\text{CO}_2$ levels
344 drop between sampling points 9 and 11, followed by a strong increase toward the Upstream exit (-11‰). The $F^{14}\text{C}$ values
345 are constant at ~ 0.93 between sample points 1 and 5, followed by more variability and generally lower $F^{14}\text{C}$ values in the

middle of the transect, coinciding with the river passage of the cave (minimum 0.89 at sampling point 11; Fig. 9c). Towards the Upstream exit, $F^{14}C$ begins to increase strongly (maximum 0.98 at sampling point 15; Fig. 8c). Sample 13 is excluded due to a ruptured bag during sampling.

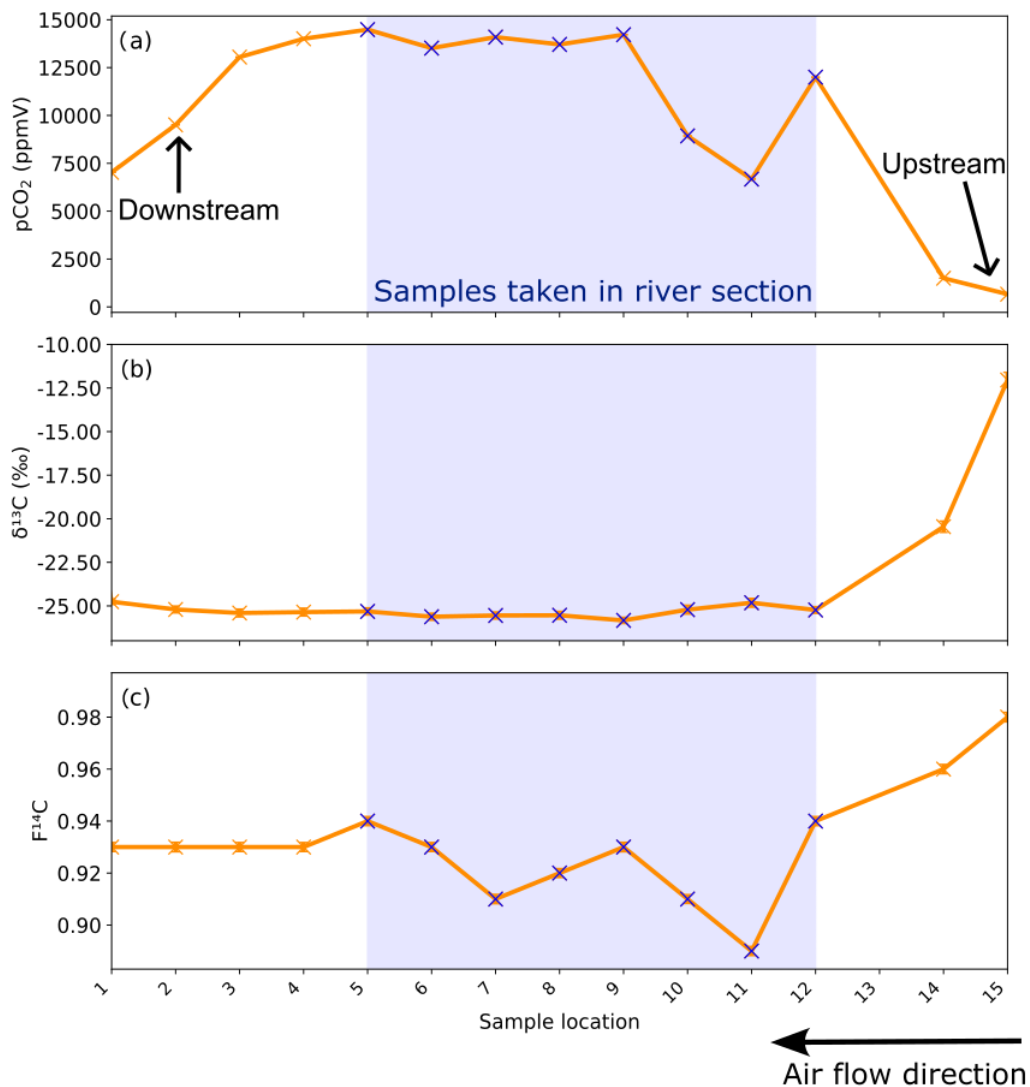


Figure 8. a) CO_2 concentration, b) $\delta^{13}C$ and c) $F^{14}C$ of cave air samples from the different sites sampled during the through trip. Sampling locations are shown in Fig. 1. The blue crosses and blue bar notate where samples were taken standing in the river. The direction of cave air ventilation is shown by the lower arrow. The approximate locations of the Upstream and Downstream sampling sites are annotated.

355 5 Discussion

356 5.1 Sources and variability of shallow depth CO₂

357 The observed fluctuations in CO₂ concentrations in the Shallow borehole gas samples vary with depth, vegetation cover, and
358 season (Fig.3), suggesting complex carbon cycling dynamics taking place in the soil zone and shallow fractured epikarst.
359 Overall, both samples from forest and meadow locations have similar mean CO₂ concentrations. In the Shallow 1 forest
360 boreholes, higher CO₂ concentrations occur during summer months (Fig. 3a), aligning with increased autotrophic (occurring
361 in plant roots, leaves, and stems) and heterotrophic (microbial) respiration rates during warmer seasons. This results in
362 higher CO₂ production compared to losses from soil efflux to the atmosphere and downward transport in gaseous or
363 dissolved form. Due to our discrete sampling approach, we cannot discern whether higher production or decreased losses are
364 responsible for higher CO₂ concentrations. The decline in CO₂ concentrations during winter suggests that reduced
365 autotrophic and soil microbial respiration in response to lower temperatures is likely an important driver of the seasonal
366 cyclicity. The lower CO₂ concentrations during winter in the forest boreholes (Shallow 1) compared to the meadow
367 boreholes (Shallow 2) could be an indication of frost effects. Soil frost induced by colder temperatures can reduce
368 heterotrophic respiration in soils. Experiments inducing deep frost in forested soils by removing snow cover caused a greater
369 decrease in heterotrophic respiration during winter months compared to snow covered control soils (Muhr et al., 2009).
370 Furthermore, frost-heave can physically damage the extraradical hyphae on plant roots (Lekberg & Koide, 2008). Due to the
371 shielding of the tree canopy, the forested areas in the Milandre catchment likely experience less snow cover compared to the
372 open meadow where snow can accumulate freely. The meadow soils may experience reduced frost effects, meaning that
373 heterotrophic respiration is not impacted to the same extent as the forest soils and can accumulate CO₂ year-round, as seen in
374 borehole Shallow 2. However, during the monitored period, the Milandre catchment experienced little snow cover,
375 suggesting that frost effects were likely minimal overall.

376

377 Though CO₂ production is reduced in winter, concentrations of several ~1,000 ppmV are measured at our sites, perhaps due
378 to: i) slow, but persisting metabolic microbial activity at low temperatures (possible even below freezing temperatures as low
379 as -5 to -7.5 °C (Kähkönen et al., 2001)), ii) reduced, but persisting autotrophic respiration of shallow roots, or iii) because of
380 reduced transportation of CO₂ due to higher soil water content (Hashimoto & Komatsu, 2006). Similar seasonal trends in
381 CO₂ concentrations have been reported in other studies (Billings et al; 1998; Pumpanen et al., 2003; Zhang et al., 2023).

382

383 The $\delta^{13}\text{C}$ values of CO₂ in shallow boreholes located in both the meadow and forest correspond to the typical isotopic
384 signature of C3 plants which dominate the catchment area, supporting our interpretation of a dominant biogenic source of
385 carbon in the soil gas (Fig. 2) (Fig. 3b). Plants using the C3 metabolic pathway (e.g. most temperate vegetation) produce
386 carbon with a $\delta^{13}\text{C}$ of ~ -23 to -34 ‰ (Staddon et al., 2004). Furthermore, due to the nature of the sampling, the soil was not
387 sampled in steady state conditions, potentially disturbing any existing CO₂ trends with depth. Another consideration is the

388 symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and plant roots which is ubiquitous globally, occurring
389 in 80 % of terrestrial plants (Fitter et al., 2000). AMF grows on plant roots and forms arbuscules within root cells, and
390 hyphae which extend into the soil (Zhu & Miller, 2003). Plants benefit from this relationship because of the increased uptake
391 of nutrients such as phosphorus and nitrogen, and improved resistance to environmental stress through more stable soil
392 structure and enhanced water absorption (Kiers et al., 2011; Boyno et al., 2024). The fungi benefit through access to plant
393 derived carbohydrates for energy, and a stable environment for growth and reproduction (Kiers et al., 2011). The respiration
394 of AMF can contribute to the subsurface sequestration of carbon (Treseder & Allen, 2008), and may indeed contribute to the
395 subsurface CO₂ which we measured at Milandre cave. There appears to be a temperature dependence of roots with AMF
396 colonisation, where respiration rates increase at higher temperatures (Atkin et al., 2008). We observe an increase in CO₂
397 concentration during the higher temperature summer months (Fig. 3a, Fig. 5a), which may be caused in part by a
398 contribution of CO₂ from enhanced respiration of AMF. The isotopic composition of the CO₂ produced by AMF respiration
399 is not currently well constrained. Studies analysing the $\delta^{13}\text{C}$ of individual AMF spores showed that the $\delta^{13}\text{C}$ broadly
400 corresponded to the C3 or C4 plant that it was associated with (Nakano et al., 1999). Therefore, it can be assumed that CO₂
401 produced by AMF respiration is similar to that of the C3 host plant dominated catchment of Milandre cave. As carbon is
402 fixed from modern atmospheric CO₂ during host plant photosynthesis, the F¹⁴C signature should be modern (~1). Hence, the
403 respiration of AMF may contribute to the modern CO₂ measured in the boreholes in both meadow and forest sites (Fig.3 &
404 5).

405

406 Though both meadow and forest boreholes have C3 plant $\delta^{13}\text{C}$ signatures, the $\delta^{13}\text{C}$ values of CO₂ in meadow soils are on
407 average lower than those in the forest soils. Previous analysis of soil CO₂ from various karstic sites in Slovenia showed a
408 similar trend, whereby the $\delta^{13}\text{C}$ values in meadow sites were ~1 ‰ lower compared to forested sites (Stickler et al., 1997).
409 The meadow and forested areas at our site have developed contrasting soil compositions, with the meadow soils deeper,
410 more compacted, and with a higher organic content than the forest soils, which are shallow, unconsolidated, and with more
411 roots. Comparisons of root free and root containing soils have suggested that root respiration contributes lower $\delta^{13}\text{CO}_2$ to the
412 soil gas (Diao et al., 2022). This is the opposite trend to what our findings would suggest. Efforts to disentangle the $\delta^{13}\text{C}$ of
413 autotrophic and heterotrophic contributions to soil CO₂ have shown a range of results, where autotrophic respiration can
414 produce the same (Wu et al., 2017), higher (Moyes et al., 2010), or lower (Risk et al., 2012) $\delta^{13}\text{C}$ than heterotrophic
415 respiration. However, different soil compositions can affect soil moisture, which can cause both increases (Unger et al.,
416 2010), decreases (Powers et al., 2010), and no change (Diao et al., 2022) in soil $\delta^{13}\text{C}$. It is therefore difficult in the context of
417 this study to determine the exact reason for the difference in $\delta^{13}\text{C}$ between surface covers. Interestingly, we also do not find
418 evidence for contributions to subsurface gas from C4 plants, possibly due to the absence of sampling locations in the fields in
419 the catchment where C4 crops are grown, suggesting limited lateral gas transport.

420

421 The CO₂ F¹⁴C of meadow boreholes in Shallow 2 shows statistically significant seasonal behaviour ($\rho = 0.67$, $p = 0.001$, n
 422 $= 30$), with more aged CO₂ dominating the winter and spring months (F¹⁴C ~ 0.88), and modern CO₂ in summer (F¹⁴C $\sim$
 423 1.00) (Fig. 3c). These seasonal shifts suggest a potential influence of temperature-sensitive processes on soil carbon
 424 dynamics, with higher autotrophic respiration rates and heterotrophic respiration of very recently fixed photosynthates
 425 dominating in the warm months (Campeau et al., 2019). Though typically the productive growing season for meadow
 426 grasses occurs in spring (Wingler & Hennessy, 2016), there is a lag in the F¹⁴C response, which peaks between June and
 427 October. This may be due to the time it takes for enough modern CO₂ to accumulate in the soil and dominate the average gas
 428 age. Similar impacts of seasonality on the contributions of young carbon pools to the soil CO₂ budget have been observed in
 429 soils of various environments whereby autotrophic respiration and heterotrophic decomposition of younger carbon dominate
 430 in warmer months (Trumbore, 2000; Chiti et al., 2011; Vaughn & Torn, 2018). A contribution of fossil carbon from the
 431 dissolution of carbonate bedrock fragments in the soil zone could also be considered to explain the low soil CO₂ F¹⁴C values
 432 in winter. However, such a bedrock contribution would result in a concomitant increase in $\delta^{13}\text{C}$, as marine carbonates, which
 433 typically constitute the karstified host rock, are characterized by $\delta^{13}\text{C}$ of values of $\sim 0\text{‰}$ (Planavsky et al., 2015) and up to $+2\text{‰}$
 434 in the region of Milandre cave (Weissert & Mohr, 1996) (Fig. 2). This would contribute a gaseous $\delta^{13}\text{C} \sim 9.54\text{‰}$ lighter
 435 when at equilibrium with DIC from carbonate dissolution at 10.5 °C (Mook et al., 1974). Since we do not observe increases
 436 in $\delta^{13}\text{C}$ of soil gas during the periods of lower F¹⁴C, this is an unlikely scenario. A further explanation for the seasonal
 437 variability could be due to the seasonal ventilation dynamics in the karst system, which promote upward movement of cave
 438 air during the winter, and downwards movement of soil air in summer (see section 5.4).

439

440 The modern F¹⁴C CO₂ signature in the Shallow 1 forest borehole (Fig. 3c) suggests a year-round dominant source of CO₂
 441 from autotrophic root respiration and the decomposition of very recently fixed soil organic matter. As the root systems are
 442 deeper and more developed in the forest soils, there is a higher input of modern carbon compared to the meadows. The soils
 443 in the forested area are shallow (in some areas $< 10\text{ cm}$ deep) and are dominated by large pebbles and fragments of the
 444 carbonate bedrock and shallow roots. Thus, shallow depth carbon sequestration may be suppressed in the forest soil,
 445 resulting in a CO₂ profile dominated by deeper root respiration (Hasenmueller et al., 2017; Tune et al., 2020).

446

447 5.2 Sources and variability of CO₂ at 5 m depth

448 CO₂ concentrations at 5 m depth vary considerably over the sampling period and between individual sites (Fig. 5a). CO₂
 449 concentrations are generally higher in the forest boreholes (Deep 1, Deep 2) than in the meadow boreholes (Deep 3, Deep 4),
 450 likely due to the high volumes of CO₂ produced by autotrophic respiration of the mature woodland root system which
 451 penetrates deep into the unsaturated zone. The CO₂ $\delta^{13}\text{C}$ values across all boreholes reflect the C3 dominated ecosystem of
 452 the catchment, and do not indicate any input from C4 plants in the nearby fields. Notably, the ecosystem composition of the
 453 overlying vegetation (meadow vs. forest) influences the isotopic composition of CO₂ at 5 m in the same way as the shallow
 454 boreholes, with a slightly lower $\delta^{13}\text{C}$ values in the meadow than the forest. The F¹⁴C values of unsaturated zone CO₂ vary

across all deep boreholes by ± 0.02 around a mean value of 1.00 (Fig. 5c), suggesting that across the catchment, autotrophic tree root respiration may be the dominant source of CO₂ at these depths (Breecker et al., 2012; Tune et al., 2020). Furthermore, the year-round modern CO₂ F¹⁴C values in the deeper meadow boreholes Deep 3 and Deep 4 compared to the seasonally pronounced signal in the shallower boreholes of Shallow 2 highlights the potential influence of the meadow doline morphology. Boreholes Deep 3 and Deep 4 were drilled on the edge of the doline beneath shallow leptosols like those found in the forest, while Shallow 2 was drilled fully within the doline, where the soil is thicker and much richer in organic matter. Unfortunately, there is no unsaturated zone depth borehole installed within the lower basin of the meadow to verify whether the seasonal signal measured at Shallow 2 translates to the deeper subsurface.

Whilst many studies point to the degradation of exported aged organic matter being the main source of karstic ground air (Breecker et al., 2012; Matthey et al., 2016; Bergel et al., 2017), unsaturated zone borehole CO₂ in the Milandre cave catchment has predominantly modern F¹⁴C values, and does not show seasonal isotopic variability. This suggests that the majority of the CO₂ at 5 m depth is likely supplied by contemporaneous C3 tree root respiration. Similarly modern (106.4 pMC (percent modern carbon, corresponding to 1.064 in F¹⁴C)) biologically sourced CO₂ was also detected in boreholes from Nerja cave, Spain (Vadillo et al., 2016). Modern, but not substantially above modern F¹⁴C also excludes the contribution of substantial amounts of decadal-aged soil material containing bomb spike carbon due the enrichment in ¹⁴C by thermonuclear weapons testing during 1950's and 1960's (Trumbore, 2000; Shi et al 2020). This result supports the body of literature stating that, in addition to the export and respiration of older carbon, large volumes of modern carbon are also transported into the unsaturated zone, likely in large part through deep root respiration (Breecker et al., 2012; Campeau et al., 2019; Tune et al., 2020). This suggests that deep roots, as found in mature forest ecosystems, might be more important than previously thought in contributing to the ground air budget.

5.3 Ventilation driven isotopic variation in cave air

The CO₂ dynamics in the cave show distinct variations in concentration and isotopic composition between the two sampling points Upstream and Downstream (Fig. 6a, b, c). Cave ventilation dynamics modulate the mixing ratio between the atmosphere and the other contributing pools, and are usually the dominant source of variability (Kukuljan et al., 2021; Buzjak et al., 2024). Recent modelling of air flow dynamics in Milandre cave found that outside temperature controls 95 % of flow variability (Garagnon et al., 2022). During colder months when the outside temperature is ≤ 8 °C, the air in the cave flows topographically upwards from the northern downstream entrance close to the Downstream site, to the higher entrance closer to Upstream. When the temperature increases above 8 °C, the ventilation regime reverses. This seasonality is reflected in the alternating concentrations and isotopic composition of the CO₂ at the cave sampling sites as they experience varying amounts of dilution with atmospheric air during the year. CO₂ degassing from the cave river at Upstream reduces the dilution effect in the upper passage. The effects of ventilation can also be observed in the higher spatial resolution sampling during the through trip (Fig. 8a, b, c) whereby the summer regime ventilation resulted in lower concentrations, more positive $\delta^{13}\text{C}$,

488 and increased $F^{14}\text{C}$ due to atmospheric mixing closer to the upper entrance (Upstream site). Spatially, a marked decrease in
489 CO_2 concentration and $F^{14}\text{C}$ occurs at sampling locations 10 and 11 (~750 to 1,000 m from the cave entrance respectively)
490 though little change is observed in the $\delta^{13}\text{C}$. This air input could be associated with an older organic matter pool which,
491 however, contributes only little to the entire CO_2 mass flux.

492

493 **5.4 Sources and variability of CO_2 in cave air**

494

495 The Keeling plot y-intercepts show that the composition of the gas pool (karst endmember) that mixes with atmospheric air
496 in the cave varies seasonally (Fig. 7c). It is important to note that, while the isotopic composition of cave CO_2 is influenced
497 by ventilation-driven dynamics, the Keeling plot y-intercept represents the composition of the subsurface gas that mixes with
498 the atmospheric air during ventilation and is not affected by it. The $\delta^{13}\text{C}$ of the karst endmember is mostly stable, varying ± 2
499 ‰ around a mean value of -26 ‰, a value typically associated with the CO_2 produced by the C3 plants which dominate the
500 catchment ecosystem. This value is also very similar to the $\delta^{13}\text{C}$ CO_2 values of soil and unsaturated zone gas and reinforces
501 the notion of the karst endmember being dominated by ecosystem respiration. Previous work at Milandre cave investigating
502 aquifer dynamics using ^{222}Rn suggested that the majority of the cave CO_2 comes from the overlying soils (Savoy et al.,
503 2011). The $F^{14}\text{C}$ of the Keeling plot y-intercept, on the other hand, shows seasonal variability with distinctly more “modern”
504 (closer to $F^{14}\text{C} = 1$) during the summer and “aged” ($F^{14}\text{C} < 1$) CO_2 during the winter. The transition from an $F^{14}\text{C}$ of 0.94 to
505 0.88 represents a change in the mean apparent age of CO_2 from ~ 100 to 1000 years, well beyond the assumed residence time
506 of water in the unsaturated zone of ~ 5.5 to 6.6 years (Affolter et al., 2020).

507

508 Several mechanisms may be responsible for the seasonal $F^{14}\text{C}$ fluctuations of the karst endmember. A contribution of carbon
509 from the oxidation of methane, either from methanotrophs or through the evolution of pore fluid waters, must be considered.
510 Methane can be produced by both thermogenic (originating from ancient organic rich sediments) and biogenic (particularly
511 through methanotrophic bacteria) processes (Suess & Whiticar, 1988). The $\delta^{13}\text{C}$ CO_2 range of published values for the CO_2
512 resulting from methane oxidation from both sources is broad, with biogenic spanning from ~ -20 to -90 ‰, and thermogenic
513 from ~ -30 to -65 ‰ (Suess & Whiticar, 1988). The $\delta^{13}\text{C}$ CO_2 produced by thermogenic sources can also evolve to less
514 negative values of ~ -25 to -34 ‰ in settings where there is near total conversion of CH_4 to CO_2 (Shoell, 1980; Frieling et
515 al., 2016). Methane that undergoes biogenic oxidation is typically sourced from the atmosphere, meaning the resulting CO_2
516 will have a modern $F^{14}\text{C}$ signature ($F^{14}\text{C} \sim 1$). In contrast, CO_2 produced by thermogenic methane oxidation will have a fossil
517 $F^{14}\text{C}$ signature, because the methane originates from ancient organic rich sediments that contain no measurable radiocarbon
518 ($F^{14}\text{C} \sim 0$). While measuring methane concentrations quantitatively was not possible with our analytical setup, qualitative
519 data shows that methane concentrations in Milandre cave air and catchment borehole samples were exceedingly low (~ 90 to
520 970 ppb), suggesting methane oxidation may occur in the Milandre subsurface environment. Methanotrophic bacteria have
521 been identified in 98 % of soils sampled from 21 North American caves at a mean bacterial abundance of 0.88 % (Webster

et al., 2022). Methanotrophic bacteria in caves significantly contribute to methane consumption, with a study in Pindal Cave (NW Spain) showing how methanotrophic bacteria associated with moonmilk deposits remove 65 % to 90 % of atmospheric methane entering the cave (Martin-Pozas et al., 2022). As our cave and borehole CO₂ samples have a $\delta^{13}\text{C}$ of ~ -26 ‰ and a variable F¹⁴C in the cave air and shallow meadow samples, we cannot rule out a contribution of methane oxidation from biogenic processes actively occurring in Milandre. However, this would not explain the depleted radiocarbon signature that we measured episodically in the cave air. Methane evolved abiogenically from pore fluids in Oxfordian marls may have an isotopic ratio similar to that measured in our samples if thermogenically derived with a dominant conversion of CH₄ to CO₂. However, it is unlikely that this process occurred in the Milandre cave sediments given the lack of organic rich sedimentary rocks in the stratigraphy. Thus, while we cannot quantify the importance of methane oxidation on the subsurface carbon budget at our site, it is unlikely that methane oxidation processes are responsible for the variation in radiocarbon ages in cave air CO₂.

533

Despite the apparent limiting factors for microbial growth in caves, it is known that different organisms are able to thrive within this niche including bacteria, archaea, algae, and fungi. In terms of bacteria, Proteobacteria is typically reported as the dominant group in caves (Zhou et al., 2007; Tomczyk-Żak & Zielenkiewicz, 2016). However, in some cases Actinobacteria is the largest group of microbes (Wischart et al., 2018). In two Chinese caves with high CO₂ concentrations (> 3,000 ppm), Proteobacteria, Actinobacteria, Bacteroidetes, and Thaumarchaeota were the dominant phyla (Chen et al., 2023). Hundreds of fungi taxa have been described in caves globally, with Ascomycota being the most commonly reported (Gherman et al., 2014). Though not yet thoroughly investigated, diverse communities of protists have also been identified within caves (Gogoleva et al., 2024), as well as archaea (Biagioli et al., 2023), and algae (Kosznik- Kwaśnicka et al., 2022). As biological processes generally favour the incorporation of lighter ¹²C compared to ¹³C, the respiration of these organisms would contribute an organic signature (lower $\delta^{13}\text{C}$ and a F¹⁴C corresponding to the age of the carbon source being consumed, which can range from modern to more aged F¹⁴C) to the CO₂ composition of cave air at Milandre cave. With the isotopic analysis used in this study, and because we did not analyse the microbial community diversity present in Milandre cave and its catchment, the CO₂ contributions from different types of microbial processes cannot be distinguished from one another. We are only able to distinguish between “organic” processes (in-cave microbial, soil, root, and fungi respiration) which produce a CO₂ with a lower $\delta^{13}\text{C}$ signature and a range of F¹⁴C depending on the age of the organic material consumed, and “inorganic” processes (carbonate degassing and atmospheric ventilation) which contribute CO₂ with a higher $\delta^{13}\text{C}$ and a range of F¹⁴C.

551

Varying contributions of host rock-derived carbon to cave air through shifts in the host rock dissolution regime can affect the isotopic composition of cave air. Dissolution of the host rock carbonate can occur under a wide range of conditions between two extreme cases: completely closed and completely open systems. In a completely closed system, the aqueous solution becomes isolated from the soil CO₂ reservoir after passing into the unsaturated zone resulting in a theoretical 50:50

contribution ratio of carbon ions in solution from the carbonate host rock and from soil air, and overall decreasing $F^{14}C$ to as low as 0.5 (Fohlmeister et al., 2011; Milanolo & Gabrovšek, 2015). On the other hand, in a completely open system the aqueous solution can continuously exchange with an unlimited soil CO_2 reservoir resulting in most carbon atoms sourced from the soil CO_2 reservoir, and the rock contribution being minimal (Fohlmeister et al., 2011). Most natural systems exist in an intermediate state between completely open and completely closed settings.

If the seasonal variability in CO_2 $F^{14}C$ in Milandre cave reflected seasonal shifts in the “openness” of the system, summers (with higher $F^{14}C$) would be characterised by a more open system with higher rates of exchange between the aqueous solution and the soil CO_2 reservoir. The observed shift to lower $F^{14}C$ in winter would reflect a more closed system with a higher contribution of the $F^{14}C$ free carbonate bedrock. Shifts in the open-closed continuum would likely result in changes in the $\delta^{13}C$ of the endmember (Fairchild & Baker, 2012) which is not seen in the cave air, with an expected lower $\delta^{13}C$ in a more open system, and an increase in a more closed system because of the higher carbonate host rock carbon contribution ($\delta^{13}C \approx 0$ ‰). However, varying amounts of isotopic fractionation can occur when CO_2 is degassed from the drip water DIC pool into the cave atmosphere, potentially influencing the isotopic composition of the measured cave air CO_2 (Mickler et al., 2019).

We constructed a mixing model which allows us to evaluate how much variability in the Keeling plot y-intercept (i.e. the second source of carbon to cave air besides the atmosphere) can be explained by variation in DIC contribution from dissolution regime changes and degassing fractionation. This model assumes that the Keeling plot y-intercept is itself a mixture of two endmembers that contribute CO_2 to cave air, the modern soil ($F^{14}C_{soil} = 1$), and the DIC ($F^{14}C_{DIC}$), where the $F^{14}C_{DIC}$ represents a mixture of dissolved soil CO_2 and DIC from carbonate mineral dissolution, which we can constrain with our measured DIC values. The $F^{14}C_{soil}$ represents direct input of CO_2 soil gas into the cave.

The mixing ratio between $F^{14}C_{soil}$ and $F^{14}C_{DIC}$ results in the $F^{14}C$ of CO_2 of the karst endmember ($F^{14}C_{cave}$). Thus, the cave air CO_2 $F^{14}C$ can be expressed as (where f is fraction, defined as between 0 and 1):

$$F^{14}C_{cave} = f_{soil} F^{14}C_{soil} + f_{DIC} F^{14}C_{DIC} \quad (3)$$

Since $f_{DIC} = 1 - f_{soil}$:

$$F^{14}C_{cave} = f_{soil} F^{14}C_{soil} + (1 - f_{soil}) F^{14}C_{DIC} \quad (4)$$

587

588 For $F^{14}C$, fractionation is generally negligible during carbonate dissolution and degassing compared to $\delta^{13}C$, due to unit
589 conversion i.e. % vs ‰ (Fohlmeister et al., 2011). We use the mixing calculation (Eq. 4) to estimate the contributions of f_{soil}
590 and f_{DIC} using the highest (0.94), an intermediate (0.90), and the lowest (0.88) measured cave CO_2 $F^{14}C$ values ($F^{14}C_{cave}$),
591 and $F^{14}C$ DIC ($F^{14}C_{DIC}$) based on the highest (0.98) intermediate (0.88) and lowest (0.78) measured values of drip water
592 DIC. We find that when $F^{14}C_{DIC} = 0.98$, the mixing model results in impossible scenarios with $f_{soil} > 1$ and $f_{DIC} < 0$ because
593 this value is higher than the highest $F^{14}C_{cave}$. When $F^{14}C_{DIC} = 0.88$ it results in a highly unlikely scenario based on our
594 observations and the literature where $f_{soil} = 0$ and $f_{DIC} = 1$, as this corresponds to the lowest $F^{14}C_{cave}$ value (Fig. 9a). Only
595 the scenario with lowest $F^{14}C_{DIC} = 0.78$ produced realistic mixing fractions. Overall, this implies that there is a discrepancy
596 between the measured $F^{14}C_{DIC}$ and what is derived by a simplified mixing model based on the $F^{14}C$ of cave CO_2 , with
597 $F^{14}C_{DIC}$ values too high to result in the $F^{14}C_{cave}$ composition that we observe. Thus, carbonate dissolution and subsequent
598 degassing of CO_2 from drip water DIC to cave air is unlikely to be the dominant process explaining the shifts in cave air $F^{14}C$
599 over time.

600

601

602 As our mixing ratios derived from measured DIC values were mostly not realistic according to the two endmember mixing
603 scenario, we considered how a wider theoretical range of $F^{14}C_{DIC}$ values (from closed to mainly open system dissolution
604 conditions, 0.50 to 0.90 $F^{14}C$) would affect the contributing fractions of f_{soil} and f_{DIC} . Variations in $F^{14}C_{DIC}$: 0.50 to 0.85
605 result in viable mixing fractions across the range of $F^{14}C_{cave}$, with the f_{soil} (20 to 76 %) and f_{DIC} (24 to 80 %) differing
606 widely (Fig. 9b).

607

608 As the mean value of cave CO_2 $\delta^{13}C$ is -26 ‰ (we take a range from -25 to -27 ‰), we created a similar two-endmember
609 mixing calculation for $\delta^{13}C$ values to test whether the mixing ratios derived from $F^{14}C$ translate to observed $\delta^{13}C$ values. For
610 this we used the mean $\delta^{13}C$ of soil CO_2 ($\delta^{13}C_{soil} = -26$ ‰), and the $\delta^{13}C$ of the DIC ($\delta^{13}C_{DIC} = -15$ ‰ (estimated from a
611 mean of the $\delta^{13}C$ produced during the $F^{14}C$ DIC AMS measurement (see Appendix B)).

612

Fractionation effects during degassing must be considered when modelling $\delta^{13}\text{C}$. If equilibrium fractionation between DIC and $\text{CO}_{2(\text{g})}$ occurs, the fractionation factor ($\Delta^{13}\text{C}_{\text{HCO}_3-\text{CO}_2,\text{eq}}$) at 10.5 °C between HCO_3^- and CO_2 is 9.54 ‰, with the gas being 9.54 ‰ depleted in ^{13}C relative to the fluid (Mook et al., 1974). Kinetic fractionation can occur during rapid degassing of CO_2 as well as during rapid precipitation of carbonate minerals (Mickler et al., 2019). If kinetic fractionation occurs during degassing, this results in a greater depletion of the dissolved CO_2 in ^{13}C compared to equilibrium fractionation (i.e. a more positive $\delta^{13}\text{C}_{\text{DIC}}$). The possible ranges reported for the composition of CO_2 generated from kinetic fractionation during degassing in caves vary widely, with some reporting $\Delta^{13}\text{C}_{\text{HCO}_3-\text{CO}_2,\text{kin}}$ close to that resulting from equilibrium fractionation (Dulinski & Rozanski, 1990), and others a range of 10 to 65 ‰ (Mickler et al., 2019). The extent of kinetic fractionation is expected to depend on the amount of degassing that occurs, which is a function of the disequilibrium between the fluid and the surrounding air (Frisia et al., 2011). In our system with limited geochemical data regarding fluid compositions, it is difficult to determine what extent of kinetic fractionation may have occurred. Hence, we first assume that the fractionation is < -9.54 ‰ (i.e., greater compared to equilibrium conditions) and take a moderate but arbitrary value ballpark of $\Delta^{13}\text{C}_{\text{HCO}_3-\text{CO}_2,\text{kin}} = 30$ ‰.

A mixing calculation is then applied to assess if the measured range of the karst endmember $\delta^{13}\text{CO}_2$ given by the Keeling plot can be consistent with the extended range of $F^{14}\text{C}_{\text{DIC}}$ (0.50 to 0.85 $F^{14}\text{C}$) and measured $F^{14}\text{C}_{\text{cave}}$ under conditions with equilibrium degassing and kinetic degassing:

Equilibrium degassing:

$$\delta^{13}\text{C}_{\text{cave}} = f_{\text{soil}} \delta^{13}\text{C}_{\text{soil}} + f_{\text{DIC}} (\delta^{13}\text{C}_{\text{DIC}} - \Delta^{13}\text{C}_{\text{HCO}_3-\text{CO}_2,\text{eq}}) \quad (5)$$

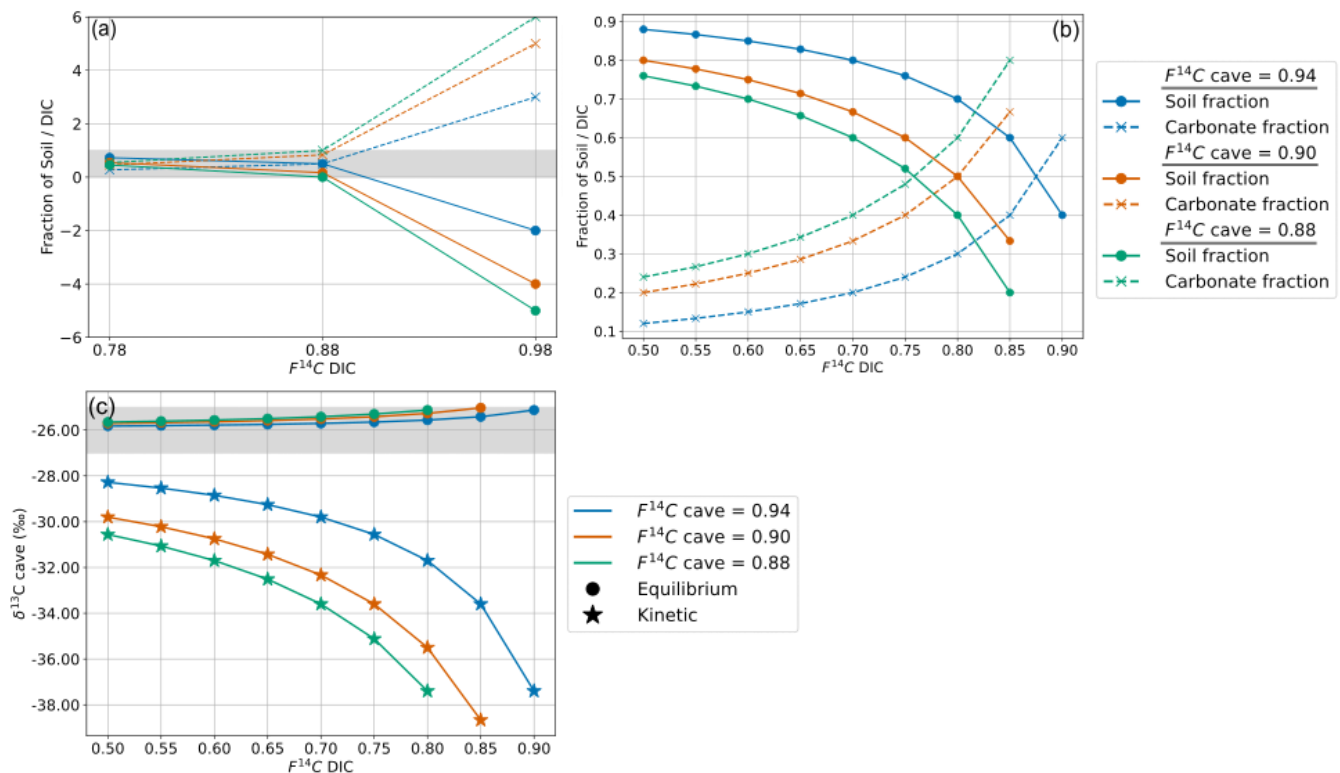
Kinetic degassing:

$$\delta^{13}\text{C}_{\text{cave}} = f_{\text{soil}} \delta^{13}\text{C}_{\text{soil}} + f_{\text{DIC}} (\delta^{13}\text{C}_{\text{DIC}} - \Delta^{13}\text{C}_{\text{HCO}_3-\text{CO}_2,\text{kin}}) \quad (6)$$

For the maximum (0.94), intermediate (0.90), and minimum (0.88) values of the endmember $F^{14}\text{C}$, only equilibrium fractionation during degassing can explain the observed $\delta^{13}\text{C}_{\text{cave}}$ value of approximately -26 ‰ (Fig. 9c). Kinetic fractionation scenarios result in very negative $\delta^{13}\text{C}_{\text{cave}}$ values which do not fit those measured, except when using very small kinetic fractionation factors similar to that of the equilibrium fractionation ($\Delta^{13}\text{C}_{\text{HCO}_3-\text{CO}_2,\text{kin}}$ of -10 ‰, see Appendix C). It is possible that kinetic fractionation occurs between Milandre cave waters and the cave air due to atmospheric ventilation

which occurs year-round. The atmospheric air which enters from the upper entrance in the summer and the lower entrance in the winter creates a concentration gradient between the CO_2 present within cave waters and the concentration in the cave atmosphere. However, the rapidly increasing pCO_2 with distance from the cave entrance (Fig. 8a) implies that the concentration gradients required for kinetic fractionation do not occur throughout most of the cave passage and that kinetic influences are probably minimal.

647



648

Figure 9. a) Mixing model between f_{soil} and f_{DIC} for the measured range of $F^{14}\text{C}_{\text{DIC}}$ values and $F^{14}\text{C}_{\text{cave}}$ values of 0.94 (blue), 0.9 (orange) 0.88 (green). The grey bar shows physically possible mixing ratios between 0 and 1. b) Mixing model between f_{soil} and f_{DIC} using the extended DIC $F^{14}\text{C}$ range and cave air $F^{14}\text{C}$ values. c) Cave air $\delta^{13}\text{C}$ for varying DIC $F^{14}\text{C}$ and cave air $F^{14}\text{C}$ values with equilibrium fractionation at -9.54 ‰ (circles) and kinetic fractionation at -30 ‰ (stars). The grey bar shows cave air $\delta^{13}\text{C}$ range which fits the measured values between -25 ‰ and -27 ‰.

654

Overall, the $F^{14}\text{C}_{\text{DIC}}$ measured beyond $F^{14}\text{C} = 0.90$ is too high to reproduce our measured cave air CO_2 $F^{14}\text{C}$ and $\delta^{13}\text{C}$. The $F^{14}\text{C}_{\text{DIC}}$ is based on the analysis of 9 drip water sites of varying drip rates and ecosystem coverage over the course of one year. It is possible that the sampling set up did not capture the true range of $F^{14}\text{C}_{\text{DIC}}$ in the drip water, though this hypothesis

658 is highly unlikely due to the diversity in drip rate and hydrological response of drips measured, and we believe our DIC data
659 to be representative of the system.

660

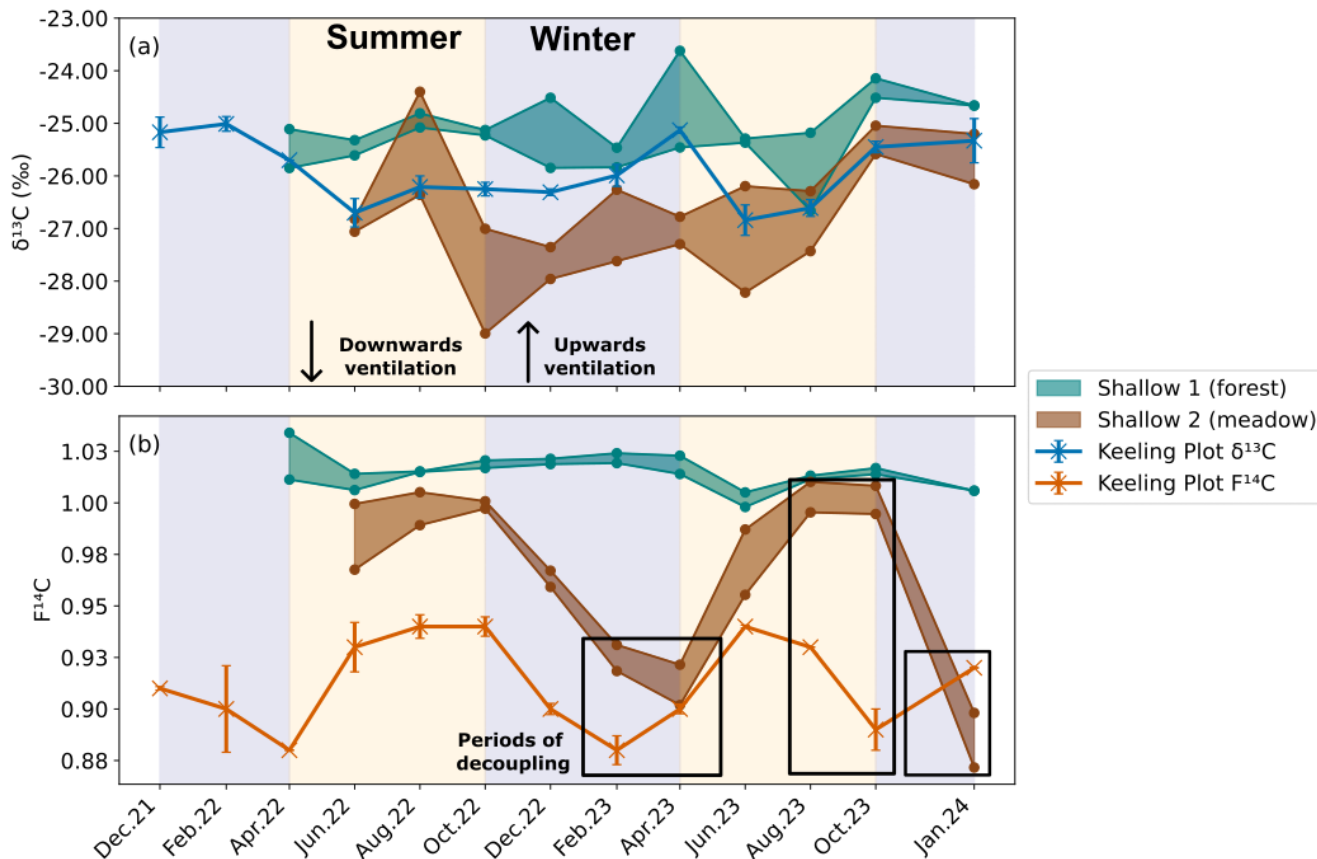
661 The $F^{14}C_{DIC}$ of the cave river was not measured, however previous studies investigating the Milandre cave river have
662 suggested that in non-flooding conditions the river is fed by slow diffuse flow as the unsaturated zone acts as a buffer,
663 storing water and dampening the signal of normal rainfall. During flooding events, the unsaturated zone aquifer is bypassed
664 and the stream is mainly fed through fracture flow (Perrin et al., 2003; Savoy et al., 2011). A diffuse flow fed river is likely
665 to have isotopic characteristics similar to that of the drip waters, which have higher $F^{14}C$ suggesting more open system
666 dissolution conditions. In contrast, times of flooding and soil saturation result in less exchange with the soil gas reservoir,
667 leading to dissolution under more closed conditions (Perrin et al., 2003; Savoy et al., 2011). However, the drip water $F^{14}C_{DIC}$
668 is relatively stable over one year of monitoring in a variety of hydrological conditions, implying that variations in host rock
669 dissolution and degassing dynamics are not the explanation for the variation in $F^{14}C$ and $\delta^{13}C$ of the endmember.

670

671 The meadow soil boreholes in the doline (Shallow 2) have similar patterns in CO_2 composition to the karst endmember, with
672 a $\delta^{13}C$ of -26 ‰ and variable $F^{14}C$ (Fig. 10a & b). In 2022, the $F^{14}C$ in the meadow soil and karst endmember align closely
673 over the seasonal cycle, but then decouple slightly in 2023 during the transitional periods between winter and spring where
674 the karst endmember $F^{14}C$ changes before the meadow. This suggests that, instead of the soils influencing the cave air due to
675 seasonal changes in soil respiration, the cave ventilation regime may be influencing the soil CO_2 $F^{14}C$. The cave ventilates
676 upwards in winter from Downstream to Upstream in a chimney effect (and vice versa in summer). Here, the lower $F^{14}C$ karst
677 endmember CO_2 may flow upwards through the highly fractured unsaturated zone of the doline and ventilate the meadow
678 soils, resulting in a mixture of low $F^{14}C$ CO_2 from the karst endmember and modern CO_2 from the soils. This possibly reflects
679 a contribution of older CO_2 from within the karst itself, likely from a “ground air” reservoir of older respiring organic
680 material in the unsaturated zone as found by several other studies (Breecker et al., 2012; Noroha et al., 2015; Matthey et al.,
681 2016; Bergel et al., 2017). It is possible that several reservoirs of older organic material exist throughout the downstream
682 catchment of Milandre which may contribute the low $F^{14}C$ CO_2 to the ventilating cave air in winter. In summer the
683 ventilation reverses, and the higher $F^{14}C$ CO_2 from the soil is transported downwards into the cave, increasing the $F^{14}C$ of the
684 cave endmember CO_2 . The $F^{14}C$ of the karst endmember is always lower than the meadow soil $F^{14}C$, implying that there is
685 always mixing between the soils and the karst endmember (which additionally also reflects the DIC contribution). Similar
686 upwards transport of CO_2 in karst boreholes has been observed in Nerja Cave, Spain (Benavente et al., 2010; Benavente et
687 al., 2015), and in the Gibraltar karst (Matthey et al., 2016). We do not observe significant effects of the winter upwards
688 ventilation in the $F^{14}C$ CO_2 in any of the deeper boreholes in the meadow or any of the boreholes in the forest. This could be
689 related to the location of Shallow 2 in the doline, whilst the rest of the boreholes are situated higher on the banks (Deep 3 and
690 Deep 4), or further away from the doline in the forest (Deep 1 and Deep 2). The topography difference between the higher

691 forest and lower meadow likely reflects influences of the secondary porosity in the bedrock, which is likely more highly
692 fractured in the meadow doline formation compared to the forest. As the doline structure likely has a higher secondary
693 porosity and is more highly fractured than the higher bedrock, ventilation effects may be more important here than at the
694 other locations.

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700 Figure 10. Comparison between the a) $\delta^{13}\text{C}$ and b) $F^{14}\text{C}$ of the shallow depth boreholes in the forest (Shallow 1, green) and meadow
701 (Shallow 2, brown), and the Keeling plot endmembers $\delta^{13}\text{C}$ (blue) and $F^{14}\text{C}$ (orange) also show in Figure 7. Summer (yellow) and
702 winter (blue) seasons are highlighted by the coloured background. The direction of cave ventilation is shown by the arrows with
703 downwards ventilation (from Upstream to Downstream) in summer, and upwards ventilation (Downstream to Upstream). Periods
704 of decoupling between the $F^{14}\text{C}$ of the meadow borehole and the cave endmember are shown in black boxes.

705

706 The accumulation of the older organic carbon in the unsaturated zone likely occurs over time. Soil layers in karst regions
707 often accumulate on bedrock with deep vertical fractures (Cheng et al., 2023). This decreases the ability of long-term storage
708 of older soil carbon pools due to preferential fracture flow, also known as subsurface leakage, which destabilises the oldest
709 soil layer and leads to export of older carbon into the unsaturated zone (Sánchez-Cañete et al., 2018; Wan et al., 2018).
710 Several studies have acknowledged this potential source of CO₂ produced autochthonously inside the unsaturated zone by the
711 microbial degradation of this old soil, plant, or root material which was previously washed into the unsaturated zone,
712 contributing older CO₂ to the ground air mix (Noronha et al., 2015; Bergel et al., 2017; Ding et al., 2023).

713 6 Conclusion

714 We conducted a comprehensive investigation over two years into the dynamics of CO₂ concentrations, $\delta^{13}\text{C}$ and F¹⁴C values
715 across different land covers and soil types within the Critical Zone at Milandre cave in northern Switzerland. Our analysis
716 found distinct seasonal fluctuations in CO₂ levels in soil boreholes in both forest and meadow areas, with higher
717 concentrations during summer compared to winter. Low $\delta^{13}\text{C}$ values of ~ -26 ‰ indicate respiration of C3 plants which
718 dominate the catchment area. The stable modern F¹⁴C of ~ 1.00 in shallow forest boreholes indicated a year-round modern
719 CO₂ contribution from tree and plant roots to the subsurface, consistent with a well-ventilated soil. On the other hand,
720 samples from meadow boreholes situated in a doline with a thick, well developed soil cover displayed seasonality in F¹⁴C
721 from ~ 0.88 in the winter and spring to ~ 1.00 in summer.

722

723 In the unsaturated zone we observed substantial variability in CO₂ concentrations across the catchment ($\sim 3,000$ to $\sim 37,000$
724 ppmV), with higher concentrations in boreholes with forest cover than those with meadow cover. Despite differences in
725 surface vegetation, the isotopic compositions of $\delta^{13}\text{C}$ and F¹⁴C remained stable in both forest and meadow environments,
726 reflecting the dominant modern C3 vegetation signature contributing to the ground air. The year-round modern unsaturated
727 zone CO₂ in meadow boreholes Deep 3 and Deep 4 contrasts with the seasonally attenuated signal in the thicker meadow
728 soils.

729

730 Large variations in cave air CO₂ concentrations (atmospheric to $\sim 30,000$ ppmV), $\delta^{13}\text{C}$ (~ -9 to -25 ‰), and F¹⁴C (0.88 to
731 0.98), are controlled by seasonal temperature-driven cave ventilation. The seasonal changes in the isotopic composition of
732 the Keeling plot derived karst endmember cannot be explained with the observed DIC F¹⁴C range, suggesting that bedrock
733 dissolution and degassing dynamics are likely not the cause of the isotopic variation in the cave air. The isotopic
734 characteristics of the cave air are comparable to those of the meadow doline soil. The seasonal variability in karst
735 endmember CO₂ F¹⁴C precedes that in the soil, and is always older. This suggests that cave ventilation contributes older CO₂
736 to the doline soils, likely sourced from a reservoir of aged organic material in the unsaturated zone, during the upward winter

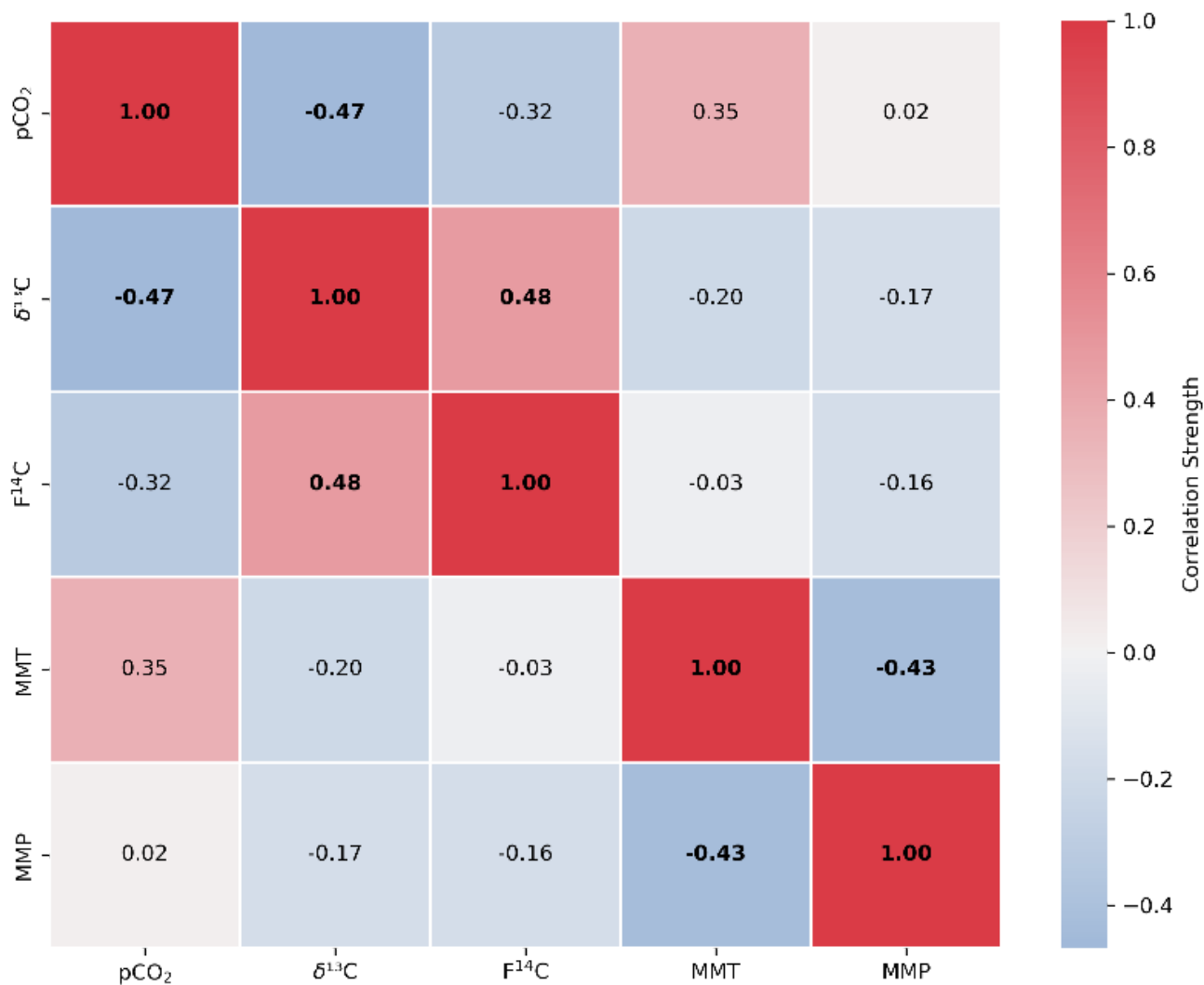
737 ventilation regime. In summer, the reversed downwards ventilation contributes younger soil CO₂ to the cave, mixing with the
738 older unsaturated zone reservoir.

739

740 Our study provides important insights into how carbon is transported to the subsurface in karstic Critical Zones.
741 Understanding these processes is crucial for accurate estimation of the size of subsurface CO₂ pools (ground air), and to
742 refine terrestrial CO₂ budgets. Moving forward, it would be beneficial to undertake more detailed investigation of CO₂
743 transport into the subsurface implementing ¹⁴C analysis to further constrain the sources of ground air. Furthermore, higher
744 resolution monitoring over periods of interest, could assist us in understanding short-term variations.

745 **Appendix A**

746



747

748 A1 Spearman's rank correlation matrix displaying the Spearman's rho of CO₂ concentration, δ¹³C and F¹⁴C, Mean Monthly
 749 Temperature (MMT) and Mean Monthly Precipitation (MMP) from Fahy weather station from samples from soil depth boreholes
 750 Shallow 1. Parameters with a significant correlation (positive or negative) are bolded and the strength of the correlation is
 751 indicated by the blue – red colour bar.

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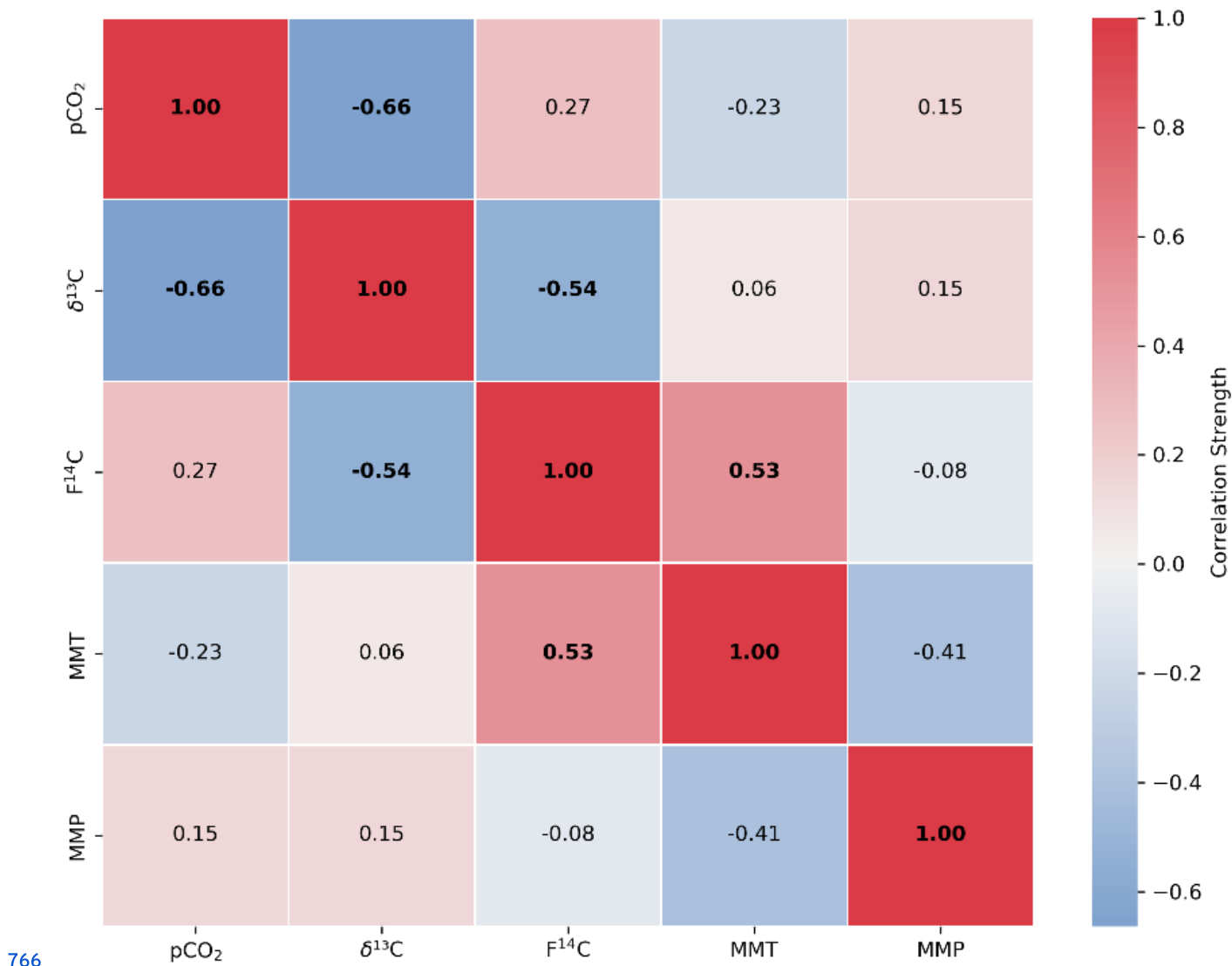
756 A2 Spearman's rank correlation matrix displaying the Spearman's rho of CO₂ concentration, δ¹³C and F¹⁴C, Mean Monthly
 757 Temperature (MMT) and Mean Monthly Precipitation (MMP) from Fahy weather station from samples from soil depth borehole
 758 Shallow 2. Parameters with a significant correlation (positive or negative) are bolded and the strength of the correlation is
 759 indicated by the blue – red colour bar.

760



761

762 A3 Spearman's rank correlation matrix displaying the Spearman's rho of CO₂ concentration, δ¹³C and F¹⁴C, Mean Monthly
 763 Temperature (MMT) and Mean Monthly Precipitation (MMP) from Fahy weather station from samples from unsaturated zone
 764 depth boreholes Deep 1 and Deep 2. Parameters with a significant correlation (positive or negative) are bolded and the strength of
 765 the correlation is indicated by the blue – red colour bar.



A4 Spearman's rank correlation matrix displaying the Spearman's rho of CO₂ concentration, δ¹³C and F¹⁴C, Mean Monthly Temperature (MMT) and Mean Monthly Precipitation (MMP) from Fahy weather station from samples from unsaturated zone depth boreholes Deep 3 and Deep 4. Parameters with a significant correlation (positive or negative) are bolded and the strength of the correlation is indicated by the blue – red colour bar.

776



777

778 A5 Spearman's rank correlation matrix displaying the Spearman's rho of CO₂ concentration, δ¹³C and F¹⁴C, Mean Monthly
779 Temperature (MMT) and Mean Monthly Precipitation (MMP) from Fahy weather station from samples from the Downstream
780 cave site. Parameters with a significant correlation (positive or negative) are bolded and the strength of the correlation is indicated
781 by the blue – red colour bar



782

783 A6 Spearman's rank correlation matrix displaying the Spearman's rho of CO₂ concentration, δ¹³C and F¹⁴C, Mean Monthly
 784 Temperature (MMT) and Mean Monthly Precipitation (MMP) from Fahy weather station from samples from the Upstream cave
 785 stie. Parameters with a significant correlation (positive or negative) are bolded and the strength of the correlation is indicated by
 786 the blue – red colour bar.

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793 B1 Drip water dissolved inorganic carbon F¹⁴C and AMS derived δ¹³C from the actively dripping galleries nearby Upstream and
794 Downstream.

Drip	Sampling date	F ¹⁴ C	F ¹⁴ C % error	AMS δ ¹³ C (‰)
GF1	Dec.21	0.86	0.92	-13.6
GF2	Dec.21	0.94	0.91	-14.3
GR2	Dec.21	0.91	0.92	-12.4
GR3	Dec.21	0.98	0.93	-13.5
GR4	Dec.21	0.91	0.97	-14.7
GR5	Dec.21	0.90	0.97	-14.2
GR6	Dec.21	0.85	1.02	-13.6
GF1	Feb.22	0.87	0.99	-12.6
GF3	Feb.22	0.95	0.96	-12.7
GR3	Feb.22	0.90	0.97	-14.1
GR4	Feb.22	0.89	0.99	-12.7
GR6	Feb.22	0.84	1.00	-11.9
GR6	Feb.22	0.88	0.99	-10.6
GF1	Apr.22	0.88	0.93	-12.8
GF2	Apr.22	0.93	0.89	-11.6
GF3	Apr.22	0.98	0.86	-12.1
GR1	Apr.22	0.87	0.90	-12.6
GR2	Apr.22	0.89	0.92	-12.1
GR3	Apr.22	0.90	0.93	-12.2
GR4	Apr.22	0.90	0.93	-13.4
GR5	Apr.22	0.89	0.98	-13.7
GR6	Apr.22	0.90	0.99	-13.6
GF1	Jun.22	0.87	1.11	-18.0
GF2	Jun.22	0.93	1.11	-18.9
GF3	Jun.22	0.96	1.07	-18.6
GR1	Jun.22	0.88	1.12	-17.4
GR2	Jun.22	0.93	1.09	-17.6
GR3	Jun.22	0.89	1.12	-17.9
GR4	Jun.22	0.89	1.12	-18.9
GR5	Jun.22	0.90	1.11	-17.9
GR6	Jun.22	0.92	1.12	-19.0
GF1	Aug.22	0.86	1.10	-17.1
GF2	Aug.22	0.92	1.12	-19.8

GR1	Aug.22	0.87	1.03	-8.9
GR2	Aug.22	0.91	1.09	-18.3
GR4	Aug.22	0.93	1.10	-19.2
GR5	Aug.22	0.91	1.10	-19.5
GR6	Aug.22	0.93	1.11	-20.8
GF1	Oct.22	0.84	1.05	-9.4
GF2	Oct.22	0.94	1.12	-20.3
GF3	Oct.22	0.95	1.11	-20.8
GR1	Oct.22	0.86	1.14	-17.2
GR2	Oct.22	0.88	1.14	-22.6
GR4	Oct.22	0.91	1.13	-18.7
GR5	Oct.22	0.89	1.13	-18.3
GR6	Oct.22	0.91	1.11	-19.1
GF1	Dec.22	0.86	1.01	-14.1
GF2	Dec.22	0.88	0.94	-14.1
GF3	Dec.22	0.94	0.95	-11.1
GR1	Dec.22	0.86	1.00	-11.5
GR4	Dec.22	0.89	0.98	-11.5
GR5	Dec.22	0.89	1.01	-12.6
GR6	Dec.22	0.78	1.02	-12.9

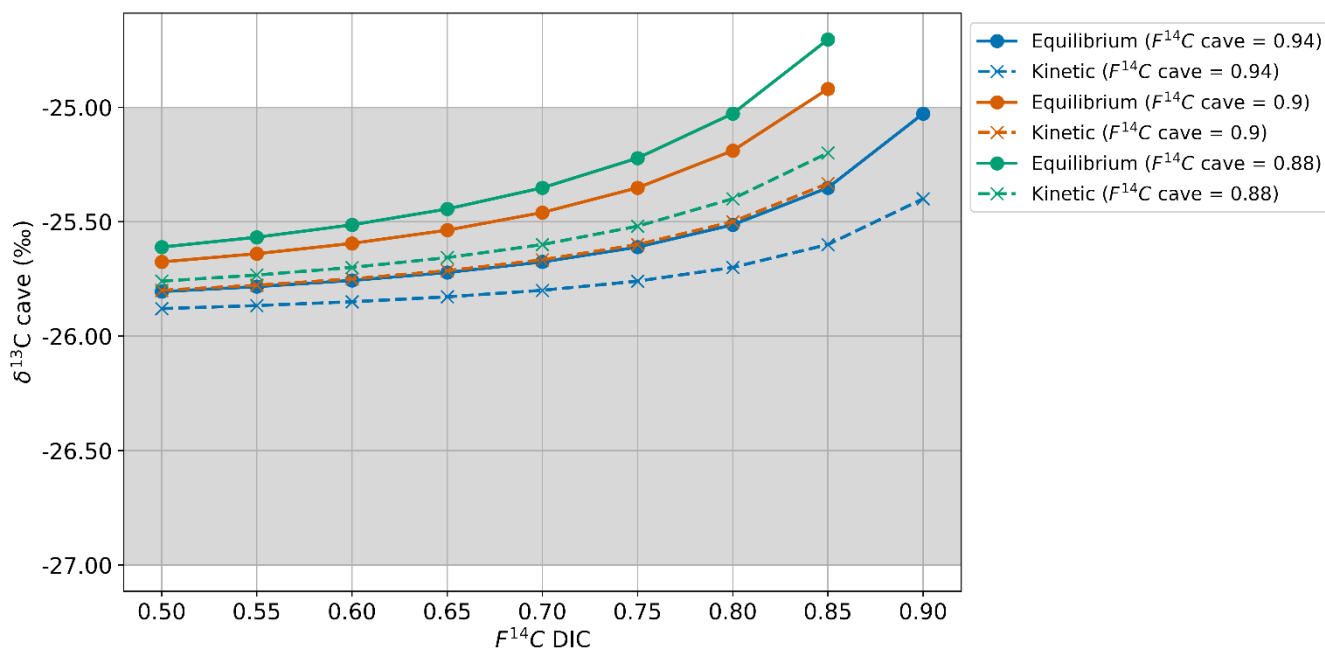
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801 C1 Cave air $\delta^{13}\text{C}$ for varying DIC $F^{14}\text{C}$ and cave air $F^{14}\text{C}$ values with equilibrium fractionation at -9.54‰ (solid lines) and kinetic
802 fractionation at -10‰ (dashed lines).

803

804 Code and data availability

805 The data, code for Spearman's rank statistics, and code to reproduce the model results are publicly available on Zenodo at
806 <https://doi.org/10.5281/zenodo.14253707>.

807 Author contributions

808 Sarah Rowan: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Software, Writing – original
809 draft preparation.

810 Marc Luetscher: Conceptualization, Resources, Visualization, Writing – review & editing.

811 Thomas Laemmel: Methodology, Validation, Writing – review & editing.

812 Anna Harris: Methodology, Writing – review & editing.

813 Sönke Szidat: Writing – review & editing.

814 Franziska Lechleitner: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing –
815 review & editing.

816 Competing interests

817 The authors declare that they have no conflict of interest.

818 Disclaimer

819

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