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RESEARCH ARTICLE

REVISED Investigative research for occurrences of hydrogen, helium, methane and carbon dioxide in West Macedonia: linking geological reservoirs to subsurface gas generation and migration

[version 3; peer review: 2 approved, 2 approved with reservations, 1 not approved]

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Abstract

Background

Exploration of subsurface gases such as hydrogen, helium, methane, and carbon dioxide can be achieved by integration of geological framework, hydrogeochemistry, and isotope analysis to understand generation, migration, and trapping mechanisms. A focused hydrogeochemical survey for the aforementioned gases was conducted in West Macedonia (Greece) to identify potentially suitable locations for geological reservoirs, gas sources and gas migration routes based on previous research. West Macedonia (Greece) is characterised by ophiolitic complexes, sedimentary basins, and active fault systems. This presents favourable geological conditions for investigating potential gas reservoirs and migration pathways.

Methods

The investigation presented in this study deployed sequential spring and borehole water sampling for geochemical analysis of trace

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elements and gas analysis for hydrogen, helium, methane and carbon dioxide to identify and characterise gaseous geological reservoirs. The investigation extended into isotope studies for $d^{13}C_{TDC}$, $\delta^{13}C_{CH4}$, $\delta^{13}D_{CH4}$, δD_{H2O}, $\delta^{18}O_{H2O}$.

Results

The analysis provided evidence for the existence of helium, biogenic methane, carbon dioxide and traces of hydrogen that need to be further investigated for validation and better understanding of the gas generation and migration routes. Variations in dissolved ions and gas composition largely reflect enrichment of resident groundwater by meteoric recharge and subsequent water–rock interaction. Fault-controlled pathways appear to facilitate multi-gas signatures in specific locations.

Conclusions

The data suggests the existence of helium, methane, carbon dioxide and validated trace concentrations of hydrogen from previous studies in the wider area. Isotopic analysis provides strong evidence for biotic generation of methane, whereas helium comes from a deeper source. Hydrogeochemical controls, structural features, and lithological variability collectively influence gas occurrence. This preliminary investigation indicates the existence of multiple gas generation and migration mechanisms and provides a scientific basis for further targeted exploration of natural hydrogen and associated gases.

Plain language summary

As our humanity evolves, we realise that our current way of converting energy is not sustainable, it creates geopolitical tensions, competition for resources and results in climate change. The future of the current generation and future ones is at stake. We have already experienced the results of climate change and its direct impacts on the food production chain, as well as water scarcity, manifesting themselves in cost and price increases in developed countries, whereas in underdeveloped and developing countries, the situation is difficult to estimate, but it is even worse. Our research focuses on alternative ways of managing energy and carbon dioxide storage to battle climate change for a just society. In addition, we investigate alternative localised natural sources of energy such as natural hydrogen and methane to support energy independence for prosperous societies.

Keywords

helium, methane, natural hydrogen, geological storage, gas migration, isotope, geological reservoirs

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REVISED **Amendments from Version 2**

The abstract has been updated to reflect the technical and scientific aspects of the research. Tables were standardised, and figures were improved in terms of geology and fault depiction. Additional conceptualisation has been incorporated to allow for the role of active faults in fluid circulation. References have been updated with the most recent ones.

Any further responses from the reviewers can be found at the end of the article

Introduction

The relatively recent political change in tackling the climate change issue has been expressed with the Paris Agreement[1] on a global scale. The Green Deal[2] in the European Union has signaled the way forward for the energy transition and decarbonisation of industry. Carbon capture and storage[3,4] technology has provided an intermediate solution to keep business as usual. Underground hydrogen storage and geological carbon storage have been developed almost in tandem, and the latter has drawn on substantial technical knowledge from the former. As the scientific society strives to find solutions the aforementioned technologies have serendipitously led to discoveries and the conceptualisation of natural hydrogen resources. In Greece, West Macedonia, HyStorIES and PilotSTRATEGY focuses on the deep saline aquifers, porous rock formations filled with brine several kilometres below ground, which promise a large capacity for storing CO_2 captured from industrial clusters or hydrogen. However, during the investigation, an additional research area related to hydrogen occurrences was identified.

The concept of natural hydrogen, although not new, still remains in its infancy with only a handful of researchers together and some pioneer industries actively pursuing the subject to identify large and constant sources that will be able to support a reliable energy transition.[5–9] Based on stochastic modelling earth is estimated to be capable of producing some 5.6×10^6 Mt of hydrogen. Most of it is unrecoverable because its accumulation is too deep, too small in volume, dispersed, or too far away offshore. Still, if a small portion of this, such as 10^5 Mt, can be recovered, it would be able to supply the global needs for 200 years with net-zero carbon emissions.[10] This will provide enough time to achieve nuclear fusion[11] and make the next revolutionary step in human civilisation history based on the Kardashev scale.[12]

Promising results for natural hydrogen have already been provided in Mali,[13] in Namibia,[14] in Colombia,[15] in Australia,[16] and in the Balkans in Albania and Kosovo.[17] Daskalopoulou *et al.*, 2018,[18] have also reported traces of natural hydrogen in West Macedonia together with helium and methane. The understanding of natural hydrogen generation mechanisms is still under development, but in general include:

1) Contact of water with reducing agents in the mantle.[19–21]

2) Reaction of water with ultrabasic rocks, serpentinization[22,23] - type reactions involving the reduction of water by iron-rich minerals have generally been regarded as requiring high temperatures (>~200°C),[24] although this has been recently challenged with observation at lower temperature conditions (<200°C).[25,26]

3) Reduction of water by iron-rich minerals in banded iron formations[27–29] or biotite-rich granites.[30]

4) High–thermal maturity organic-rich rocks.[31,32]

5) Decomposition of organic matter[19] of NH_3 to N and H_2 at high temperature (up to 600°C).

6) Natural electrolysis of water.[33]

7) Natural radiolysis of water.[16,19,34]

8) Mantle degassing.[19,35]

9) Biological activity[22] via microbes in soils and coal beds.

10) Cataclasis[19,20,36] during earthquakes.

Hydrogen is highly reactive and susceptible to redox conditions, making long-term preservation during migration challenging.[10] In addition, microbial communities are capable of utilising and producing hydrogen that exists in the subsurface.[37] Thus, understanding the mechanisms of hydrogen generation, seepage systems,[38] residence time in

geological reservoirs, biotic and abiotic loss are essential for guiding its exploration and sustainable production. Exploration is currently based on research and development of natural hydrogen conceptual geological models that will serve as geological analogues to identify prospective place for exploitation.

Past preliminary investigations in West Macedonia, Greece, have detected the presence of dissolved helium and traces of hydrogen in groundwater and spring emissions, alongside significant methane and carbon dioxide.[18] While geochemical and isotopic evidence indicated that the methane is largely biogenic in origin, helium and hydrogen when occur together there are high chances that are derived from abiotic process.[39]

Based on these past findings, a scientific hypothesis is formulated on the grounds that West Macedonia, Greece, hosts active geological processes based on serpentinisation of ultramafic rocks and radiogenic decay that are capable of generating natural hydrogen and helium. These gases may migrate along fault-controlled pathways and where suitable porous and permeable geological formations and conditions exist, could accumulate in subsurface compartments.

To test the scientific hypothesis the following objectives are investigated:

1. Confirm and map gas occurrences especially hydrogen and helium by targeting sampling in West Macedonia.

2. Determine biotic versus abiotic gas origins using isotope evidence.

3. Investigate gas migration pathways such as faults[38] via structural and hydrogeological analysis of the study area.

4. Characterise geological settings and rock reservoirs by assessing the potential of geological formations to generate, host and trap hydrogen and helium.

5. Assess resources potential and energy implications by estimating the natural hydrogen and helium volume available for use.

The geological conceptual model to be used as an analogue for testing the aforementioned hypothesis is as follows. Areas with potential for natural hydrogen exploration/exploitation require a source of sufficient hydrogen generation, a mechanism for vertical and lateral fluid migration, porous rock reservoirs for storage, and low permeability rocks (seals) to prevent leakage and allow for economically viable reserves. An additional component that relates to preservation needs to be considered due to hydrogen's high reactivity and microbial consumption. Natural hydrogen is generated in high depths, while it is consumed in shallow ones.[40] Detection of abiotic methane, brucite and carbonates is a useful indicator for the presence of natural hydrogen.[14,41] These indicators may be found together or individually in the field with closely related ultrabasic rocks. Helium is also generated by decay of radiogenic nuclides in similar rocks and can be related to natural hydrogen.[40]

In general, the presence of talc indicates higher silica activity in serpentinising systems, which diverts reactions away from magnetite formation and consequently diminishes the capacity to generate H_2. In contrast, low silica activity favours H_2 production.[24] Indicators of low-silica, H_2-generating conditions include the formation of abundant magnetite as well as brucite, which both are formed during serpentinisation reactions that release molecular hydrogen.[42–44] Serpentinisation often produces hyperalkaline fluids with pH > 10. These fluids are rich in calcium Ca^{2+} and OH^- but depleted in silica and Mg^{2+},[45] reflecting the hydration of olivine and pyroxenes in a deeper and closed system.[44]

Hyperalkaline springs found in ophiolites are related to serpentinisation[17] via the following general chemical Equation 1 where olivine is the source rock of Fe^{2+} that oxidises to magnetite (Fe^{3+})[33]:

$$Fe^{2+} + H_2O \xrightarrow{\text{yields}} Fe_3 + \frac{1}{2}H_2 + OH^- \qquad \text{(Equation 1)}$$

These hyperalkaline springs are typically geologically linked with faults, which act as upwelling pathways from deep aquifers.[45]

In terms of the geological setting of interest, ophiolites that are thrusted and overlain by carbonate rocks, can provide ample quantities of CO_2, leading to the following very slow reaction (Equation 2) but nonetheless important over geological time[26]:

$$CO_2 + 4H_2 = CH_4 + 2H_2 \text{ with } T < 100°C (\text{Sabatier reaction}) \qquad \text{(Equation 2)}$$

whereas thrust and other related faults allow for fluid percolation from deep aquifers below or from rainwater above to enable the above reactions to take place. Reaction described in Equation 2 can be catalysed by magnetite (Fe_3O_4), chromite ($FeCr_2O_4$) and awaruite (Ni_3Fe), at temperatures above 200°C.[44,46]

Supra regional geological context

Before focus on West Macedonia, Greece, a description of the regional Balkan geological setting with relevance to natural hydrogen research is provided for the reader's benefit. Research conducted by Levy *et al.*, 2023[17] has identified hot spots of hydrogen occurrences in Albania and Kosova with laboratory water sample analysis from natural springs against C and H isotopes of CH_4 and H_2. Four springs out of 21 sites provided strong evidence for H_2 occurrence. These recent finding in Albania, that are further enhanced by the finding of Truche *et al.* 2024,[47] given the geological proximity and affinity to North-West Greece, suggests that similar results may appear in Greece in the Mesohellenic basin. The Mirdita ophiolite in Albania presents interest and a potential analogue as it is located in the Albanides mountain belt that is a segment of the Dinarides–Albanides–Hellenides orogen. It is bounded on the east by the Pelagonian block and on the west by the Krasta, Cukali, and Kruja zones. The latter is well-known for its geothermal potential due to thrust faults that allow hot fluids percolation. The eastern and particularly western massifs of the Mirdita ophiolite are mantle domes of harzburgite (peridotite composed of olivine and orthopyroxene and small amounts of chromium-rich spinel) capped by mylonitic plagioclase-amphibole peridotite of 400 m thickness.[48] The northeastern part of the ophiolite complex can reach 14 km in thickness, whereas the west and southeast are estimated to be 2 km thick.[17] In Kosova, the ophiolites of the West Vardar zone part of the Dinarides are in continuity of Mirdita ophiolite. Of similar interest is the central ophiolite of Kosova that has hydrothermally metamorphosed, leading to the economic precipitation of magnesite.[17] The subsequent paragraphs provide the Greek regional geological setting.

The Othrys ophiolite complex in central Greece, specifically within the Dinaric–Hellenic ophiolite belt and lies uncomfortably into the Pelagonian carbonate platform. It represents an obduction event where an oceanic lithosphere has been thrust onto continental crust. These are complemented by dunite forming thin layers, a few cm to a few meters thick, intercalated in the harzburgite. A remnant doleritic sheeted-dyke complex is tectonically overlain by peridotites. Whitish rodingite dykes are found in serpentinised harzburgites very close to the Archani spring.[26] The peridotites are in tectonic contact with an ophiolitic mélange, which in turn tectonically overlies a Late Cretaceous-Tertiary flysch. An active fault zone, along the Leontari-Anavra line, is found north of Ekkara, at the south border of the Thessaly Plain.[26,44,49] Tsikouras *et al*. (2013)[49] and Etiope *et al.* (2013)[44] investigated 21 ophiolitic springs in the serpentinised Othrys ophiolite. Four hyperalkaline springs (pH 10.7–11.3) with calcium-rich (Ca–OH) waters indicated the presence of methane and hydrogen. These springs, one in Archani and three in Ekkara, are located along creeks and tectonically related to faults that are directly linked to the main regional active fault system. An estimation revealed that collectively the springs produce some 100 kg/year of methane.[44] The remaining 17 springs, which had pH < 8.7 values, did not show any detectable CH_4 or H_2.

The water spring isotope chemical analysis of CH_4 has revealed that the origin of the methane is not biotic. Thus, in hyperalkaline high silica fluids can positively influence the production of H_2 whereas low silica fluids may result in low H_2 production. Othrys springs albeit hydrogen positive, still are releasing low amounts of H_2 compared to other similar places.[26,44,49] This can be attributed to:

i). Normal hydrogen generation due to interaction with low-silica fluids alone, followed by consumption through CO_2 hydrogenation and/or loss to the surface as water degasses. Note that H_2 solubility is much less compared to CH_4, by about an order of magnitude.

ii). Direct methanation processes involving both low- and high-silica fluids.[10,44,49] High-silica activity promotes the formation of talc or other silica-rich phases, which consumes Mg and reduces the production of brucite (Mg $(OH)_2$, thereby lowering H_2 generation.

iii). Microbial activity that consumes hydrogen.

iv). Temperature decrease or modifications in water–rock ratios[44]

Daskalopoulou *et al.*, 2018[18] from their research in West Macedonia, Greece, and particularly in Katakali (Mesohellenic Basin) and Mesohori (Florina Basin), idenitified traces of helium and hydrogen with significant values of methane. Katakali provided 4 ppm He, 11 ppm H_2 and 880.000 ppm CH_4. The Mesohori sample registered 4 ppm He, 1.5 ppm H_2 and 479 ppm CH_4. These values albeit low are indicative and worth investigating to understand the underlying fluid generation and migration.

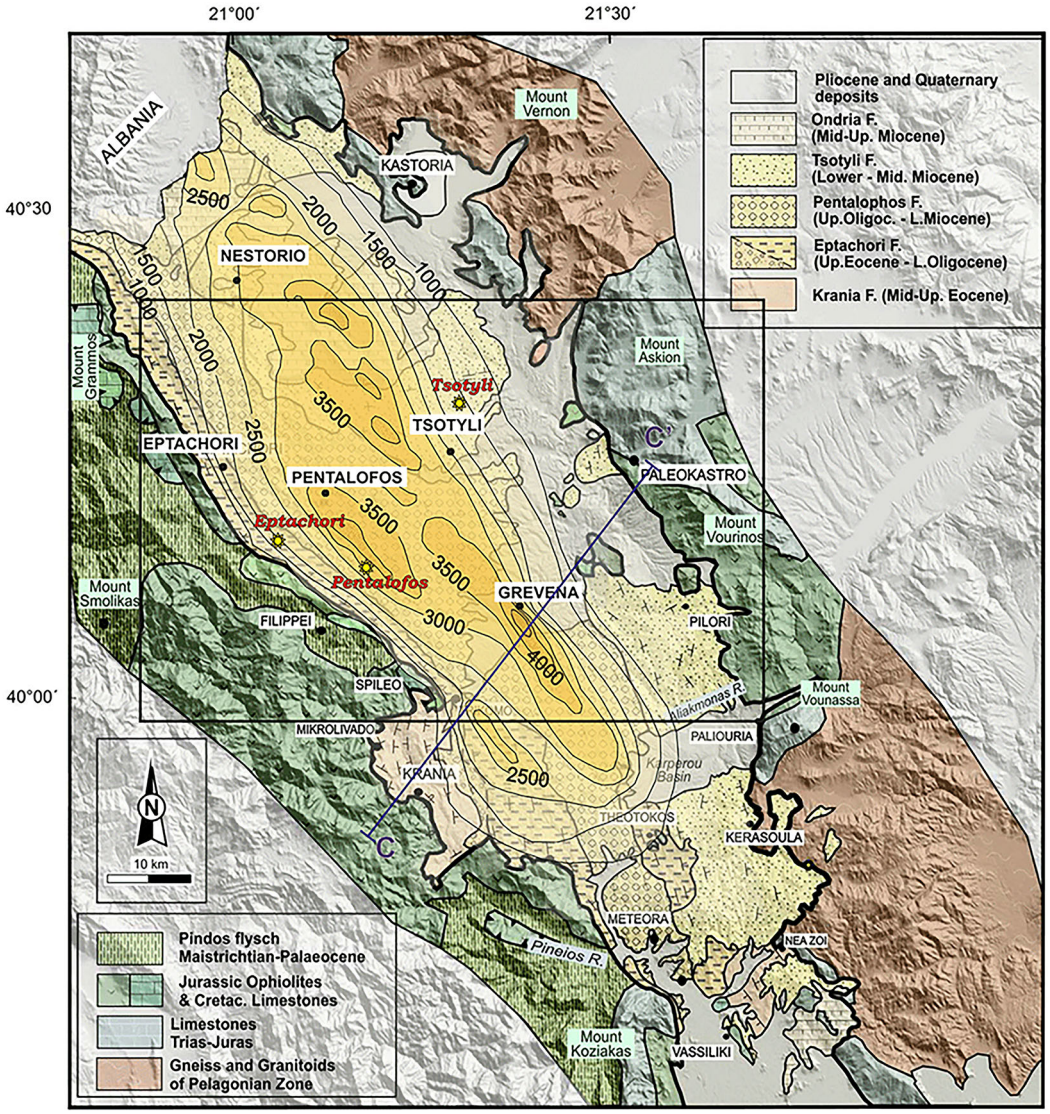

Figure 1. Modified Geological map and isodepths of the basement rocks of the Mesohellenic Basin,[61] licence: CC-BY 4.0.

Regional geological setting for Mesohellenic and Florina basin, West Macedonia, Greece

Based on the Daskalopoulou *et al.*, 2018[18] findings further research on natural hydrogen was ensued on the Mesohellenic and Florina basin. The geological setting for both basins is given further below. The Mesohellenic Basin (MHB), depicted in Figure 1, extends over 200 km in a NNW-SSE direction, is 20 to 40 km wide and has a thickness of 4500 metres. It is a late-orogenic, molassic-type basin that developed from the Middle Eocene to Middle Miocene in northwestern Greece and southern Albania.[50] It lies along the suture between the Apulian plate and the Pelagonian nappe pile and marks the boundary between the Internal and External Hellenides.[51–53]

MHB is a favourable area with a potential for hydrogen accumulations associated with the long-distance lateral flow migration that commonly occurs in sedimentary basins. Long-distance lateral migration is defined as migration across map distances of up to 250 km.[40] Ophiolites (Figure 2) are abundant in the area, offering promising potential as a source rock for natural hydrogen through serpentinization. The underlying Triassic and Jurassic limestones can contribute significant quantities of CO_2, mobilised by migratory fluids that percolate vertically and laterally through the rock mass because of the prevailing tectonic setting and associated strain.

The Jurassic oceanic lithosphere was obducted during the closing of the Mesozoic Tethys Ocean, forming the Vourinos and Pindos ophiolites that are exposed along the edges of the MHB.[52,54] The Vourinos ophiolite is a well-preserved

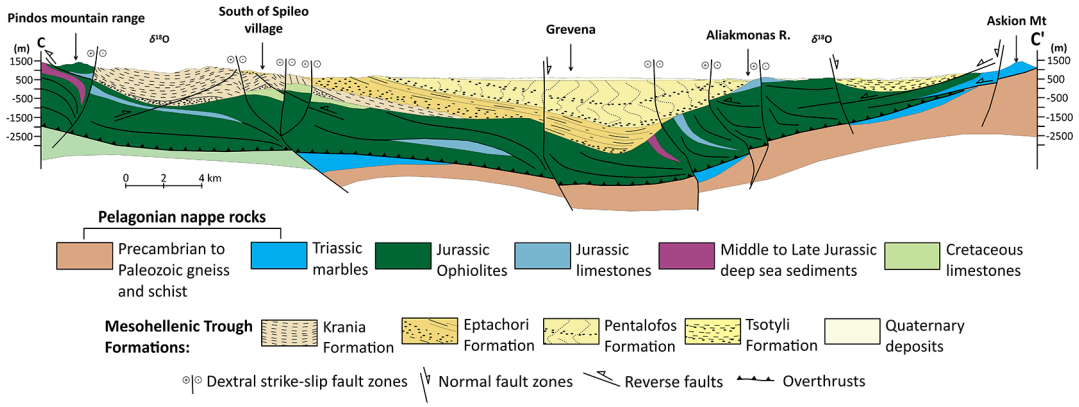

Figure 2. Modified geological cross-section of Mesohellenic Basin,[61] licence: CC-BY 4.0. The illustrated fault architecture represents the inherited Miocene structural framework developed during basin opening. Fault geometries shown in the section include syn-rift and post-rift structures and do not necessarily reflect the present-day active faulting style, which is dominated by normal faulting under the current extensional stress regime.

Penrose-type section made up of pillow lavas, sheeted dikes, a thick ultramafic-mafic cumulate sequence, and mantle peridotites. Hydrothermal activity during seafloor spreading is indicated by features including epidotized dikes, sulfide mineralisation, and jaspers.[55] A continuous magnetic anomaly beneath the Mesohellenic Trough suggests a common ophiolitic root, supporting a shared tectonic origin.[51,55]

The basin formed atop westward-emplaced Tethyan ophiolites following their final emplacement between Middle and Late Eocene during the Alpine orogeny, with subsidence driven by crustal loading and flexure.[50,56] The stratigraphy of the MHB is subdivided into seven major lithostratigraphic units.[57–60] These include: 1) the Middle-Upper Eocene Krania Formation (Fm) incorporating conglomerates and olistoliths (lower sequence) and turbiditic beds (upper sequence) with a thickness of 1500 m, 2) the Oligocene Eptachorion Fm with silty marls and very-fine sandstone beds overlying thick conglomerates (~1000 m in thickness), 3) the Upper Oligocene-Lower Miocene Taliaros/Tsarnos Fm and the equivalent Pentalofos Fm, showing sequences from sandstones to conglomerates (~2500 m in thickness), 4) the Lower to Middle Miocene Tsotyli Fm with marl-sandstone alternations in the north and conglomerates in the south (~600 m in thickness), and 5) the Ondria Fm and the equivalent Orlias Fm (Lower to Middle Miocene) comprising fossiliferous limestones, marls, and sandstones (≥350 m in thickness). These MHB thick sedimentary strata, as a geological reservoir, offer the benefit of (1) sufficient burial to compact rocks to low porosity and (2) a higher likelihood of one or more potential sealing formations throughout the stratigraphic sequence.[40] Tyrologou *et al.* 2023 have confirmed with field surveys and laboratory investigation the existence of favourable rock sealing conditions related to the Pentalofos, Eptachori and Tsotyli sedimentary formations[61] due to their low porosity and permeability values.

Major tectonic unconformities define the bases of the Krania, Eptachorion, and Tsotyli Formations. Lithologies within these formations include conglomerates of fan-delta and alluvial fan origin, turbiditic sandstones and shales, as well as deltaic, floodplain, and shelf sandstones and siltstones.[57–59] These sediments show a general coarsening trend from north to south and were deposited during a progressive shallowing of the basin, with a maximum sedimentary thickness reaching 4.5 km near Grevena.[51,62] These sedimentary facies hold potential as porous media capable of economically accumulating hydrogen while being protected from overlain low permeability rocks described previously. Upper Miocene unconformably overlies the molassic sequence to Quaternary deposits.

The Vourinos ophiolitic complex within the MHB[63] is the most profound potential source for natural hydrogen. The Theopetra-Theotokos Structure in the southern portion of the basin exposes the basement ophiolitic and carbonate units and runs almost parallel to the main basin axis.[53,60] A complex tectonic development influenced by both brittle and semi-ductile deformation occurred in the basin. Along NW-SE to NNW-SSE faults, dextral strike-slip tectonics dominated the Early to Late Oligocene, allowing for lateral basin displacements and subsidence.[64]

The Florina Basin is part of a large NW-SE trending graben system in NW Macedonia, Greece, that formed during the Lower Miocene following the Alpine Orogeny. This graben system (~150 km in length) contains the Florina, Ptolemaida-Amynteo, Kozani-Servia, and Sarandaporo Basins.[65] Depicted in the synthesised related stratigraphic column in Figure 3, the basement rocks of the basin system include mainly Triassic to Jurassic crystalline limestones, marbles and dolomites, as well as Palaeozoic to Mesozoic schists, phyllites, gneisses, and granites of the Pelagonian Zone.[65]

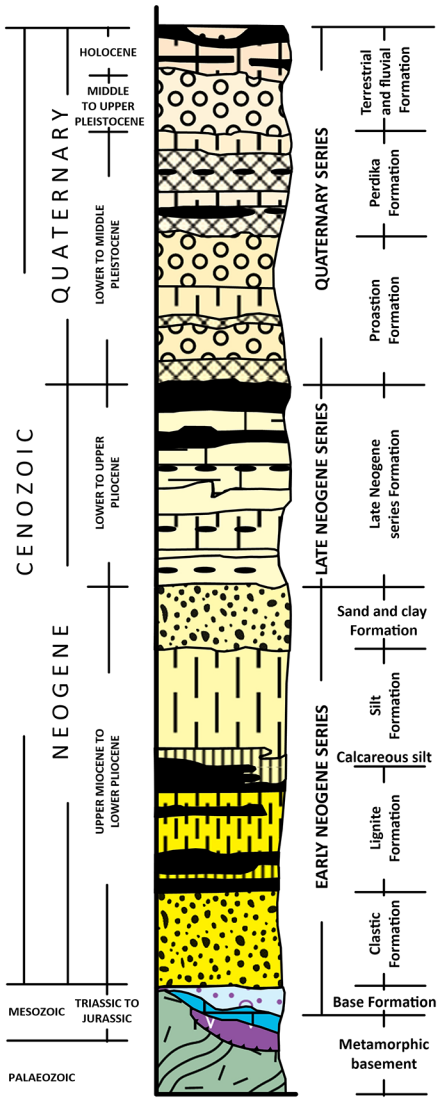

Figure 3. Synthesized stratigraphic column of the Sarantaporos, Ptolemaida-Amynteo -Florina basins, licence: CC-BY 4.0.

Locally, ophiolitic rocks with serpentinites and serpentinized peridotites occur[66] that present the potential to generate natural hydrogen and can be considered as source rock.

The sedimentary fill of the Florina Basin reaches a total thickness of about 560 m, 21 km width and 17 km length. The basement rocks of the Florina Basin comprise Palaeozoic gneiss, schist and Middle Triassic to Lower Jurassic crystalline limestone, marble and dolomite, which are overlaid by forty-one distinct sedimentary cycles described in three separate formations.[67]

The Base Formation (Upper Miocene) found first at the bottom (~251–548 m) consists mainly of alternating sand, clay, and conglomerate beds, interpreted as stacked alluvial fan sequences that grade upward into fluvial deposits. A change from active tectonism and a high sediment supply to a quieter regime with less accommodation space is indicated by this vertical transition. CO_2 accumulations have been found in the fine sand layers of these river deposits, suggesting that they could serve as traps also for natural hydrogen.[68]

The overlying Vevi Formation (Upper Miocene – Low Pliocene, ~25.1–124 m) records a lacustrine depositional system characterised by 21 distinct sedimentary cycles, where marl, clay and sand dominate. Several sedimentary cycles are capped by lignite seams with a cumulative thickness of up to 6.6 m. These lignite deposits can also generate natural hydrogen.[19,69]

The Lophon Formation (0–124 m) is the uppermost unit and represents a return to fluviolacustrine sedimentation during the Early Pleistocene.[65] Lithology is composed of sand, clay, and silt found as beds and marly limestone as lenses. Cross-bedded conglomerates and sands appear in the upper parts of the stratigraphy. Lophon Formation is distinguished by coarser fluvial interbeds interspersed with fine-grained lacustrine clays and silts.[65] This change indicates a decrease in accommodation space brought on by either increased sediment influx or decreased subsidence, which is probably related to regional elevation and the Quaternary fragmentation of the Florina-Ptolemais-Servia Basins system.[70]

The sedimentary architecture of the Florina Basin supports the presence of natural gas trapping systems.[71] The river sand deposits of the Base Formation have been shown to naturally accumulate CO_2, providing a useful analogy for subsurface storage behaviour. With alternating permeable sands and impermeable layers like clays and lignites, the lithological variability of the basin offers efficient natural trapping mechanisms that hold natural CO_2 and may also be able to hold natural hydrogen accumulation. Hydrogeochemical analyses show that groundwater quality in regions impacted by natural CO_2 seepage has not significantly declined[71] indicating the existence of low permeable rocks in the vicinity exhibiting good seal capabilities.

Unlike CO_2, which is soluble and easily immobilised by dissolution and mineral reactions,[72] H_2 is far more mobile and weakly soluble.[24,73] Any natural H_2 detected at the surface should not be interpreted as seal failure but rather as evidence of active deep generative zones with focused leakage pathways. Thus, in this case, the CO_2 preservation demonstrates sealing capacity, whereas H_2 seepage may reflect deeper accumulation.

The CO_2 emissions at Florina are ascribed to the regional Quaternary volcanism, and are primarily of crustal origin, including also a restricted mantle input, while NE-SW trending faults serve as fluid migration conduits.[74,75] Between 2012 and 2014, the Florina region experienced a significant rise in micro-seismicity, with over 2000 events documented and an Mw 4.1 main shock in February 2013. With certain episodes displaying migration patterns suggestive of pore-fluid pressure diffusion, seismicity seemed to be episodic and clustered. Deep CO_2 fluids may play a part in fault zone reactivation, as evidenced by shear-wave splitting investigations that showed anisotropy compatible with the local stress field and fault orientations.[76–78]

Across western Macedonia and the MHB, active and probably active faults are dominantly expressed as normal fault scarps and flexures controlling Quaternary sedimentation patterns, indicating ongoing extensional deformation rather than strike-slip kinematics.[79] This geomorphology-based inventory confirms also the presence of NE–SW-oriented active normal faults in the broader Florina region, consistent with Quaternary extension. Independent constraints from earthquake focal mechanism inversions and from high-resolution GPS-derived strain rate fields further support this interpretation.[80,81] Although minor shear components may exist across western Macedonia and the Florina Basin, active normal faults act as primary loci of present-day deformation.[80] This active tectonic regime as indicated by the 1995 Grevena-Kozani earthquake sequence[66,82]; may enhances permeability and facilitates fluid and gas migration.

Local geology setting of promising sites for natural hydrogen

Based on collected data from a) previous research conducted by Daskalopoulou *et al.*, 2018[18] b) observations investigation in the field using anecdotal data, c) from publicly available media of water springs with the elevated gas yield, the authors considered the local geology and concluded that the springs presented in Table 1 are promising places for an initial investigation related to natural hydrogen.

To shed light on potential gas generation and migration mechanisms, the local geology of each water spring and sampling area is summarised based on published geological maps and field observations. Water springs at Katakali and Kivotos emerge through formations of the Mesohellenic Trough, while spring emanations at Tropeouhos, Ammohori, Itea, Marina, Mesohori, Mesocampos and Neos Kafkasos are found at the Florina Basin (Figure 4).

At Katakali, the near surface layers include Pliocene to Lower Pleistocene marls, sandstones, sandy marls, clays, and conglomerates of the Karperon Basin.[83] A small lignite layer within the marls near the Sioutsa river indicates local marsh conditions. Near basin margins, these sediments transition into unbedded fluvial-torrential deposits. The lower stratigraphy includes:

1) the Miocene (Upper Aquitanian to Tortonian) Tsotyli Fm of the MHB (<2200 m in thickness). It is composed of ophiolitic conglomerates at the base, gradually transitioning into sandstones, marls, and sandy marls,

2) the Middle to Upper Oligocene Eptachori Fm of the MHB, consisting of ophiolitic conglomerates with lateritic lenses at the base and interbedded sandstones and marls above. This formation lies unconformably over the metamorphic basement.[83]

Table 1. Locations of water springs observed and suspected with a high possibility of natural hydrogen.

N°	Name	N (WGS84)	E (WGS84)	X (Greek Grid, EGSA 87, m)	Y (Greek Grid, EGSA 87, m)	Z (m)
S1	Tropeouhos	40° 44' 25,45"	21° 26' 23,87"	283693	4512806	695
S2	Mesocampos	40° 53' 39,57"	21° 30' 41,36"	290218	4529721	598
S3	Neos Kafkasos	40° 54' 14,50"	21° 29' 37,49"	288754	4530841	585
S4	Ammohori_1	40° 46' 39,93"	21° 28' 50,51"	287251	4516854	633
S5	Itea	40° 50' 1,64"	21° 30' 59,80"	290459	4522988	613
S6	Mesohori	40° 53' 12,92"	21° 29' 16,50"	288208	4528956	589
S7	Ammohori_2	40° 46' 54,80"	21° 28' 57,69"	287433	4517308	631
S8	Marina	40° 51' 44,29"	21° 29' 34,15"	288543	4526211	595
S9	Kivotos	40° 14' 35,55"	21° 25' 34,62"	280926	4457643	547
S10	Katakali	39° 55' 31,15"	21° 40' 37,5"	301337	4421760	404

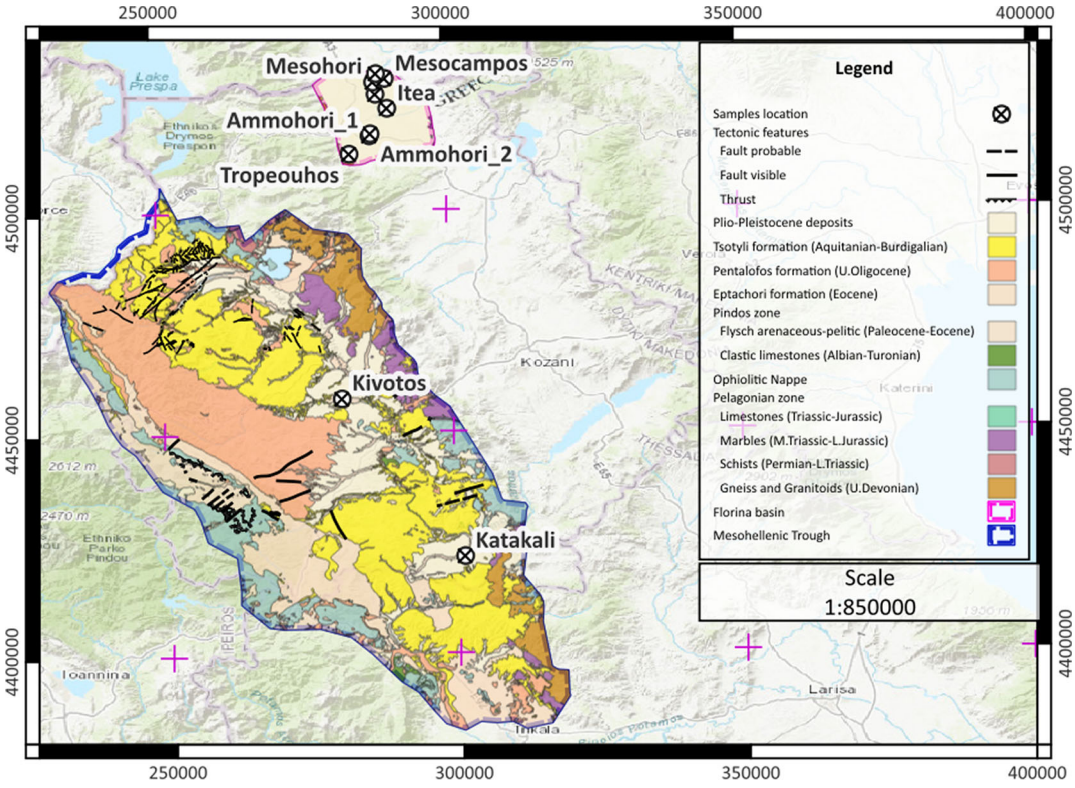

Figure 4. Map with the sample location in EGSA 87 grid system, scale 1:800.000, licence: CC-BY 4.0.

The stratigraphy at Kivotos sampling site comprises Quaternary alluvial sediments, such as loose sands and clays, which are underlain by Pliocene to Pleistocene fluvial and lacustrine deposits forming terrace sequences composed of loose conglomerates, blue to greenish clays, sands, and friable sandstones.[84] The upper parts of this unit are characterised by red clays and conglomerates, suggesting alternating oxidising and reducing conditions in a dynamic depositional setting. The Tsotyli Fm (Aquitanian to Burdigalian) with <500 m in thickness is composed of conglomerates, and intercalated sandstones and clastic limestones, that exhibit rapid lateral facies changes and thinning.[84] The lowermost unit in the area consists of brecciated Middle to Upper Cretaceous limestones with rudists and marly limestones.

The stratigraphy at Tropeouhos, Ammohori, Itea, Marina, Mesohori, Mesocampos and Neos Kafkasos comprises Neogene sands, clays, and conglomerates that grade downward into massive, whitish-brown to whitish-yellow

fossiliferous marly limestones, marls, and clays (<100 metres in thickness).[67,85] The presence of thin lignite and xylite layers in the deeper parts indicates episodes of organic-rich deposition in marshy, low-energy environments. Beneath these Neogene sediments lies the Pelagonian metamorphic basement of Palaeozoic age, composed predominantly of orthogneiss and paragneiss with intercalations of schists in the form of layers and lenses, and minor occurrences of amphibolites.[67,85]

Additionally, at Itea and Mesohori, the local stratigraphy includes Pleistocene conglomerates, sandstones, sands, and red clays, indicating deposition in fluvial to alluvial environments under oxidising conditions (<200 metres in thickness).[67]

Materials and methods

Water samples for gas measurements were collected in accordance with the UK Environment Agency Methods for sampling and analysing methane in groundwater: a review of current research and practice,[86] as well as Capasso and Inguaggiato, 1998[37] and Inguaggiato and Rizzo, 2004.[87] The sample collection method deployed is described below.

The sampling bottles were 100/125/250 ml vials crimped using a Teflon septum (Figure 5). Vial size was determined by the target analysis: 100 ml for carbon isotopes, ≥125 ml for noble gas isotopes.

As prerequisite actions, before filling the vials with water sample, the vials were fully filled and rinsed with the same water to be sampled from the spring samples 2 or 3 times to reduce contamination.

Prior to the sampling, the spring water was measured against dissolved O_2 (mg/L), Conductivity (μS/cm), Temp (°C) and pH. Physico-chemical conditions of the groundwater (pH, temperature, salinity) can contribute to CO_2 dissolution or release. Differences in He and CO_2 solubility and reactivity can determine the recorded variations in the He/CO_2 ratio.[37] The collection should come in two (2 nr) bottles of 100 mL, or 125 mL per location or sampling area. The first bottle is used for carbon, oxygen, hydrogen and helium isotopes, whereas the other bottle is used for gas chromatography (GC). The exact size of the vials is dictated by the analysis to be performed. Prior conduct with the laboratory to perform the analysis is highly recommended.

In bodies of water such as lakes, pools, seawater, or drainage galleries, as well as any location facilitating direct sample collection beneath the water's surface, it is imperative to submerge and crimp the bottles. This entails placing the bottles underwater, allowing them to fill completely while ensuring that the caps remain submerged at all times to prevent any contact with the atmosphere to avoid any air contamination during the sampling phase. A standard stainless-steel crimper able to cap 8–32 mm aluminium plastic/full aluminium and stainless steel/tight caps was used for sealing the sampling bottles.

Both the rubber closure and aluminium cap (Figure 5) were applied and the vials were moved sideways to check if any air bubbles were visible to the naked eye. In any sampling action, it was ensured that the bottom of the bottle and the mouth of the crimper were levelled before applying force to the crimper's tongues (Figure 6). Otherwise, the aluminium cap will not be sealed perfectly.

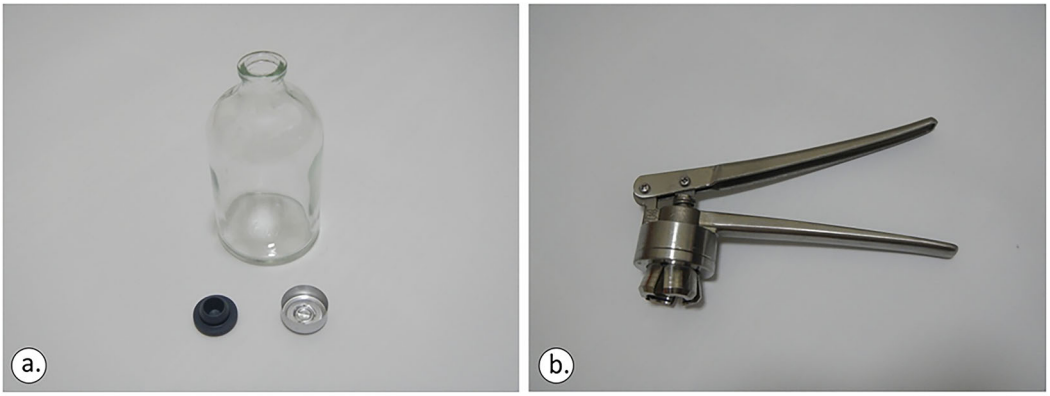

Figure 5. The 100 ml crimp-top glass vial, the 20 mm rubber closure (lower left) and aluminium cap (a) and the standard stainless-steel crimper (b), licence: CC-BY 4.0.

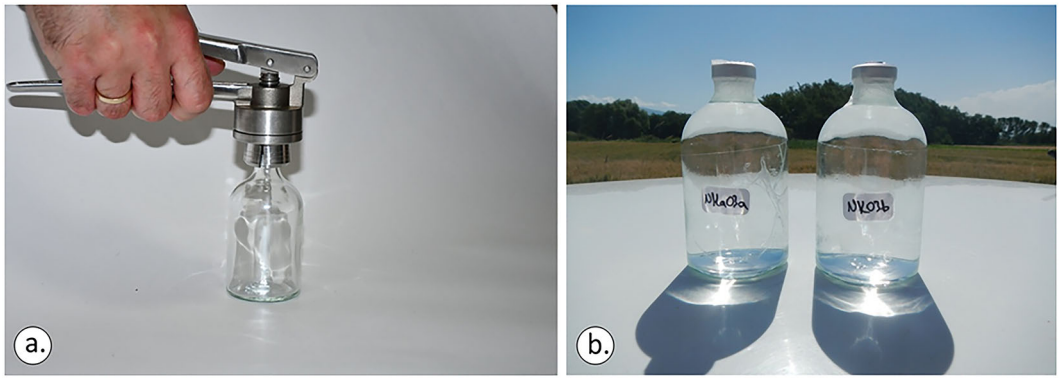

Figure 6. Ensuring that the bottom of the bottle and the mouth of the crimper are levelled before applying force to the crimper's tongues (a). Sampling vials should be fully filled (b), licence: CC-BY 4.0.



Figure 7. Plastic (Falcon) 50 ml sample filters, license: CC-BY 4.0.

For water sampled from taps, a tube of suitable diameter, crafted from either silicone or Teflon, is required. This tube was inserted into the bottles until reaching the bottom, enabling the water to fill the container from bottom to top. Subsequently, the tube is slowly removed, ensuring the vial is entirely filled up to the upper portion of the bottle neck. Extreme care was taken to eliminate any air bubbles during this process, followed by sealing the vial securely to prevent air contamination. It is recommended that the final procedure be conducted within a cylinder containing an identical sample of water extracted from the tap. This approach ensures the vial is fully submerged upon reaching its capacity, facilitating underwater sealing and crimping with water acting as a barrier to prevent air entrapment.

For inductively coupled plasma mass spectrometry (ICP-MS) analysis, employing plastic sterilised (Falcon) 50ml sampler filters (Figure 7), which have been appropriately acidified beforehand, is deemed sufficient.

After the sampling took place, a visual observation of the existence or nonexistence of bubbles was noted. Samples were appropriately numbered on a pre-agreed sampling system and metadata such as location name, coordinates, time and data of sampling, related geological formation and any other observed data were properly recorded. Recording sample numbers in each sample bottle is vital to minimise analytical problems during the laboratory investigation. All data collected, recorded and subsequently uploaded in SESAR, the System for Earth and Extraterrestrial Sample Registration. Each sample was allocated an International Geo Sample Number (IGSN) to ensure that they are Findable, Accessible, Interoperable, and Reusable (FAIR).[88] The IGSN persistent identifier can link each sample with any analyses performed in any laboratory. Published data can be accessed via hyperlinked journals online. All data can be located via online geological data services or the Zenodo platform.

Four sampling campaigns were distributed across different seasons, with collections in summer 15–19 July 2023, spring 21–22 April 2024, late spring/early summer 5–6 June 2024, and late autumn/early winter 29 November–3 December 2024, enabling the assessment of potential seasonal effects such as temperature changes, precipitation patterns, organic matter input, and hydrological dynamics. In each case, the samples were shipped to an external accredited laboratory and specifically to the Laboratory for Stable Isotopes, Istituto Nazionale di Geofisica e Vulcanologia Sezione di Palermo in Italy for geochemical laboratory analysis that followed the below provided protocol.

Samples were analysed for He, H_2, O_2, N_2, CH_4 and CO_2 by gas chromatography (Perkin Elmer Clarus500 equipped with a double Carboxen 1000 packed column system, TCD-FID detectors) using Ar as the gas carrier. TCD detection limit for O_2, N_2, and CO is 10–100 µmol/mol (ppm_v) and for H_2 and He is 1–10 µmol/mol (ppm_v). For FID detection limit for CH_4 is 0.1–1.0 µmol/mol (ppm_v). Ar was analysed with a Perkin Elmer XL gas chromatograph with MSieve 5A column, TCD detector having He as carrier. Detection limit for Ar is 10–100 ppm and analytical uncertainties are ± 5%. Hydrocarbon analyses were performed with a Shimadzu 14a gas chromatograph equipped with a Flame Ionization Detector (FID) using He as the carrier gas. The analytical error is ≤5% with a minimum detectable quantity of MDQ ≤ 3×10^{-12} gC/sec.

Isotope determinations of $\delta\,^{18}O_{H_2O}/^{16}O$ and δD_{H_2O} in water samples were performed using the equilibration technique for oxygen and water reduction (hydrogen production using granular Zn) for hydrogen. Measurements were carried out using a FinniganDelta Plus mass spectrometer (Hydrogen) and an automatic preparation system coupled with an AP 2003 IRMS (Oxygen). The $\delta^{18}O_{H_2O}/^{16}O$ and δD_{H_2O} ratios are reported relative to the V-SMOW (Vienna Standard Mean Ocean Water) scale. Isotope ratios are determined by comparing three in-house water standards that have been previously calibrated using IAEA international reference standards such as VSMOW2 and SLAP2 (Standard Light Antarctic Precipitation 2).[89,90] Analytical precision computed as 1σ on ten measurements of the same sample, for each measurement is better than 0.2‰ for $\delta^{18}O_{H_2O}$ and 2‰ for δD_{H_2O}.

Carbon isotope composition of CO_2 was determined by using a Thermo Delta Plus XP, coupled with a Thermo TRACE Gas Chromatograph (GC) and a Thermo GC/C III interface. The TRACE GC is equipped with a Poraplot Q (25 m × 0.32 mm) column and uses Helium (5.6) as carrier gas at a constant flow of 0.9 cm^3/min. Undesired gas species, such as N_2, and CH_4, are vented to the atmosphere using back-flush of He and a Sige valve. The $^{13}C/^{12}C$ ratios are reported as $\delta^{13}C_{CO_2}$ values relative to the V-PDB standard (Vienna Pee Dee Belemnite).[91] Carbon isotope ratios were determined by comparing three in-house standards $\delta^{13}C_{CO_2}$ ranging from +0.3 ± 0.1‰ to −28.5 ± 0.3‰ vs V-PDB calibrated using a CO_2 standard (RM8564[92]) with known isotopic composition ($\delta^{13}C_{CO_2}$ = −10.45 ± 0.04‰ vs V-PDB) and two international standards (NBS 18 and NBS 19[91]). External precision, computed as 1σ (standard deviation) on ten measurements of the same sample, is 0.1‰. The RM8564, NBS 18 and NBS 19 standards can be obtained from the Terrestrial Environment Radiochemistry Laboratory of the International Atomic Energy Agency (https://analytical-reference-materials.iaea.org/catalogs, web page accessed on 21/08/2025).

Carbon ($\delta^{13}C_{CH_4}$) and hydrogen (δD_{CH_4}) isotopes of CH_4, both in free gases and in dissolved gases, were measured using a Thermo TRACE GC interfaced to a Delta Plus XP gas source mass spectrometer and equipped with a Thermo GC/C III (for Carbon) and with GC/TC peripherals (for Hydrogen). The gas chromatograph was equipped with an Rt-Q Plot column (Restek 30 m × 0.32 mm i.d.) and the oven was held at a constant temperature (50°C for carbon and 40°C for Hydrogen). The flow rate of carrier gas (He 5.6 grade) was held at a constant flux of 0.8 cm^3/min. A split/splitless injector with a split ratio from 10:1 to 80:1 was used for sample introduction, except for diluted samples (CH_4 concentration lower than 10 mmol/mol) when direct on-column injection was performed.

The inlet system consists of a stainless steel loop with a known volume (50 µl), connected to a two-position six-port Valco® valve. Before the introduction of the sample, a vacuum of 10^{-2} mbar measured with an EBRO pressure gauge is ensured by a rotary vane pump. Once CH_4 was separated from the gas mixture, it was quantitatively converted to CO_2 by passing through a combustion oven (T = 940°C) for $^{13}C/^{12}C$ ratios analysis or to H_2 by passing it through a reactor set at a temperature of 1440°C for $^{2}H/^{1}H$ ratios analysis. Each sample analysis took about 500 s.

The $^{13}C/^{12}C$ ratios are reported as $\delta^{13}C_{CH_4}$ values for the V-P standard and $^{2}H/^{1}H$ ratios are reported here as δD_{CH_4} values with respect to the V-SMOW standard. Carbon isotope ratios were determined by comparing an in-house standard ($\delta^{13}C_{CH_4}$ = −49.5 ± 0.2‰) calibrated using four CH_4 standards (Isometric Instruments) with known isotopic composition ($\delta^{13}C_{CH}$ ranging from −23.9 ± 0.3‰ to −66.5 ± 0.3‰ vs V-PDB).

Hydrogen isotope ratios were determined by comparing an in-house standard ($\delta^{13}C_{CH_4}$ = −200 ± 2.0‰) with a CH_4 standard with known isotopic composition δD_{CH_4} = −186.1 ± 3.0‰ vs V-SMOW). External reproducibility, estimated as 1σ (standard deviation) on ten measurements of the same sample, is 0.2‰ and 2.0‰ for carbon and hydrogen isotopes,

respectively. The measured δD_{CH_4} values were normalised using the standards to ensure they were accurately reported on the V-SMOW scale. The estimated external accuracy (or overall measurement uncertainty) for the final reported δD_{CH_4} values is better than ± 2.5‰.

In CO_2-dominated gases having CH_4 concentrations lower than 1000 µmol/mol, the analyses of the isotope ratios of methane were carried out in the headspace gas samples collected using pre-evacuated 60 mL glass flasks filled with 20 mL of a 4 N NaOH solution.

The abundance and isotope composition of He, and the $^4He/^{20}Ne$ ratios, were determined by separately admitting He and Ne into a split flight tube mass spectrometer (Helix SFT). Helium isotope compositions are given as R/RA, where R is the ($^3He/^4He$) ratio of the sample and RA is the atmospheric ($^3He/^4He$) ratio (RA = 1.386×10^{-6}). The analytical errors were generally <1%.

Location names, sampling date and coordinates of all new sampling sites together with raw chemical results can be found as supplementary material at https://doi.org/10.5281/zenodo.16914636.[93]

Results

Below are presented the results from all four sampling campaigns, starting with the isotope data analysis (Table 2–Table 5). As the samples are from groundwater, the Global Meteoric Water line was deployed to analyse the data rather than the Local Meteoric Water line, which relies on precipitation samples only[94] (Figure 8).

Table 2. Water samples collected between 15-19/07/2023, laboratory data analysis provided at 06/09/2023, first sample campaign, rounded to two significant figures.

N°	Spring location name	δD_{H_2O}	$\delta^{18}O_{H_2O}$	Dissolved O_2 (mg/L)	Conductivity (µS/cm)	Temp (°C)	pH
S1	Tropeouhos	-72	-10.0	7.9	-	17.5	6.04
S2	Mesocampos	-50	-7.4	0.19	1686	16	6.06
S3	Neos Kafkasos	-59	-8.5	0.76	575	14.1	6.08
S4	Ammohori 1	-64	-9.0	8.3	920	17.5	6.07
S5	Itea	-66	-9.6	2.73	1017	16.5	6.03
S6	Mesohori	-62	-9.1	1.09	478	14	6.03
S7	Ammohori 2	-70	-9.9	7.1	319	20.5	6.01
S8	Marina	-68	-9.6	6.3	338	25.6	6.04
S9	Kivotos	-57	-8.2	0.24	850	15.3	5.4
S10	Katakali	-74	-10.5	0.25	1447	18	6.0

Table 3. Water samples collected between 21-22/04/2023, laboratory data analysis provided at 15/05/2024, second sample campaign.

N°	Spring location name	δD_{H_2O}	$\delta^{18}O_{H_2O}$	Dissolved O_2 (mg/L)	Conductivity (µS/cm)	Temp (°C)	pH
S1	Tropeouhos	-75.0	-11.9	8.85	1803	14.4	7.5
S2	Mesocampos	-59.0	-10.1	2.59	1984	13.5	5.67
S3	Neos Kafkasos	-64.0	-11.0	1.60	468	14.5	5.42
S4	Ammohori 1	-66.0	-10.7	7.06	971	12	6.49
S5	Itea	-62.0	-10.0	2.91	972	13	5.61
S6	Mesohori	-66.0	-10.8	2.50	450	13	6.5
S7	Ammohori 2	-69.0	-11.2	7.78	318	14.8	5.7
S8	Marina	-67.0	-11.2	8.15	347	14.1	6.29
S9	Kivotos	-61.0	-10.3	1.75	803	14.9	7.25
S10	Katakali	-76.0	-12.0	1.40	1440	16.1	7.5

Table 4. Water samples collected between 05-06/06/2024, laboratory data analysis provided at 24/07/2024, third round campaign.

N°	Spring location name	δD_{H_2O}	$\delta^{18}H_{H_2O}$	Dissolved O_2 (mg/L)	Conductivity (µS/cm)	Temp (°C)	pH
S1	**Tropeouhos**	-80	-10.1	0.78	1694	20.0	7.04
S2	**Mesocampos**	-58	-8.8	1.72	1809	20.3	6.01
S3	**Neos Kafkasos**	-65	-9.7	1.05	477	17.0	5.83
S4	**Ammohori 1**	-70	-8.9	4.87	968	21.0	6.76
S5	**Itea**	-66	-9.3	2.63	712	16.1	5.83
S6	**Mesohori**	-62	-9.2	2.27	485	16.0	6.22
S7	**Ammohori 2**	-71	-9.6	7.33	301	21.4	6.20
S8	**Marina**	-67	-9.2	7.00	329	22.9	6.56
S9	**Kivotos**	-63	-8.2	1.05	781	18.8	7.65
S10	**Katakali**	-73	-9.6	7.22	1468	18.0	8.48

Table 5. Water samples collected between 29/11/2024 – 3/12/2024. Laboratory data analysis provided at 06/03/2025, fourth campaign. Samples marked with suffix (-2) represent duplicates. BW = bottled water.

N°	Spring location name	δD_{H_2O}	$\delta^{18}O_{H_2O}$	Dissolved O_2 (mg/L)	Conductivity (µS/cm)	Temp (°C)	pH
S1	**Tropeouhos**	-11.5	-78	0.41	1862	14.2	7.12
S3	**Neos Kafkasos**	-9.8	-66	1.22	487	14.3	6.03
S9	**Kivotos**	-8.6	-58	0.25	855	14.3	7.75
S10	**Katakali**	-11.1	-75	0.26	1505	15.1	8.04
S1	**Tropeouhos-2**	-11.4	-79	0.41	1862	14.2	7.12
S9	**Kivotos-2**	-8.8	-59	0.25	855	14.3	7.75
S10	**Katakali-2**	-11	-75	0.26	1505	15.1	8.04
BW	**Blank (bottled water)**	-8.4	-49	-	-	-	-

The stable isotope results (Table 2–Table 5; Figure 8) show that $\delta^{18}O_{H_2O}$ and δD_{H_2O} values from all four sampling campaigns plot close to the Global Meteoric Water Line (GMWL), indicating a predominantly meteoric origin of the sampled waters. Deviations observed in selected sites suggest evaporation effects and/or local water–rock interaction. No systematic seasonal shift outside the meteoric domain was observed, indicating relative isotopic stability of the groundwater system throughout the monitoring period.

In all sampling campaigns, the pH measured was either slightly alkaline, with values around 7.5 or slightly acidic and values around 5.4. There was only one exception in the third sample campaign, in the Katakali water sample, which registered an alkaline pH of 8.48.

The ICP chemical analysis of the second round presented in Table 6 revealed that all samples had high detectable levels of strontium (Sr) and barium (Ba) in the scale of µg/L. The Si concentrations varied from 7.7 to 59.6 mg/L, indicating variable rock silicate interaction.[95,96]

Regarding the rest of the chemical elements identified Tropeouhos and Ammohori 1 had high levels of Fe (1010 and 2807 µg/L), Mn (37 and 444 µg/L), and Al (92 and 1455 µg/L), respectively. Ammohori 1 also showed elevated Ti (86 µg/L) and Zn (176 µg/L), while Tropeouhos had significantly high concentrations of Li (406 µg/L), B (7160 µg/L), and Br (303 µg/L).

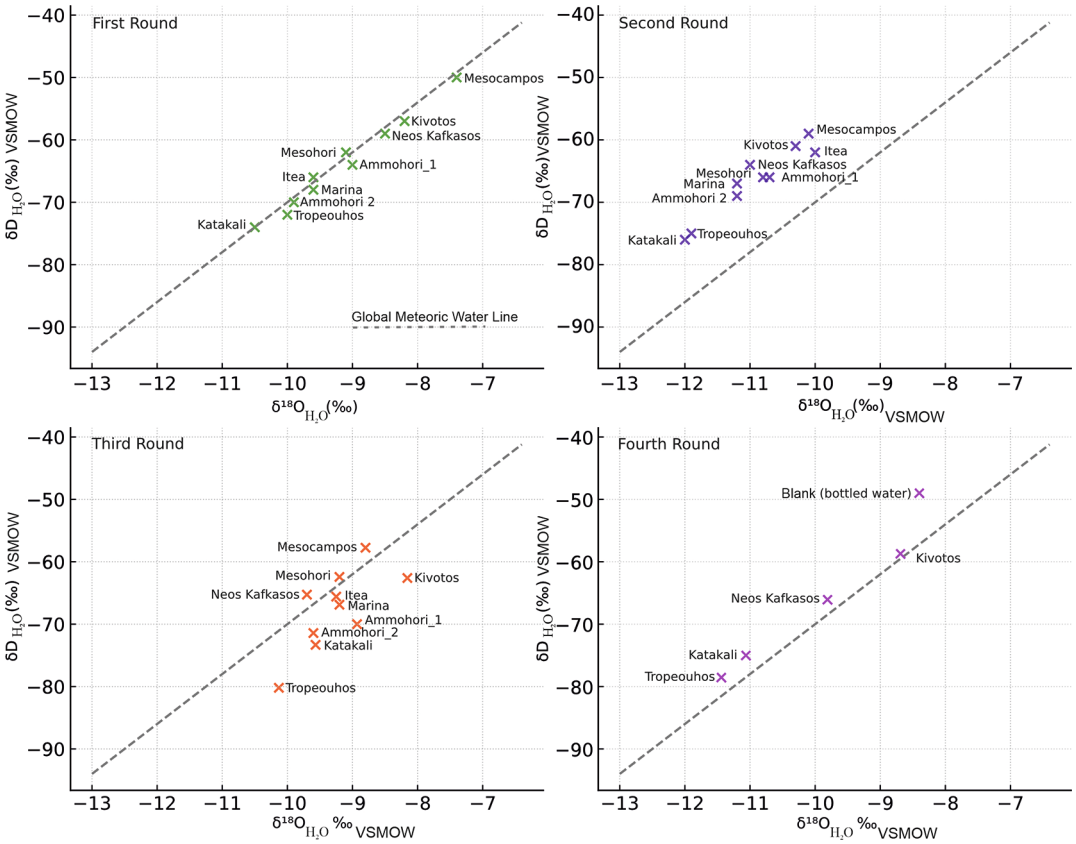

Figure 8. Stable Isotope composition of water samples collected from June 2023 to December 2024 with reference to the Global Meteoric Water Line (GMWL), licence: CC-BY 4.0.

The Mesohori water sample had notable values of 132 μg/L of Al, 682 μg/L of Mn and 247 μg/L of Fe. The Neos Kafkasos water sample showed Mn values of 436 μg/L. The Katakali sample had prominent values of 132 μg/L of Li, 933 μg/L of B, 220 of Fe μg/L, 185 μg/L of Br. Mesokampos had 363 μg/L of Br. All other chemical elements detected from the second water sampling campaign were of considerably lower value.

The geochemical analysis from the third sampling campaign presented in Table 7 revealed further information related to the gas component of the water samples. The Katakali water sample analysis provided values 4.4 ppm of H_2 coupled with 3704 ppm of CH_4, suggesting active geochemical processes related to either organic degradation or serpentinisation. The Kivotos sample showed values of 29 ppm of He, 1.7 ppm of H_2, and 31,400 ppm of CH_4. The latter consists of anecdotal public observations suggesting strong methane generation potential and possible mantle-derived gas contribution to gas mix phase.[43] In both Katakali and Kivotos samples, H_2 was detected at concentrations near the analytical detection limit, confirming its presence but introducing uncertainty in the reported values.

The Tropeouhos sample registered notable He levels (1403 ppm), accompanied by low CH_4 concentrations at 89 ppm. This could be associated with deep-seated helium degassing coupled with some microbial or thermogenic methane input.[97,98]

Samples Ammohori_1 and Ammohori_2 registered elevated CO_2 concentrations of approximately 7–8%, while methane remained low at around 1 ppm. In addition, the Itea sample contained 60.7% CO_2 and 4 ppm of CH_4, suggesting a dominant CO_2 component in the gas phase. This is consistent with the accumulation of natural CO_2 in the Florina basin in the Tertiary formation and its leakage through fractures and permeable rocks.[71] The Mesohori sample registered 9 ppm of CH_4, while Mesokampos contained 34 ppm of CH_4 along with approximately 60% CO_2, pointing to variable organic or volcanic degassing influence.[99] Finally, the Neos Kafkasos sample exhibited 6 ppm of He, 2 ppm of H_2, and a substantial 40% CO_2, indicating multi-gas signatures possibly linked to deep fluid migration through faulted rocks.[74,99]

Table 6. ICP chemical analysis of water samples from Kivotos Ammohori 1, Ammohori 2, Itea, Marina, Mesohori, Neos Kafkasos, Katakali, Tropeouhos, Mesocampos locations, second round campaign. All concentrations are reported in μg/L.

Spring location	Li	Be	B	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Br	Rb	Sr	Mo	Cd	Sn	Sb	Cs	Ba	Tl	Pb	Bi	U	Si
Kivotos (S9)	8	<0.02	269	4	0.10	0.05	0.19	5	7	0.01	<0.1	<0.1	<0.1	0.02	0.11	466	0.97	591	0.14	<0.01	0.01	0.00	0.01	67	<0.01	0.01	0.01	0.18	15000
Ammohori 1 (S4)	3	0.16	41	1455	87	5	5.01	37	2807	1	7.6	6	177	0.44	1.2	128	4.4	660	0.24	<0.01	0.23	0.03	0.25	89	0.03	4.77	0.02	1.99	18000
Ammohori 2 (S7)	13	0.22	15	7	0.20	0.75	0.86	10	53	0.03	9.7	47	65	0.05	0.27	88	0.31	281	0.12	<0.01	0.02	0.02	0.00	24	<0.01	1.22	<0.01	0.08	27000
Itea (S5)	3	0.03	37	40	0.15	0.92	0.63	18	22	0.12	3.7	9	30	0.31	3.2	47	0.57	260	1.09	<0.01	0.04	0.04	0.00	61	0.01	0.23	<0.01	6.07	8000
Marina (S8)	8	0.04	15	25	0.63	2	2.64	0.29	16	0.03	4.1	1	0.34	0.66	0.41	41	0.39	208	0.19	<0.01	<0.01	0.01	0.00	38	<0.01	0.01	<0.01	0.52	26000
Mesohori (S6)	7	0.02	90	146	11	0.71	0.58	682	247	2	8.1	15	76	0.15	0.37	58	0.99	214	0.18	0.02	6.03	0.18	0.02	54	0.01	7.89	0.05	0.21	11000
Neos Kafkasos (S3)	8	0.04	60	30	0.12	0.79	0.12	436	10	2.03	7.3	1	4.7	0.44	0.80	44	0.94	260	0.31	<0.01	0.01	0.01	<0.01	99	0.01	0.09	<0.01	0.32	14000
Katakali (S10)	132	<0.02	933	5	0.41	0.19	0.61	8	221	0.40	9.0	<0.1	<0.1	0.13	0.17	185	1.1	544	0.10	<0.01	<0.01	<0.01	0.02	156	<0.01	<0.01	<0.01	0.00	60000
Tropeouhos (S1)	407	1.03	7160	92	1.8	0.25	0.23	444	1010	0.30	0.2	<0.1	0.9	1.5	0.33	303	39	902	6	<0.01	<0.01	0.01	31.12	11	<0.01	0.20	<0.01	0.09	17000
Mesocampos (S2)	5	0.03	268	17	0.34	5	0.47	50	57	1	23.6	21	41	1.7	2.5	362	42	970	3	<0.01	0.06	0.08	0.05	183	0.05	0.31	<0.01	1.17	34000
error %	*11*	*8.90*	*3*	*10*	*11*	*7*	*2.30*	*3*	*12*	*2.00*	*12.8*	*14*	*2.6*	*5.1*	*14.3*		*2.2*	*2*	*5*	*12*	*8.20*	*6.70*	*1.70*	*4*	*4.80*	*10.10*	-	*2.10*	11000

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Table 7. **Gas chemical analysis as a result of the third water sampling campaign, n.a denotes not enough sample quantity to perform the chemical analysis for that specific element.**

Spring location	He (ppm)	H_2 (ppm)	O_2 (%)	N_2 (%)	CO (ppm)	CH_4 (ppm)	CO_2 (%)	Gas volume (cc/120 cc)	He ($\times 10^{-4}$ cc/L STP)	H_2 (cc/L STP)	O_2 (cc/L STP)	N_2 (cc/LSTP)	CO ($\times 10^{-6}$ cc/L STP)	CH_2 ($\times 10^{-6}$ cc/L STP)	CO_2 (cc/L STP)
Katakali (S10)	n.a	4.4	5.22×10^{-2}	18.14	n.a	3704	0.79	6.4	0.0	0.00	0.04	17.0	<LOQ	362.5	8.90
Kivotos (S9)	29	1.7	4.41×10^{-2}	19.87	n.a	31400	1.58	6.4	16.4	0.00	0.02	11.3	<LOQ	1778.4	0.90
Tropeouhos (S1)	1403		3.66×10^{-2}	24.81	n.a	89	2.16	6.4	794.6	0.00	0.02	14.0	0.0	5.0	1.20
Ammohori_1 (S4)	n.a		1.65	19.02	0.2	1.2	7.08	6.6	0.0	0.00	0.96	11.1	12.5	0.7	4.10
Ammohori_2 (S7)	n.a		5.99	19.88	0.2	1.1	7.87	7.2	0.0	0.00	3.82	12.7	13.9	0.7	5.00
Itea (S5)	n.a		1.60×10^{-1}	2.19	n.a	4.1	60.68	14.4	0.0	0.00	0.20	2.8	0.0	52.3	77.30
Marina (S8)	n.a		4.35	16.34	0.2	0.8	4.13	6.2	0.0	0.00	2.39	9.0	7.3	0.4	2.30
Mesohori (S6)	n.a		3.1×10^{-1}	16.42	n.a	9	3.3	6.8	0.0	0.00	0.19	10.0	0.0	54.2	2.00
Mesocampos (S2)	n.a		7.01×10^{-2}	2.02	n.a	34	60.22	12.4	0.0	0.00	0.08	2.20	0.0	373.1	66.10
Neos Kafkasos (S3)	6	1.9	1.00×10^{-1}	9.48	2.2	n.a	40.01	10	5.3	0.00	0.09	8.40	779	0.0	35.40

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Following the results of the third campaign, a fourth one commenced with a focus on four promising locations of Katakali, Kivotos, Tropeouhos and Neos Kafkasos. To further elucidate the origin of the gas phase, the geochemical investigation considered the following analysis:

1. He
2. H_2
3. CH_4
4. CO_2
5. Helium isotopes (^3He, ^4He)
6. Deuterium of hydrogen ($\delta^2D_{H_2}$)
7. Deuterium of methane ($\delta^2H_{CH_4}$)
8. Deuterium of water ($\delta^2H_{H_2O}$)
9. Oxygen-18 of carbon dioxide ($\delta^{18}O_{CO_2}$)
10. Oxygen-18 of water ($\delta^{18}O_{H_2O}$)
11. Carbon-13 of methane ($\delta^{13}C_{CH_4}$)
12. Carbon-13 of carbon dioxide ($\delta 1^8O_{CO_2}$)
13. Carbon-13 of DIC ($\delta^{13}C_{DIC}$)
14. Carbon-14 of DIC ($^{14}C_{DIC}$)

This time, a blind sample system with duplicates and blanks was used during the laboratory analysis. The duplicates were two field-independent samples collected as close as possible to the same point in space and time per site. Blanks were simple water table samples included in the analytical batches. All samples, including blanks, were labelled with a coding system unknown to the laboratory analysts (blind sampling system). None of the blind samples were analytical blanks or matrix spikes. The purpose of the duplicate samples was to serve as a quality control measure for analytical consistency, to ensure operator objectivity, and to assess spatial or micro-scale variability within the same hydrogeological system. The water table blanks were included to monitor potential contamination during sample preparation and analysis. They contained no analytes and was processed alongside the regular samples to ensure quality control and identify any background interference.

The Katakali and Katakali-2 samples, collected independently from the same spring, showed good agreement in $\delta^{13}C_{CH_4}$ values (–69.5‰ vs –69.8‰), which falls within expected analytical precision (±0.2‰). However, the $\delta^2H_{CH_4}$ values (–251‰ vs –230‰) differed by approximately 21‰, which may reflect small-scale natural heterogeneity at the sampling site or isotopic fractionation during gas release. Similarly, the Kivotos and Kivotos-2 samples showed a 6.0‰ difference in $\delta^{13}C_{CH_4}$ (–75.5‰ vs –69.5‰) and a 15.0‰ difference in $\delta^2H_{CH_4}$ (–107‰ vs –92‰), again suggesting site-specific isotopic variability rather than analytical inconsistency.

The duplicate samples Tropeouhos and Tropeouhos-2 demonstrated satisfactory analytical reproducibility in $\delta^{18}O_{H_2O}$ (–9.2‰ vs –9.1‰, a 0.1‰ difference) and δD_{H_2O} (–62‰ vs –60‰, a 2.0‰ difference), further supporting the consistency of the dataset. Chemical concentrations showed strong consistency; Fe (2807 μg/L vs 2750 μg/L), Mn (444 μg/L vs 439 μg/L), Al (1455 μg/L vs 1418 μg/L), and Li (406 μg/L vs 397 μg/L), with negligible deviation between duplicates. On the same note, elevated B (7160 μg/L vs 7095 μg/L), Br (363 μg/L vs 357 μg/L), and Ba (121 μg/L vs 119 μg/L) values confirmed both geochemical signatures and instrumental consistency. For the Neos Kafkasos samples it was not possible to analyse its duplicate.

Table 8. **Targeted Gas chemical analysis as a result of the fourth water sampling campaign.**

Spring location	He (ppm)	H_2 (ppm)	O_2 (%)	N_2 (%)	CH_4 (ppm)	CO (ppm)	CO_2 (%)	Gas volume (cc/120 cc)	He ($\times 10^{-4}$ cc/L STP)	H_2 (cc/L STP)	O_2 (cc/L STP)	N_2 (cc/L STP)	CH_4 ($\times 10^{-3}$ cc/L STP)	CO ($\times 10^{-3}$ cc/L STP)	CO_2 (cc/L STP)	total Alk	$\delta^{13}C_{-TDC}$ (‰)	$\delta^{13}C_{-CH4}$ (‰)	δD_{CH4} (‰)
Tropeouhos (S1)	-	-	0.21	22.56	81		2.4	6	0.0	0.0	0.15	20.44	4.0	0.0	27.40	2.00	3.9	-	-
Neos Kafkasos (S3)	10	-	0.58	8.45	7		49.9	10	9.8	0.0	0.62	10.65	3.3	0.0	587.10	4.2	-1.2	-	-
Kivotos (S9)	43	-	0.19	19.08	43000		1.67	6.2	27.9	0.0	0.14	17.63	1833	0.0	19.10	6.3	-14.2	-75.5	-107
Katakali (S10)	-	-	0.12	4.07	300370		2.33	7.4	0.0	0.0	0.10	4.19	14558.3	0.0	26.90	15.8	13.9	-69.5	-251
Tropeouhos-2 (S1)	1.4	-	0.21	22.47	81		2.49	5	0.9	0.0	0.15	20.36	4.04	0.0	28.40	2.1	1.9	-	-
Kivotos-2 (S9)	44	-	0.24	20.04	44100		1.73	5.8	27.0	0.0	0.17	17.81	1903.3	0.0	19.70	6.4	-15.8	-69.5	-92
Katakali-2 (S10)	-	-	0.1	6.08	312300		2.44	7.6	0.0	0.0	0.09	6.37	15440.5	0.0	28.20	15.7	14.4	-69.8	-230
Blank (bottled water)	-	-	3.3	19.86	2.7	19.5	0.94	5.6	0.0	0.0	2.26	17.29	0.1	1.8	10.70	4.0	-11.2	-	-

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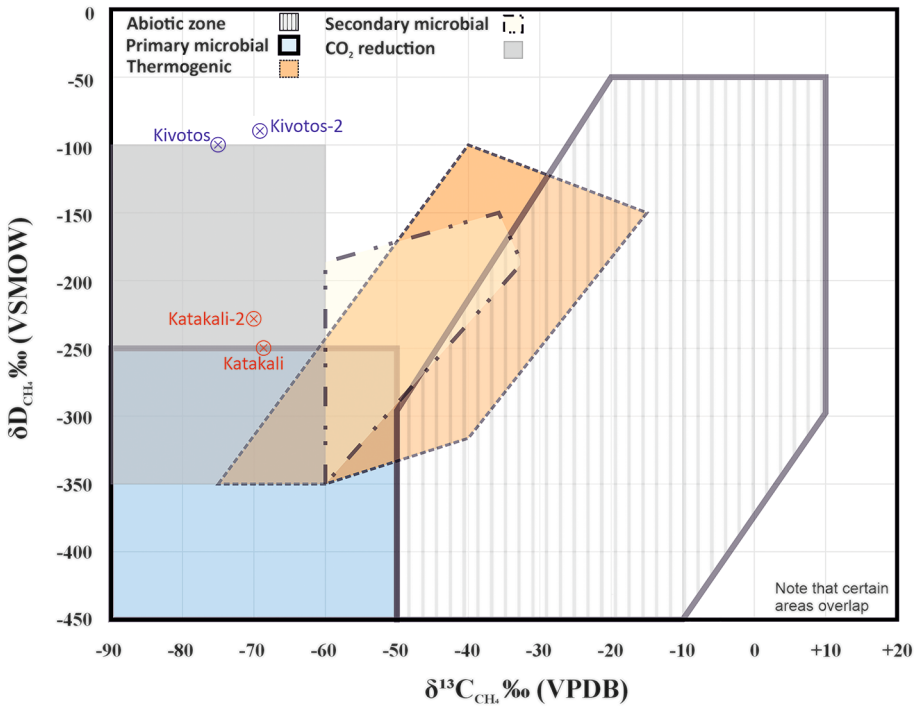

Figure 9. Methane genetic diagram for Kivotos and Katakali samples based on the Milkov and Etiope,[96] reproduced with CCC permission from Elsevier, modified samples addition and simplified accordingly.

In all cases, the duplicate samples showed almost similar values. The results of the fourth campaign are presented in Table 8. It should be noted that although all listed chemical elements were analysed, some were either not detected or present in quantities insufficient for further measurements.

In this final fourth round, no hydrogen gas was registered. However, the Katakali samples showed a mean value of 306335 ppm CH_4 with $\delta^{13}C_{CH_4}$ values around –70‰ and δD_{CH_4} near –230‰. On the same note, the Kivotos samples registered a mean of 43.5 ppm of He coupled with a mean 43550 ppm CH_4 with $\delta^{13}C_{CH_4}$ at –75.5‰ and and δD_{CH_4} near -100‰. Both family of samples (Katakali 1&2 and Kivotos 1&2) are plotted in methane genetic diagram (Figure 9) based on $\delta\ ^{13}C_{CH_4}$ versus δD_{CH_4} to provide an interpretation based on the integrated geological data and geochemical analysis.[100] The Tropeouhos samples showed a mean of 81 ppm of CH_4, in this case, the first sample showed no He values, whereas the second sample 1.4 ppm of He. The Neos Kafkasos, although sampled in duplicates, only one sample survived and was analysed. The sample that survived showed a value of 10 ppm of He and 7 ppm of CH_4. For the Neos Kafakasos sample, there was no $\delta\ ^{13}C_{CH_4}$ available. The $\delta\ ^{13}C_{TDC}$ was at –1.2‰ and δD_{H_2O} was at –66‰.

An exploratory Spearman rank correlation has been implemented between $\delta^{13}C_{CH_4}$ and δD_{CH_4} for the four samples with complete isotopic data. The correlation coefficient was low ($\rho \approx 0.10$), indicating no monotonic relationship between the two isotopic parameters.

Discussion and results interpretation

The discussion text is structured on the scientific hypothesis presented in the introduction section against the five objectives formulated for its investigation.

Hydrochemistry and isotopic evidence supporting gas occurrences

To confirm the presence and occurrences of gas-bearing waters, related to the first objective of the scientific hypothesis presented in the introduction, isotopic and hydrochemical results from all sampling campaigns are considered below.

In the third and fourth sampling campaigns, gas-bearing waters were identified, with helium, hydrogen, methane, and carbon dioxide detected in multiple springs (Table 7, Table 8). Their presence provides the first line of evidence for active subsurface gas generation and accumulation.

However, several of the hydrogen concentrations measured in this research are in proximity to the lower end of the equipment's detection range and therefore require careful interpretation. On the same note, the repeated detection of H_2 at the same sites across independent sampling campaigns (Katakali, Kivotos, Neos Kafkasos), including those conducted by Daskalopoulou *et al.*, 2018,[18] and Daskalopoulou, 2017,[101] combined with its systematic absence in others, argues against random analytical noise. The spatial consistency of H_2 with mapped ultramafic lithologies and fault-controlled migration pathways further supports a geological signal rather than an instrumental artefact. In contrast, helium concentrations, particularly the high values recorded at Tropeouhos (up to 1403 ppm) are well above detection limits, reproducible across campaigns, and therefore are considered analytically robust. The combined occurrence of low-level H_2 with independent geochemical indicators (e.g., alkaline pH, elevated Li and B, reducing Fe–Mn conditions) provides further confidence that even near-detection-limit H_2 reflects real subsurface processes.

Isotopic composition of δD_{H_2O} and $\delta^{18}O_{H_2O}$ of groundwater provides insights into the origins of the detected gases by shedding light on the recharge conditions, groundwater residence time and mixing with deeper fluids.[87,94] Seasonal recharge variations alter hydraulic gradients, pore pressures and redox conditions. These changes can influence the transport and dilution of gases generated at depth, facilitate the upward migration of deep CO_2 and He rich fluids[102] and regulate Fe–Mn redox cycling.[103]

In the first sample campaign (Table 2, Summer 2023), most samples plot in proximity to the Global Meteoric Water Line,[104,105] indicating a meteoric origin with little evaporation enrichment[106] (Figure 8). Katakali and Tropeouhos in the first round (Table 2) are the most depleted in both isotopes, indicating a colder recharge. Mesocampos and Kivotos are relatively enriched, possibly indicating partial evaporation.[105] Since the elevations of all sampling locations are relatively similar, altitude differences are unlikely to be the primary factor driving the observed isotopic variability. This original investigation did not provide a distinct chemical signature; however, during the Katakali sample collection, gas bubbles were observed in the water sample. This is due to a decrease in pressure, which causes these dissolved gases to come out of solution and form bubbles.

The second round of data (Table 3) indicated that Tropeouhos, Mesocampos and Neos Kafkasos had a shift towards isotope depletion, indicating hydrologic change. This can be explained by seasonal input.[107] The first batch was collected in Summer (June 2023) during a low rainfall season, whereas the second batch was collected in Spring (April 2024) during a high rainfall season. This is consistent with a well-mixed aquifer or steady-state recharge conditions typical of early spring. This sample location above the GMWL (Figure 8) suggests that precipitation during the winter had sufficient time to infiltrate deeper into the ground and homogenise with groundwater before sampling.[94,104,108,109]

The third round of samples collected were notably more depleted in δD_{H_2O} and $\delta^{18}O_{H_2O}$ and showed higher deviation from GMWL related to rainfall patterns and evaporation, which is consistent with the June sampling period (Table 4). Still, most of the samples are in proximity to the GMWL, indicating that rainfall is still the prevailing factor (Figure 8).

The fourth batch of samples collected mostly in Winter (December) plots above the GMWL, suggesting winter recharge from isotopically lighter sources indicated by the isotope depletion (Table 5). The dispersion of the samples may reflect heterogeneity in recharge sources, mixing with deeper groundwater or longer water to rock contact time,[94,108] Figure 8.

The chemical composition of groundwater samples collected during the second campaign reflects a range of geochemical processes influenced by water–rock interaction and rock differences (Table 6). The Tropeouhos and Ammohori_1 sample chemical results indicate active water and rock interaction involving iron-bearing minerals that undergo redox processes with Fe^{3+} (insoluble ferric) → Fe^{2+} (soluble ferrous) in anoxic environments.[95,110] High concentration of Fe can be associated with dissolution of iron bearing minerals or the serpentinization process.[71] Both processes favour natural hydrogen production.[22,33,111] The same applies to a lesser extent for the Mesohori and Katakali samples.

High CO_2 concentrations in Itea (60.7%), Mesokampos (~60%), and Neos Kafkasos (~40%), including Ammohori_1 and _2 (~7–8% CO_2) are related to the Florina basin, where natural CO_2 of volcanic origin has accumulated in the Miocene fluvial sandstones.[68,99] The accumulations migrating via faults have been detected from depths of 296–338 m and 366–372 m below the surface and are well reported.[68] They also provide CO_2 analogues for CO_2 storage, which is also related to the Pilot Strategy Project.[61]

In addition to shallow mixing processes, the role of active normal faulting in controlling permeability and fluid circulation should be considered. Recent geomorphological and geodetic datasets indicate that western Macedonia is dominated by Quaternary normal faulting.[79–81] Such active fault systems may significantly influence subsurface fluid flow by enhancing vertical permeability along fractured fault zones. In this context, meteoric water may not be restricted to

shallow aquifer mixing but could infiltrate deeper parts of the basin along structurally controlled pathways, interacting with ophiolitic and sedimentary units before returning to the surface. Accordingly, temporal and spatial variations in gas and water chemistry may reflect not only precipitation-driven dilution in near-surface aquifers, but also structurally mediated recharge and circulation at greater depths. The interaction between active extensional faulting, inherited basin structures, and lithological contrasts likely governs the connectivity between deep gas-generating horizons and surface discharge points.

Origins of gases: biotic, abiotic and radiogenic signatures

To evaluate whether the detected gases have biotic or abiotic origins, as addressed by the second objective of the scientific hypothesis, pH conditions, isotopic signatures, and dissolved element concentrations were examined. These parameters provide diagnostic fingerprints that allow one to distinguish between microbial gas production, water–rock reactions that generate abiotic gases, and crustal or mantle sources that contribute radiogenic signatures.

The pH values of all samples ranged from 5.4 to 8.48 (Table 2–Table 5). Most samples were slightly acidic (around pH 5.4), suggesting interaction with silicate-rich lithologies, likely weathered ophiolites, or the influence of organic-rich soils. In contrast, the Katakali sample exhibited a distinctly alkaline pH (8.48), potentially indicating interaction with carbonate rocks (e.g. Triassic–Jurassic limestones) or with serpentinized ultramafic rocks undergoing low-temperature alteration, which commonly release hydroxide ions and elevate pH.[7,22,33]

Hydrogen concentrations provide further insight into abiotic gas-generating processes. In the third sampling campaign, detectable H_2 was identified at Katakali (4.4 ppm), Kivotos (1.7 ppm), and Neos Kafkasos (2 ppm). Although H_2 values are close to instrument detection limits, their repeated occurrence at the same sites, except for Katakali, suggests genuine geological signals rather than analytical artefacts. Hydrogen is an indicator of water–rock reactions, typically associated with a) serpentinization of ultramafic rocks (e.g., ophiolites), b) radiolysis of water in fractured rocks under the influence of natural radioactivity and c) anaerobic corrosion of Fe-bearing minerals, particularly under reducing conditions. These processes are common in tectonically active zones, where fluid pathways are enhanced[7,13,14,16,22,33,40] and discussed further in the next section.

The Katakali sample's combination of high pH, elevated Li and B (Table 6), and relatively low transition metal concentrations points toward interaction with serpentinized ultramafic rocks. These lithologies commonly generate hyperalkaline fluids through serpentinization reactions and are known potential sources of natural hydrogen.[112,113] However, methane pattern at Katakali diverges from this abiotic H_2 pattern and suggests a different origin.

Methane patterns show a contrasting behaviour. In the third sampling round, Katakali contained 3,704 ppm CH_4, whereas the fourth sampling campaign revealed high concentrations (>300,000 ppm) in both duplicates, along with a slightly alkaline pH (~8). These values suggest methane generation under anoxic,[73] closed-system conditions, consistent with deep microbial environments[114] rather than shallow aquifers, which are constantly replenished. This interpretation is supported by the isotopic composition of δD_{H_2O} and $\delta^{18}O_{H_2O}$ from the fourth round, which indicates mixing with deeper, older groundwater. The absence of helium and hydrogen, combined with $\delta^{13}C_{CH_4}$ values around –70‰ and δD_{CH_4} near –230‰, strongly supports biogenic methane via microbial CO_2 reduction[43,115] (Figure 9). A secondary, less likely mechanism may involve abiotic H_2 generated by serpentinization that is subsequently oxidised while CO_2 is reduced to methane under oxic conditions by cyanobacteria[73] during migration through mixed groundwater zones. Further investigation may be needed to properly distinguish between bacterial imprint and diffusive fractionation.[97] For reference, Daskalopoulou *et al.* 2018,[18] previously detected 4 ppm He and 11 ppm H_2 at Katakali, suggesting spatial or temporal variability in gas inputs.

In contrast to Katakali's microbial methane signature, helium distributions reveal a strong radiogenic component at other sites. Tropeouhos (1403 ppm He) and Kivotos (29 ppm He) samples show significant helium enrichment, which is not produced in shallow processes. Such high He levels suggest input from a radiogenic source in crystalline basement rocks (U/Th decay),[116] mixing with mantle-derived fluids migrating through deep-seated fault systems.[116] Even the low but measurable helium at Neos Kafkasos (6 ppm) aligns with this interpretation and reflects its structural proximity to Tropeouhos, indicating shared deep fluid pathways.

While gas compositions provide direct evidence of source processes, dissolved element concentrations complement these data by revealing the redox and lithological conditions that influence gas generation and migration. Samples from Ammohori 1 and Tropeouhos displayed high levels of Fe (up to 2807 µg/L), Mn (up to 444 µg/L), and Al (up to 1455 µg/L), indicating reducing conditions that mobilise these elements from iron and manganese oxides and aluminosilicates. Such elevated concentrations are related to reductive dissolution of Fe/Mn oxides under anoxic

conditions,[71,117] contribution from ophiolitic or volcanoclastic host rocks[112,113] and weathering of feldspars[118] and/or clay-rich layers typically found in flysch or schist rocks.[71,119,120]

Migration pathways and fluid circulation

The third objective of the scientific hypothesis presented in the introduction is to understand how gases migrate and accumulate in the study area. This is achieved by integrating trace-element geochemistry, stable isotopes, and gas compositions to infer the depth, direction, and mechanisms of subsurface fluid flow. The presence of Li, B, Br, noble gases and reducing conditions provide critical constraints on circulation depth, residence time, lithological interactions, and the degree of confinement of the system.[43,113,121]

The Katakali and Tropeouhos samples from the third sampling campaign show elevated concentrations of Li, 131 and 406 µg/L, respectively, indicating deep water circulation interacting with igneous or silicate rocks.[43] This interpretation is consistent with the isotopic compositions shown in Figure 8 for the third-round section, depicting evolved signatures characteristic of prolonged water–rock interaction. Such conditions also favour the preservation of natural hydrogen generated by serpentinization or radiolysis.[22]

Tropeouhos, Katakali, and Mesokampos also show high values of B (up to 7160 µg/L), and Br (up to 363 µg/L). These elements are typically enriched in hydrothermal systems,[71] marine sedimentary or evaporite-hosted aquifers[122] or fluids with long residence times and extensive water–rock contact.[113] In Mesokampos, concurrent B–Br enrichment and high CO_2 (~60%) are consistent with a marine or connate water signature, suggesting mixing with residual basinal brines or deep-seated formation waters.[122,123] Slight enrichment in δD and $\delta^{18}O$ supports a history of evaporation and prolonged water–rock interaction rather than modern meteoric recharge. All the aforementioned information points toward deep fluid circulation and long water–rock interaction.

At Katakali, moderately enriched δD_{H_2O} and $\delta^{18}O_{H_2O}$ values indicate some evaporation, but the dominant control appears to be long residence time and water–rock interaction with organic-rich strata (possibly lignites or deep carbonate strata). The interpretation from the data received from the sample reflects a closed-system[124] microbially dominated environment with limited gas migration, depicted in Figure 10.

Despite high CH_4 (>300,000 ppm), the absence of He and H_2 implies limited upward migration of mantle-derived or serpentinization-related gases.[43,115,125] This contrasts with Daskalopoulou *et al.* (2018),[18] who reported minor amounts of He and H_2. Such variation is not contradictory and may reflect differences in sampling procedure, sample preservation, analytical uncertainty, or transient pore-water flushing.[126,127] However, both studies converge on identifying high methane concentrations that point towards a biotic origin. This study indicates a closed-system microbial gas generation and limited vertical migration for the Katakali site.

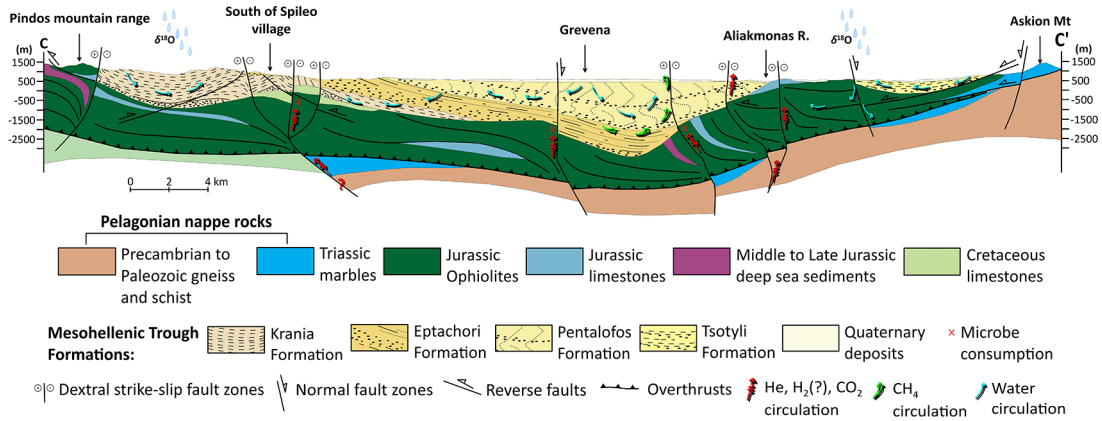

Figure 10. Geological cross section of Mesohellenic Basin with interpretative subsurface gas generation and migration mechanisms, licence: CC-BY 4.0. The structural framework depicted corresponds to the inherited basin architecture formed during Miocene extension and associated deformation phases. Although some pre-existing faults may be selectively reactivated under the present extensional stress field, the current Quaternary tectonic regime in western Macedonia is characterized predominantly by normal faulting rather than strike-slip kinematics.

Kivotos presents a mixed gas system in which He (29–44 ppm) and H_2 (1.7 ppm) co-occur with substantial methane (~43,000 ppm). Helium and hydrogen indicate abiotic sources, such as serpentinisation of ultramafic rocks or radiolysis in fractured basement lithologies.[115,116] CH_4 may derive from either microbial or abiotic origin the low $\delta^{13}C_{CH_4}$ values (–75.5‰ to −69.5‰,) remain firmly within the microbial range,[43,128] whereas δD_{CH_4} values (–107 to –92‰) are less depleted than typical biogenic methane (Figure 9). These characteristics suggest transitional or mixed methane, possibly microbial CH_4 modified by thermogenic influence or by hydrogen exchange during migration. Slight δD_{H_2O} and $\delta^{18}O_{H_2O}$ enrichment supports an intermediate residence time and moderate water–rock interaction (Figure 8, fourth round). Kivotos, therefore, may represent a hybrid gas system where microbial CH_4 coexists and possibly interacts with deeper, abiotic fluid inputs.

Tropeouhos is dominated by high helium values (up to 1403 ppm), low CH_4 (81 ppm), with no detectable H_2. This signature is characteristic of radiogenic He from U–Th decay in basement rocks or mantle-derived He transported along deep-seated fault structures.[19–21] The low CH_4 and absence of H_2 suggest minimal microbial or abiotic CH_4 generation.[30,43,115,125] Tropeouhos possibly represents an active degassing zone, where He migrates vertically along tectonic conduits[38] rather than accumulating in a confined reservoir.

The Neos Kafkasos with low but detectable He (10 ppm) and H_2 (2 ppm) combined with CO_2 at ~50% suggest (a) interaction with deep carbonate reservoirs undergoing dissolution, (b) magmatic CO_2 inputs associated with known volcanic activity in the Florina basin[99] and c) mildly reducing conditions that promote CO_2 accumulation.[65,68,76–78] Although $\delta^{13}C_{CH_4}$ was not obtained due to the low concentration of CH_4, the available data on $\delta^{13}C_{TDC}$ (–1.2‰) and δD_{H_2O} (–65‰) suggest thermogenic or mixed gas influence.[129]

Based on the proximity of Mesokampos and Itea, both with elevated CO_2% values, a regional CO_2 dominated system is suggested, with deep-origin CO_2 migrating upward along structural pathways. He and H_2 values indicate connectivity to deeper sources.

Trapping mechanisms and localized reservoir behaviour

The fourth objective of the scientific hypothesis is to evaluate how geological formations within the Mesohellenic Basin generate, host, and trap hydrogen and helium. In this study, the objective is approached by integrating mineralogical compositions, sedimentary facies, petrophysical properties, and redox behaviour to assess both (a) the potential for hydrogen formation through serpentinisation, radiolysis, or Fe-bearing mineral alteration, and He through U–Th decay and (b) the stratigraphic and structural conditions that permit gas accumulation and preservation.

The Mesohellenic Basin's tectonic inheritance from Tethyan ophiolite obduction, its thick multilayered siliciclastic stratigraphy, and its extremely low-permeability greywacke and marl successions have the potential for gas generation, retention, and long-term preservation of geogenic gases. The ophiolitic basement (Figure 2) provides the mineralogical combination for abiotic H_2 and radiogenic He production; the basin sediments and caprocks provide confinement; and structural reactivation governs migration.

It is noted that the fault geometries illustrated in Figures 2 and 10 do not aim to represent exclusively the present-day active tectonic regime, but primarily the inherited structural architecture developed during the Miocene opening and evolution of the MHB.[51,53] Several basin-bounding and intra-basin structures record earlier deformation phases, including oblique and locally strike-slip components associated with basin formation. Under the current extensional stress field documented for western Macedonia,[79–81] such pre-existing weaknesses may undergo selective reactivation, predominantly in normal-slip mode, depending on their orientation relative to the present-day σ3 direction. Therefore, while the dominant Quaternary faulting style in western Macedonia is normal faulting, inherited MHB structures may locally accommodate extension through reactivation, enhancing structural permeability and contributing to fluid and gas migration pathways without implying an active regional strike-slip regime.

All samples contained detectable Sr and Ba, consistent with the dissolution of carbonates and feldspathic minerals across the Mesohellenic Trough. Sr indicates interaction with marls, limestones, plagioclase-rich sandstone units,[130] and mixed litharenites. The third-round water samples from West Macedonia show a complex mixture of dissolved gases, reflecting interactions between geological formations, redox conditions, and possibly deep-seated fluid sources.

The mineralogical framework of the basin is dominated by the Eptachori, Pentalofos, and Tsotyli Formations, deposited in a high-energy environment with significant siliciclastic input. Their petrophysical and structural properties exert strong control on gas migration and confinement. Unhealed discontinuities within these formations can act locally as fluid migration pathways, whereas major faults provide a significant migration mechanism.

Eptachori Formation greywackes are fine-grained, strongly cemented, and composed of quartz, calcite, albite, muscovite and accessory titanite, biotite, actinolite and zircon.[131] Their Fe–Mg silicates and U–Th–bearing minerals enable radiolytic H_2 and radiogenic He generation, while extremely low permeability (<0.01 mD) and low porosity (~7.4%)[61] favour long-term gas retention.

Pentalofos Formation sandstones and greywackes contain higher-energy, mixed lithic detritus, including chromite, chlorite, pyrite and actinolite, derived from the underlying ophiolitic and metamorphic massifs.[131] With porosities of 4.9–10.8% but similarly low permeability (<0.01 mD),[61] these units offer only limited reservoir potential, although their mineralogy may support some localised serpentinisation-derived H_2 and radiogenic He formation.

Tsotyli Formation marly sandstones and conglomerates exhibit moderate porosity (~6%), extremely low permeability, and strong mechanical strength, functioning as regional sealing units[61] that inhibit vertical migration.

The non-lignite interbeds of the Florina Basin (marl and sand) consist primarily of illite, mica, kaolinite, quartz, ferroan chlorite and albite, with negligible carbonates.[131,132] These fluvio-lacustrine sediments form a redox-active matrix capable of buffering Eh, promoting Fe–Mn mobilisation, and supporting microbial methanogenesis or hydrogen consumption. Their silicate-dominated composition enhances water–rock reactions and contributes Sr, Ba, Fe and Mn to groundwater, consistent with the hydrochemical signatures observed at Tropeouhos and Ammohori. Gemeni *et al.*, 2015[71] demonstrate that Fe–Mn–Br enrichment in this region derives from interaction with deeper igneous and ophiolitic substrates rather than hydrothermal activity, reinforcing the role of the ophiolitic basement and potential its derived detritus in H_2 and He generation.

As discussed in the previous sections, elevated values of Li (up to 406 μg/L), B (up to 7160 μg/L), and Br (up to 363 μg/L) in Katakali, Tropeouhos, and Mesokampos indicate deep fluid residence, prolonged circulation through silicate and evaporitic strata, and potential mixing with connate brines. Stable isotopes (δD_{H_2O}, $\delta^{18}O_{H_2O}$) show displacement from modern meteoric signatures toward more evolved compositions, confirming long residence times, deeper infiltration and significant water–rock interaction.[19–21,30,43,65,68,76–78,115,125] The geochemical data support the interpretation that the area hosts mixed deep–shallow circulation systems, where ultramafic, carbonate, and organic-rich lithologies influence gas composition.

Petrophysical data from across the basin show very low permeability (<0.01 mD) in all formations, ensuring strong confinement; low porosity (4–10%), limiting storage capacity but enhancing residence times for helium; high clay-bound water fractions (87–97%), reducing effective pore space and increasing sealing efficiency, high mechanical strength, particularly in Eptachori and Tsotyli, supporting their behaviour as regional caprocks.[61]

It is suggested that the Mesohellenic Basin behaves as a multilayered, strongly confined system. In this geological setting helium accumulates preferentially in low-permeability horizons near U–Th-bearing minerals (e.g., ophiolitoc basement, Eptachori, Pentalofos) and migrates along deep reactivated faults (Tropeouhos), producing high He anomalies. Hydrogen is generated via serpentinisation of ultramafic detritus (ophiolite-derived), radiolysis of U–Th minerals, or Fe-bearing mineral alteration. It is preserved only where reducing, low-permeability compartments exist (e.g., occasional signatures at Kivotos), but is absent where microbial or redox consumption dominates (Katakali). Methane is preserved in closed or semi-closed microbially dominated pockets (e.g., Katakali) and it is scarce in structurally open or fault-connected systems (Tropeouhos).

Geological and exploration implications

The fifth objective of the scientific hypothesis is to assess resource potential and energy implications by estimating the natural hydrogen volume available for use. The geochemical investigation has identified in Kivotos and Neos Kafkasos detectable H_2, He, evolved δD–$\delta^{18}O$ signatures and B–Br enrichment. These data indicate that deep fluid circulation interacts with ultramafic substrates, confirming the existence of generative processes and active migration pathways. However, the absence of He and the limited presence of H_2 at Katakali, despite serpentinisation-compatible geochemistry, highlight that if hydrogen is generated, it is susceptible to redox consumption in microbially dominated or organic-rich settings. Thus, resource-grade accumulations are unlikely in reducing compartments but may be preserved in more oxidised or structurally open domains where hydrogen escape is constrained by stratigraphy rather than biology.

From an energy perspective, the basin configuration is favourable for small- to medium-scale accumulations concentrated along fault-proximal compartments that overlie the ophiolitic basement Figure 10. These areas show the most substantial overlap between generative lithologies, evidence of deep fluid ascent (He anomalies), and trapping conditions capable of preserving geogenic gases across geological timescales. While the current data do not permit formal volumetric

estimation, the combination of source potential, seal integrity, long residence times, and structural compartmentalisation suggests that high-resolution structural imaging and targeted hydrogeochemical surveys could delineate zones with recoverable natural hydrogen.

Conclusion

The West Macedonia, Greece, presents the complex basement geology with felsic rocks ideal for radiolysis, ophiolites for serpentinisation and pervasive crustal faulting for degassing. The geochemical investigation supports the interpretation of multiple water sources or flow paths, including both shallow, oxygenated meteoric water and deeper, more evolved fluids enriched in light elements. These findings fulfil the first objective of the scientific hypothesis by demonstrating that the lithological and tectonic framework required for geogenic H_2 and He production is present in the region. They also partially satisfy the second objective by showing that both biotic and abiotic processes may contribute to gas generation, even though direct evidence for sustained hydrogen production remains limited due to low measured concentrations and potential microbial consumption.

Overall, the results point toward a migration-dominated system in which gases undergo chemical modification along their ascent, with no confirmed large accumulations to date aside from the known natural CO_2 reserves. This constrains the fifth objective of the scientific hypothesis, indicating that while a generative geological setting exists, present data do not yet support volumetric estimates of recoverable natural hydrogen.

In such cases, isotopes still point to dominantly biogenic CH_4 even if the gas migration system involves ophiolitic or deep rocks. Specifically, biogenic methane dominates in confined organic-rich environments (Katakali), while He and H_2 signals at Kivotos and Tropeouhos suggest deep crustal or mantle-derived fluids, potentially linked to serpentinization or radiogenic decay. Sites like Kivotos are most promising for mixed hydrogen generation, with some abiotic gas potential. Tropeouhos may serve more as a gas migration indicator, while Neos Kafkasos indicates natural CO_2 degassing rather than accumulation.

Where He and H_2 were detected (e.g., Kivotos), the system likely involves both deeper lithological interaction (ultramafics/fracture pathways) and microbial methane generation in overlying sediments[121] or mixed geological reservoirs.[73] In such cases, isotopes still indicate biogenic CH_4 even if the gas migration mechanism involves ophiolitic or deep rocks. On the same note H_2 is difficult to find in the surface springs due to easy microbial consumption in the water[133–138] and rapid oxidation along shallow circulation pathways. These limitations directly affect the evaluation of reservoirs and trapping behaviour which falls under the investigation of the third objective.

However, the present chemical analysis from Katakali samples reinforicing previous research from Daskalopoulou *et al.*, 2018[18] that also reported helium and hydrogen occurrences. A pausible explanation may be that abiotic H_2 could be generated at depth but microbially consumed before reaching the sampling point.[139] Thus, biogenic isotopic carbon signature masks the hydrogen abiotic origin.[140–143] The presence of helium indicates deep crustal or mantle fluid origins.[73] For instance, microbial communities relying on a geologic supply of H_2 have been identified in Precambrian cratons where ancient waters trapped in deep fractures that undergo radiolysis.[144] Given the natural carbon dioxide abundance in the area[68,74,132] it is possible that H_2 is consumed in higher depths by microbes (Equation 3)[145] and thus sustain rock supported ecosystems[146] to produce methane. In this way the abiotic hydrogen isotopic fingerprint may be suppressed.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \text{(Hydrogenotrophic Methanogenesis)} \quad \text{(Equation 3)}$$

In such a case methane isotopes alone can be misleading about the hydrogen origin[73] and additional tracers such as helium, clumped isotopes,[147] dissolved hydrogen monitoring and sulphur isotopes will need to be taken into account. For instance, $\delta^{13}C_{CH_4}$ can be composition insensitive to mixing with microbial gases[38] while microbial SO_4^{2-} reduction to H_2S is a process that occurs in anaerobic environments (Equation 4) in high depths including lacustrine sediments and groundwater[148] that can also alter the hydrogen's abiotic origin.

$$SO_4^{2-} + 4H_2 \rightarrow H_2S + 2H_2O \text{ (Hydrogenotrophic Sulphate Reduction)} \quad \text{(Equation 4)}$$

Thus, sulphur isotopes must be investigated to detect an abiotic hydrogen source.[73] Therefore to properly understand the origins of the hydrogen and methane in the area a combined geochemical–microbiological approach is needed. This directly aligns with the fourth, which is partially fulfilled by demonstrating the role of mixed biotic–abiotic processes but requires further evidence to fully resolve gas origins.

The afforementioned findings and discussion demonstrate the need for further investigation, particularly dedicated gas flux monitoring, mineralogical sampling, and structural mapping to delineate potential H_2-prone zones and assess their economic relevance. This preliminary study sheds light on the complex but dynamically evolving systems that dominate the Mesohellenic and Florina basins. Whereas hydrogen and helium may exist in economically viable quantities in the area, the question remains to be answered. Still, the current fluid generation and flow mechanisms existing in the Mesohellenic and Florina basin can shed light and provide analogues for exploration and discovery of economic accumulations.

It is well known that hydrogen seepage on the surface is not constant and may change the point of escape over time or flow rate[149] while sampling in water comes with its drawbacks.[133–138] Thus, future work should focus on data collection from Katakali, Kivotos, Tropeouhos and Neos Kafkasos over a long period of time rather than being spontaneous. Since the data collection has been conducted on water samples rather than in soil, it is suggested that the aforementioned sites be investigated with a handheld GA5000 or similar over a period of six months or a year on a weekly basis. The day of the visit, the operator should collect at least 6 hourly readings of hydrogen. Should the operator register hydrogen readings, water samples with vials should be taken for the following analysis:

1. ICP for a chemical suite analysis
2. He
3. H_2
4. CH_4
5. CO_2
6. Helium isotopes (^3He, ^4He)[146]
7. Deuterium of hydrogen ($\delta^2H_{H_2}$)
8. Deuterium of methane ($\delta^2H_{CH_4}$)
9. Deuterium of water ($\delta^2H_{H_2O}$)
10. Oxygen-18 of carbon dioxide ($\delta^{18}O_{CO_2}$)
11. Oxygen-18 of water ($\delta^{18}O_{H_2O}$
12. Carbon-13 of methane ($\delta^{13}C_{CH_4}$)
13. Carbon-13 of carbon dioxide ($\delta^{13}O_{CO_2}$)
14. Carbon-13 of DIC ($\delta^{13}C_{DIC}$)
15. Carbon-14 of DIC ($^{14}C_{DIC}$)
16. Sulphur isotopes ($\delta^{34}S$)
17. Clumped isotopes ($\Delta^{13}CH_3D$, $\Delta^{12}CH_2D_2$)[73,150]

Microbial sequencing to evaluate consumption pathways and metabolic signatures.

To fully understand the local system, it is necessary to sample precipitation and surface water, in addition to groundwater.[94] If any of the sites provide satisfactory results, then, provided that the appropriate permits are granted, permanent equipment should be installed in the springs to continuously monitor for hydrogen and helium. Further to this, a full exploration program with geological mapping and targeted geological soil[19–22,24–28,30–33,35,37,40,41,43,44,46,48–71,74–88,91–99,104–113,115–120,122–125,128–138,140,151–157] and water sampling with

intrusive investigation should then take place as a prerequisite for addressing the fifth objective, which remains only partially fulfilled due to insufficient data to estimate natural hydrogen volumes.

Ethics and consent

Ethical approval and consent were not required.

Data and software availability

Underlying data

Zenodo: https://doi.org/10.5281/zenodo.16914636[93]

This project contains the following underlying data:

1. Water_samples_1stround_100 ml
2. Water_samples_2ndround_250 ml
3. Water_samples_3rdround_100 ml
4. Water_samples_4thround_100 ml
5. Field_Dataentry_form_1st round
6. Field_Dataentry_form_2nd round
7. Field_Dataentry_form_3rd round
8. Field_Dataentry_form_4th round
9. Assigned codes_1st-3rd rounds
10. Assigned codes_4th round
11. Geochemical analysis_1st round
12. Geochemical analysis_2nd round
13. Geochemical analysis_3rd round
14. Geochemical analysis_4th round
15. Stratigraphy

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Current Peer Review Status: ✗ ✓ ✓ ? ?

Version 3

Reviewer Report 16 April 2026

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✓ **John Begg**
National Observatory of Athens, Institute of Geodynamics, Athens, Greece

I am satisfied that the authors have addressed most of my comments adequately. Paper approved.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 10 March 2026

https://doi.org/10.21956/openreseurope.23969.r69397

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? **Rebecca Tyne**
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This paper uses dissolved gas and gas measurements (primarily H2, He and CO2) in springs across West Macedonia and combine this with the lithological and structural setting to identify different gas sources and migration within the region. The authors have generated an impressive dataset of gas chemistry, ions and methane isotopes across different springs and also through time,

which will benefit the whole community. Despite this, I think that the paper could benefit with some further work on its structure and clarity in order to make more impactful conclusions, which I think are currently a bit lost (See below for more details).

Major comments
You have structured your discussion to assess each spring individually- while good I find it is lacking a bit of cohesion on the bigger picture. Which makes it hard to see overall trends (in space and time in the region) and draw conclusions especially relating to linking to the geology. It would be great to structure the discussion in a way that the bigger picture and trends are also consideration.

Supra regional and regional context could be shortened in geological context and moved to sup- currently distracts a bit form message and the area context more relevant.

Moderate comments
- In the abstract, the conclusion section is incredibly vague. For example, “the preliminary investigation indicates the existence of multiple gas generation and migration mechanisms”, such as what?
- Water isotopes, suggest you see seasonable variability; however, ions and noble gases suggest that there has been significant water rock reaction and a longer residence time. I’m a bit confused by this as it seems contraindicatory.

Minor comments

- Check for consistent super- and sub- scripting, as well as the use of d not just d.
- Easily replicate methods, but Figure 7 of sampling containers not necessary.

Introduction:
- Page 4 para 1, line 13 - West Macedonia should come before Greece
- Page 4, para 3, bullet 1 – I assume you mean in contact with mantle fluids within the crust? I think it's probably better just to say reducing agents, especially because the references used for the mantle don’t actually back up the statement you have made (Bendall et al., talks about mantle degassing as a source, Lefuerve is focused on mantle derived rocks being serpentinised and Mao is focused on the mantle core boundary- and any H2 is the curst from this would be degassing mantle (bullet 8).
- Page 4, paragraph 6 (last paragraph) – I find this paragraph difficult to follow.
- Page 5, paragraph 2 - Hydrogen formation doesn’t always have to happen at high depths, for example in Oman there is significant H2 formation at 100-1250m depth (Templeton et al., 2024 *refer to reference 3)*
- Page 5, paragraph 2 - why is abiotic methane, brucite and carbonate indicators for the presence of natural hydrogen? (For example, one could argue that the methane could actually be an indicator that the any natural H2 that was present has been converted to CH4).
- Page 5, paragraph 2 - I don’t follow the last sentence of this paragraph.
- Page 5 paragraph 6- Could you be more explicit here? For example, “In this study, the

ophiolite has been thrusted on top of carbonates, which act as a source of CO2 to the system, which can subsequently reaction with any H2 produced (e.g. equation 2). Although this reaction is slow in its abiotic form, it can still be significant over geological time. ***I would also like to note this reaction can be significantly quicker if it is biotically driven, see Gray et al., 2009 (refer to reference 1), Tyne et al., 2021(refer to reference 2)*

Supra regional geological context

- Page 5, para 7 line 19 – I think you mean eastern not easter?
- Page 6, para 2 – you seem to have slipped into results here talking about the origin of methane from its isotopic composition?
- Page 6, para 3 – dissolved CO2 can still migrate when dissolved so it is technically not immobilised. Not sure what you mean in the next sentence of this paragraph.

Results

- Table 7- more significant figures are needed to show results the He and H2 results.
- Table 7 – what are the errors on all these data?

Discussion

- Table 8 – can you go to more sig fig again so you can see actual values not just 0? Also make sig figs consistent between the tables.
- From the tables there quite a significant chang in the gas composition between campaign 3 and 4, it would be good if this could be further explained in the text.

Discussion origin of gases:

- Page 21, para 3 – have you thought about looking at the He/H2 ratio to determine if it is form radiolysis?
- Page 21, para 4 - why does microbial methane mean there is no abiotic H2?
- Page 21, para 6 - what other sites?

Discussion, migration pathways:

- It would be worth redrafting this section to make your point clearer as I find it a bit hard to follow.
- Page 22, para 3 – why does the absence of He and H2 imply limited upward migration of mantle gases? The amount of He is the mantle is low compared to that generate in the crust and already in the water, so while mantle He could substantially change the 3He/4He ratio (which does not seem to have been measured) in the sample, the actual concentration of He is not likely to substantially change. H2 may also be migration from below and then being converted to methane or methane could be forming at depth then migrating up? In addition, He could have been lost due to the type of sampling container used

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Isotope tracing of CO2, He and H2

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 05 March 2026

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?

Biying Chen
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This study provides a comprehensive geochemical analysis of water and dissolved gases in West Macedonia, aiming to identify potential geological reservoirs, gas sources, and migration routes, with a specific focus on helium and hydrogen. While it is evident that the authors have put significant effort into revising the manuscript and improving its structural flow, several fundamental concerns remain regarding the core contribution of this study.
The primary issue relates to the focus on natural hydrogen exploration. Given that only trace levels of hydrogen were detected in the study areas, the rationale for selecting this region as a

target for hydrogen resource evaluation remains unclear. The authors suggest in their response that the study focuses on processes rather than volumes in the revised version. However, the hydrogen generation mechanisms discussed do not appear to move significantly beyond established general knowledge. Furthermore, the proposed generation mechanisms appear to be inferred indirectly from trace elements and CH4, CO2, and He data. Consequently, the constraints on hydrogen generation remain poorly defined. The authors should more clearly articulate the novel contribution of this work and how it advances the existing literature.
Minor comments
There appears to be a discrepancy in the interpretation of the water sources for the third round of sampling. The authors first attribute the signature to rainfall and evaporation. However, the discussion of trace element content later shifts the explanation to water-rock interaction.
The authors suggest that high Fe concentrations in the Tropeouhos and Ammohori_1 samples may be linked to mineral dissolution or serpentinisation, which are processes that theoretically facilitate H2 production. However, no measurable H2 was detected in these specific samples.
The manuscript attributes the high CO2 content in the samples related to volcanic CO2 in the Florina Basin. Please provide further evidence or geochemical justification to support this link.
The term "TDC" and "DIC" are not defined.
The authors conclude that the elevated helium levels originate from a mixture of radiogenic and mantle sources. However, no helium isotope data has been provided. In the absence of this data, it is difficult to definitively determine the origin.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: natural gas geochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 02 March 2026

https://doi.org/10.21956/openreseurope.23969.r69398

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John Begg
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Review of the manuscript “Investigative research for occurrences of hydrogen, helium, methane and carbon dioxide in West Macedonia: linking geological reservoirs to subsurface gas generation and migration” by Tyrologou, P.; Koukouzas, N.; Couto, N.; Stergiou, C.L.; Carneiro, J. in Open Research Europe.

This manuscript presents a valuable contribution to initial investigations into the prospectivity of natural hydrogen (and other gaseous species) in Western Macedonia, northern Greece. The paper incorporates discussion on the geology of the region with respect to identifying potential sources of natural hydrogen generation, fluid migration pathways, reservoir potential and possible biotic modification of gas species. Thorough geochemical analysis of gas and water samples from ten localities represents the scientific core of the paper with enhanced value provided by repeated sampling to assess seasonal/temporal variation. This reviewer commends the authors for their use of a multi-disciplinary toolkit to investigate gas and fluid origins and storage potential.

Overall, this manuscript provides a significant contribution to the geochemical understanding of the occurrence of natural hydrogen, helium, methane and carbon dioxide in West Macedonia and is worthy of indexing subject to the (minor) revisions proposed below.

1. Abstract and PLS: I believe that both the Abstract and the Plain Language Summary can be improved by appropriately representing the scientific contribution of the paper. Currently, they both incorporate substantial sections that amount to political justifications for the work undertaken, an unnecessary distraction. I would prefer to see summaries of the scientific approach, data presented and conclusions. Further, I am not familiar with the style of presenting headings within an Abstract. Is this something that the Open Research Europe manuscripts accept?

2. Organization and length of introductory sections: The five stated objectives in the Introduction may have been ambitious, resulting in a lengthy manuscript. For example, the geological description of the region and area of interest occupies five and a half pages (over three sections). I believe that more focused summaries of potential source rocks, storage and cap rocks and their structure would help prepare readers adequately for what follows. Especially the last of the three introductory geological sections, under the heading “Local geology setting of promising sites for natural hydrogen”, adds little to the work at this stage, as the sampling and chemistry has not yet been discussed. Instead, this section should be relocated towards the end of the paper (Discussion?), once the authors have demonstrated prospectivity. Further, Figure 4 is rather empty

and would benefit from some geological context (including faults; see comments below).

3. Active faulting: While the section on geology is disproportionately long, there is no discussion on the active faulting and fold structures that may contribute to gas and fluid migration, and also to storage structures. In fact, a 2025 database, which was probably not available to the authors at the time of writing, provides this information and I encourage the author to consult it (Begg et al. 2025. Scientific Data.). The reason is that both Katakali and Kivotos sites lie close to NE-SW-orientated faults that have been active during the Quaternary (Begg et al. 2025). Similarly, a suite of active faults oriented NE-SW and a more limited set of NW-SE-oriented active faults are present in the Florina area (see Begg et al. 2025). Interestingly, it is unrealistic in my opinion that the authors of this manuscript favour strike-slip faulting in their figures 2 & 10, as neither geomorphic indicators (Begg et al. 2025) nor GNSS analysis (Chousianitis et al. 2024) or earthquake moment tensors (Konstantinou et al. 2017) support such mechanisms. Instead, the dominant faulting style in western Macedonia is normal faulting, which may result in the opening of fluid/gas migration pathways along active faults within the MHB.

4. Standardization of Tables: The geochemical data presented in Tables 2-8 are a primary output of this work and I congratulate the authors on this contribution. However, I was a little confused by the lack of continuity between the tables. In Table 1 the first column is spring names while the following columns locate them. In Tables 2-5, the first column is a number, but the numbers and names and the sequence of the springs sampled varies between these tables. This creates some confusion for the reader (and future users of the data). Then, the following tables revert to the spring names in the first column, but also incorporate the additional complexity of adding a second samples for some sites. Without carefully reading the text, readers could be forgiven for believing that these are different springs. Further complicating the data is that it appears that two Ammohori springs are sampled, Ammohori 1 and Ammohori 2. If springs are given consistent numbers and names, it will be intuitively clear to readers that the suffixes -1 and -2 refer to the sample numbers. Before the manuscript is accepted for indexing, the authors should make some effort to standardize their tables and nomenclature.

5. Results: I found this section to be rather disjointed. The first sentence states that isotope data will be discussed first and there is reference to Figure 8, but no presentation of the results. The results of ICP analyses that follows refers to Table 6; the text states that ICP analyses here are of water from the second round of sampling, but this is not stated in the caption. The presentation of water chemistry doesn't appear to have much coherent description and there are embedded elements of interpretation within this discussion. In the Discussion and results interpretation section, there are some important observations that warrant inclusion within the Abstract, including that detected variations in water and gas chemistry probably reflect enrichment of resident groundwater by precipitation.

6. Migration pathways and faults: the authors discussion regarding migration pathways and fluid circulation is useful but founded on geochemical analysis without reference to disruption of circulation related to active faulting. While this is understandable in the context of the data they have assembled, it leaves something of a question mark regarding the impact of active faulting on the system. I strongly recommend the authors discuss migration pathways also with respect to the active faulting in the region (Begg et al. 2025). Changes in gas and water chemistry at sample sites are interpreted as indicative of variability attributable to precipitation. However, some discussion of whether this is entirely attributable to shallow mixing of groundwater with rainwater

influx, or whether the meteoric water infiltrates deeper parts of the basin, would benefit the manuscript.

References

1. Begg J, Mouslopoulou V, Heron D, Nicol A: AFG - Active Faults Greece: a comprehensive geomorphology-based 1:25,000 fault database. *Scientific Data*. 2025; **12** (1). Publisher Full Text
2. Chousianitis K, Sboras S, Mouslopoulou V, Chouliaras G, et al.: The Upper Crustal Deformation Field of Greece Inferred From GPS Data and Its Correlation With Earthquake Occurrence. *Journal of Geophysical Research: Solid Earth*. 2024; **129** (4). Publisher Full Text
3. Konstantinou K, Mouslopoulou V, Liang W, Heidbach O, et al.: Present-day crustal stress field in Greece inferred from regional-scale damped inversion of earthquake focal mechanisms. *Journal of Geophysical Research: Solid Earth*. 2017; **122** (1): 506-523 Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and does the work have academic merit?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: geological mapping, tectonics, active faulting, hydrogeology, natural hydrogen

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Mar 2026

Pavlos Tyrologou

Response to reviewers Review of the manuscript "Investigative research for occurrences of hydrogen, helium, methane and carbon dioxide in West Macedonia: linking geological reservoirs to subsurface gas generation and migration" by Tyrologou, P.; Koukouzas, N.; Couto, N.; Stergiou,

C.L.; Carneiro, J. in Open Research Europe.

This manuscript presents a valuable contribution to initial investigations into the prospectivity of natural hydrogen (and other gaseous species) in Western Macedonia, northern Greece. The paper incorporates discussion on the geology of the region with respect to identifying potential sources of natural hydrogen generation, fluid migration pathways, reservoir potential and possible biotic modification of gas species. Thorough geochemical analysis of gas and water samples from ten localities represents the scientific core of the paper with enhanced value provided by repeated sampling to assess seasonal/temporal variation. This reviewer commends the authors for their use of a multi-disciplinary toolkit to investigate gas and fluid origins and storage potential.

Overall, this manuscript provides a significant contribution to the geochemical understanding of the occurrence of natural hydrogen, helium, methane and carbon dioxide in West Macedonia and is worthy of indexing subject to the (minor) revisions proposed below. **Response:** We thank the reviewer for the time invested to support our scientific research **1. Abstract and PLS: I believe that both the Abstract and the Plain Language Summary can be improved by appropriately representing the scientific contribution of the paper. Currently, they both incorporate substantial sections that amount to political justifications for the work undertaken, an unnecessary distraction. I would prefer to see summaries of the scientific approach, data presented and conclusions. Further, I am not familiar with the style of presenting headings within an Abstract. Is this something that the Open Research Europe manuscripts accept?** **Response:** We thank the reviewer for the support, dedicated time and this thoughtful comment. We fully agree that the primary function of both the abstract and the plain language summary is to clearly present the scientific approach, data, and conclusions of the study. At the same time, we considered it important to situate the work within its broader scientific and societal relevance. Our intention was not to provide political justification, but to clarify the scientific timeliness and contextual significance of the research question. We have revised the abstract to ensure that the description of methodology, analytical results, and principal findings are more prominent. Regarding the structured headings and the plain language summary format, these follow the Open Research Europe author guidelines and are consistent with the journal's editorial requirements. **2. Organization and length of introductory sections: The five stated objectives in the Introduction may have been ambitious, resulting in a lengthy manuscript. For example, the geological description of the region and area of interest occupies five and a half pages (over three sections). I believe that more focused summaries of potential source rocks, storage and cap rocks and their structure would help prepare readers adequately for what follows. Especially the last of the three introductory geological sections, under the heading "Local geology setting of promising sites for natural hydrogen", adds little to the work at this stage, as the sampling and chemistry has not yet been discussed. Instead, this section should be relocated towards the end of the paper (Discussion?), once the authors have demonstrated prospectivity. Further, Figure 4 is rather empty and would benefit from some geological context (including faults; see comments below).**
Response: Thank you for your valuable suggestions. The structure of the manuscript was

adjusted in response to earlier peer review comments and reflects the way to establish the geological framework prior to presenting the geochemical results. We, thus, consider the stratigraphic, structural, and lithological context essential for understanding the sampling strategy, gas occurrence, and the interpretation of potential migration and trapping mechanisms. As such, the section "*Local geology setting of promising sites for natural hydrogen*" is positioned within the introductory framework to provide the necessary geological basis for the subsequent analytical results. Figure 4 has been enhanced to include additional geological context and structural features, including faults, as suggested.
3. Active faulting: While the section on geology is disproportionately long, there is no discussion on the active faulting and fold structures that may contribute to gas and fluid migration, and also to storage structures. In fact, a 2025 database, which was probably not available to the authors at the time of writing, provides this information and I encourage the author to consult it (Begg et al. 2025. Scientific Data.). The reason is that both Katakali and Kivotos sites lie close to NE-SW-orientated faults that have been active during the Quaternary (Begg et al. 2025). Similarly, a suite of active faults oriented NE-SW and a more limited set of NW-SE-oriented active faults are present in the Florina area (see Begg et al. 2025). Interestingly, it is unrealistic in my opinion that the authors of this manuscript favour strike-slip faulting in their figures 2 & 10, as neither geomorphic indicators (Begg et al. 2025) nor GNSS analysis (Chousianitis et al. 2024) or earthquake moment tensors (Konstantinou et al. 2017) support such mechanisms. Instead, the dominant faulting style in western Macedonia is normal faulting, which may result in the opening of fluid/gas migration pathways along active faults within the MHB. **Response:** We thank the reviewer for this insightful and constructive comment regarding the present-day tectonic regime of western Macedonia and its implications for fluid and gas migration. We agree that the dominant active faulting style in the region is normal faulting and that this should be clearly reflected in our interpretation. In response, we have revised the geological section to incorporate recent publications on active faulting and crustal deformation, (Begg et al., 2025), regional stress inversion results (Konstantinou et al., 2017), and GPS-derived strain rate analyses (Chousianitis et al., 2024). A new paragraph has been added at the end of the geological chapter explicitly stating that active and probably active faults in western Macedonia and the Florina Basin are dominantly normal, with NE–SW-oriented structures controlling Quaternary sedimentation and deformation. Importantly, we clarify in the Discussion that the fault geometries illustrated in Figures 2 and 10 do not aim to represent exclusively the present-day active tectonic regime, but primarily the inherited structural architecture formed during the Miocene opening and evolution of the Mesohellenic Basin. We now explicitly state that some of these pre-existing basin-bounding and intra-basin faults (as documented by publications studying the geometery of the MSB) may undergo selective reactivation under the current extensional stress field, predominantly in normal-slip mode, depending on their orientation relative to the present-day stress axes. This distinction separates the inherited tectonic framework from the Quaternary deformation regime and avoids implying an active regional strike-slip system. In addition, Figures 2 and 10 have been slightly modified and their captions revised to clarify that the depicted structures represent the inherited basin architecture and not necessarily the current kinematic regime. Together, these revisions align the manuscript with the geomorphic, seismological, and geodetic evidence cited by the reviewer, while preserving the structural controls relevant to subsurface gas generation and migration.

4. Standardization of Tables: **The geochemical data presented in Tables 2-8 are a primary output of this work and I congratulate the authors on this contribution. However, I was a little confused by the lack of continuity between the tables. In Table 1 the first column is spring names while the following columns locate them. In Tables 2-5, the first column is a number, but the numbers and names and the sequence of the springs sampled varies between these tables. This creates some confusion for the reader (and future users of the data). Then, the following tables revert to the spring names in the first column, but also incorporate the additional complexity of adding a second samples for some sites. Without carefully reading the text, readers could be forgiven for believing that these are different springs. Further complicating the data is that it appears that two Ammohori springs are sampled, Ammohori 1 and Ammohori 2. If springs are given consistent numbers and names, it will be intuitively clear to readers that the suffixes -1 and -2 refer to the sample numbers. Before the manuscript is accepted for indexing, the authors should make some effort to standardize their tables and nomenclature.**

Response: We thank the reviewer for this constructive comment and for recognising the importance of clarity and consistency in the presentation of the geochemical dataset. In response, we have thoroughly revised Tables 1–8 to standardize nomenclature and structure throughout the manuscript. All spring names are now presented uniformly in lowercase and bold font across all tables to ensure visual consistency and facilitate cross-referencing. In addition, we have assigned fixed identification codes (S1–S10) to the ten distinct springs, and these codes are used consistently across Tables 1–5. In Table 1, the listings of the springs were revised to be the same as in Tables 1 to 5. In Tables 6 and 7, the same S1–S10 labels have been added alongside the spring names to maintain continuity between datasets and sampling campaigns. To further eliminate ambiguity between distinct springs (e.g., Ammohori 1 and Ammohori 2) and duplicate samples collected from the same location, we have clarified in the captions of Tables 5 and 8 that “Samples marked with suffix (-2) represent duplicates”. We believe these revisions resolve the continuity issues identified by the reviewer and significantly improve the clarity, traceability, and usability of the dataset for readers and future users.

5. Results: **I found this section to be rather disjointed. The first sentence states that isotope data will be discussed first and there is reference to Figure 8, but no presentation of the results. Response:** We thank the reviewer for the comment. A paragraph has been added for text coherence. **The results of ICP analyses that follows refers to Table 6; the text states that ICP analyses here are of water from the second round of sampling, but this is not stated in the caption. Response:** Corrected **The presentation of water chemistry doesn’t appear to have much coherent description and there are embedded elements of interpretation within this discussion. Response:** Thank you for your comment. In the results section, we intentionally provide limited interpretative context alongside the presentation of water chemistry data to facilitate text coherence and continuity. Given the multidimensional nature of hydrogeochemical datasets, purely numerical reporting without brief explanatory linkage may obscure the relevance of observed spatial variability and elemental associations. Thus, the interpretative remarks included are restricted to immediate data description. Broader interpretation and synthesis are reserved for the Discussion section. **In the Discussion and results interpretation section, there are some important observations that warrant inclusion within the Abstract, including that detected variations in water and gas chemistry**

probably reflect enrichment of resident groundwater by precipitation.
Response: Thank you for your comment. The abstract has being modified according to the reviewer's suggestion.
6. Migration pathways and faults: the authors discussion regarding migration pathways and fluid circulation is useful but founded on geochemical analysis without reference to disruption of circulation related to active faulting. While this is understandable in the context of the data they have assembled, it leaves something of a question mark regarding the impact of active faulting on the system. I strongly recommend the authors discuss migration pathways also with respect to the active faulting in the region (Begg et al. 2025). Changes in gas and water chemistry at sample sites are interpreted as indicative of variability attributable to precipitation. However, some discussion of whether this is entirely attributable to shallow mixing of groundwater with rainwater influx, or whether the meteoric water infiltrates deeper parts of the basin, would benefit the manuscript. **Response:** We thank the reviewer for this valuable comment. We agree that the role of active faulting in controlling fluid circulation and gas migration pathways should be more explicitly addressed. In response, we have expanded the Discussion section (within *Hydrochemistry and isotopic evidence supporting gas occurrences*) to incorporate the influence of Quaternary normal faulting in western Macedonia, following Begg et al. (2025), Konstantinou et al. (2017), and Chousianitis et al. (2024). The revised text clarifies that active normal faults may enhance vertical permeability through fractured fault zones, thereby facilitating structurally controlled fluid circulation. We also refined our interpretation of hydrochemical variability to acknowledge that changes in gas and water chemistry may not be solely attributable to shallow mixing with meteoric water, but could also reflect deeper meteoric infiltration along active fault zones and inherited basin structures. This addition strengthens the linkage between geochemical observations and the regional active tectonic framework, without overextending beyond the constraints of the available data.

Competing Interests: No competing interests were disclosed.

Reviewer Report 30 December 2025

https://doi.org/10.21956/openreseurope.23969.r66097

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Princewill M. Ikpeka
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No further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hydrogen Production and Storage

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 03 November 2025

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Princewill M. Ikpeka
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[2] Brunel University London, London, England, UK
[3] Brunel University London, London, England, UK

Overview
The paper is interesting, and the subject area is indeed very important to the energy storage/production discussion. However, I do not recommend indexing in its current form. It needs substantial revision in the following areas:

- The introduction is quite long, which makes the research aim unclear. After reading through, I recommend synthesizing the sections: "Current state of the art of natural hydrogen" and "Previous research related to West Macedonia and neighbouring areas" into "Introduction" section rather than separate sections.

- There is an issue with data reliability. The first two sampling campaigns did not yield reliable gas measurements due to sample preservation issues. This means that only the third and fourth campaigns provided usable gas data, which **greatly limits the evidence** available.

- The central hypothesis of the paper is not convincingly supported by the data presented. Measured hydrogen concentrations were extremely low, **only on the order of a few parts per million (ppm)** in the third campaign, and in the final (fourth) campaign **no hydrogen at all was detected**. For example, one water sample showed just 4.4 ppm H_2 (with thousands of ppm methane in comparison), and another had ~1.7 ppm H_2 – essentially trace levels. In the last round, the authors state “no hydrogen gas was registered”. Such minimal or absent H_2 results make it difficult to support the hypothesis of significant **natural hydrogen generation** in the area.

- The authors propose that hydrogen might be getting consumed by microbes before detection

(see Discussion), but without direct evidence (e.g. microbial analyses or consistent H_2 findings), this remains largely speculative. The data **do not provide clear proof** of natural hydrogen in these reservoirs, so the main premise of the study is left on uncertain footing.

- In addition, the paper is largely exploratory and observational, and as such it does not employ statistical analyses like hypothesis testing, regression modelling, or p-value significance tests. This means the conclusions rely on expert judgment calls not on quantitative analysis.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and does the work have academic merit?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hydrogen Production and Storage

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Dec 2025

Pavlos Tyrologou

Response to report 2 Overview
The paper is interesting, and the subject area is indeed very important to the energy storage/production discussion. However, I do not recommend indexing in its current form. It needs substantial revision in the following areas:
Response: Many thanks for your time, support and comments which are dully noted. We have now restructured and revised the manuscript. We have explicitly clarified that preservation issues affected the integrity of gas analyses from these campaigns. While part of the first sampling campaign was affected by preservation-related degassing, the subsequent datasets were collected under controlled conditions and show internally

consistent geochemical patterns. Interpretations rely on multiple independent indicators (aqueous chemistry, geological context, spatial trends) that remain robust even considering potential underestimation in early gas values. We ensure transparency regarding data quality and avoid overinterpretation. This revision strengthens the scientific credibility of the manuscript and aligns the study with accepted geochemical sampling standards.

- The introduction is quite long, which makes the research aim unclear. After reading through, I recommend synthesizing the sections: "Current state of the art of natural hydrogen" and "Previous research related to West Macedonia and neighbouring areas" into "Introduction" section rather than separate sections.
Response: Thank you for your suggestions. The authors acknowledge the reviewers comment and have shortened the introduction while also merged the subsections as it was pointed out

- There is an issue with data reliability. The first two sampling campaigns did not yield reliable gas measurements due to sample preservation issues. This means that only the third and fourth campaigns provided usable gas data, which greatly limits the evidence available.
Response: We thank the reviewer for this observation. We fully acknowledge that the first two sampling campaigns were affected by preservation issues. This was stated for transparency reasons but also to draw the attention to any future research on sample preservation and its importance. In the original and revised manuscript, we state that only the third and fourth campaigns yielded reliable helium measurement and confirmed hydrogen presence. Although this reduces the total number of usable gas datapoints, the third and fourth campaigns were conducted under controlled preservation conditions and show internally consistent compositions. Moreover, the geological context, aqueous geochemistry and spatial patterns provide independent lines of evidence that support the evaluation of subsurface processes and gas migration behaviour. The conclusions are therefore based on conservative, well-constrained data and remain scientifically robust within the defined scope of an exploratory study. The manuscript takes all these into account. While the available evidence is therefore more restricted, the dataset remains sufficient to evaluate geological processes and gas migration behaviour within the scope of an exploratory study.

- The central hypothesis of the paper is not convincingly supported by the data presented. Measured hydrogen concentrations were extremely low, only on the order of a few parts per million (ppm) in the third campaign, and in the final (fourth) campaign no hydrogen at all was detected. For example, one water sample showed just 4.4 ppm H_2 (with thousands of ppm methane in comparison), and another had ~1.7 ppm H_2 – essentially trace levels. In the last round, the authors state "no hydrogen gas was registered". Such minimal or absent H_2 results make it difficult to support the hypothesis of significant natural hydrogen generation in the area.
Response: We acknowledge the reviewer's point that measured hydrogen concentrations were low, ranging only from 1.7 to 4.4 ppm in the third campaign and falling below detection in the fourth campaign. Indeed, our revised text emphasises an evaluation of the geological potential for hydrogen generation and confinement within the Mesohellenic Basin. Thus, the revised manuscript distinguishes between (a) hydrogen generation

mechanisms that may operate within the basin due to its mineralogical and tectonic characteristics, and (b) the detectable accumulation of hydrogen in measurable quantities. This distinction is now made explicit in the Introduction, Objectives, and Discussion. The low measured concentrations are acknowledged transparently. Our interpretation focuses instead on geological capacity, using measured trace concentrations to infer processes rather than volumes. This is consistent with the early exploratory nature of natural hydrogen research. Kindly also note that the research considers not only natural hydrogen but also helium which has been detected with analytical accuracy and confidence.

- The authors propose that hydrogen might be getting consumed by microbes before detection (see Discussion), but without direct evidence (e.g. microbial analyses or consistent H_2 findings), this remains largely speculative.
Response: We thank the reviewer for highlighting the need for careful interpretation regarding microbial consumption of hydrogen. In the revised manuscript, we present microbial consumption only as a possible explanation, supported by geochemical indicators such as reducing redox conditions, elevated Fe–Mn concentrations, and the presence of organic-rich strata in the Florina Basin. The text emphasises that this is a hypothesis grounded in analogous studies, not a confirmed mechanism for the sites investigated here. We state that future work involving microbial community characterisation would be required to confirm or refute this interpretation.

The data do not provide clear proof of natural hydrogen in these reservoirs, so the main premise of the study is left on uncertain footing.
Response: We appreciate the reviewer's concern and agree that the measured hydrogen concentrations are low and do not constitute proof of substantial natural hydrogen accumulations. In the revised manuscript, we now explicitly state that the evidence reflects incipient or low-level generative processes rather than resource-grade hydrogen reservoirs. Our interpretation focuses on identifying geological conditions capable of producing hydrogen, such as serpentinisation, radiolysis, and Fe-bearing mineral alteration, and assessing whether these processes are consistent with the geochemical signals observed. rather than asserting the presence of economically significant accumulations. Although hydrogen concentrations are modest, they form one component of a broader, multi-line evidence framework that includes geological context, mineralogical controls and aqueous geochemistry. The central theme of the study is to mostly evaluate geological potential. We have revised the text accordingly to further clarify the affroementioned.

- In addition, the paper is largely exploratory and observational, and as such it does not employ statistical analyses like hypothesis testing, regression modelling, or p-value significance tests. This means the conclusions rely on expert judgment calls not on quantitative analysis.
Response: Thank you again for this constructive comment. We agree that the study is primarily exploratory; however, we emphasise that all analytical procedures followed rigorous QA/QC protocols, including certified reference materials, duplicate measurements, instrument calibration with internationally traceable standards, and documented external reproducibility (0.2‰ for $\delta^{13}C_{CH_4}$ and 2‰ for δD_{CH_4}). These controls ensured that the geochemical patterns we report are robust even in the absence of formal hypothesis-testing statistics. Regarding statistical treatment, the dataset structure, characterised by small

sample numbers per site and non-replicated measurements for several parameters, does not meet the assumptions for regression modelling or significance testing. Instead, we applied the only statistically defensible quantitative test available: an exploratory Spearman rank correlation between $\delta^{13}C$-CH_4 and δD-CH_4 for the four samples with complete isotopic data. The correlation coefficient was low ($\rho \approx 0.10$), indicating no monotonic relationship between the two isotopic parameters. This outcome is consistent with our interpretation that the samples fall within a narrow biogenic isotopic range, with limited variability relative to analytical uncertainty. Importantly, the absence of strong statistical structure does not weaken the geological interpretation. Multiple independent lines of evidence, reproducible gas occurrences, stable isotope systematics, He-rich signatures, water chemistry and mapped fault–ultramafic contacts, converge toward a coherent conceptual model of subsurface gas generation. In this context, the repeated detection of hydrogen across campaigns suggests a genuine geological signal, even though concentrations lie near detection limits. The presence of ultramafic lithologies, reducing conditions, radiogenic helium and fault-controlled migration pathways is compatible with low-flux abiotic H_2 generation. Microbial consumption is also plausible and could explain the subdued surface values. As with the statistical limitations discussed above, confirming natural hydrogen systems requires expanded temporal and spatial monitoring, which we highlight as a clear direction for future work. This integrative method is standard practice in subsurface gas prospecting and is well suited to the reconnaissance phase of a developing geochemical system. Regarding hydrogen, the evidence is similarly robust within the constraints of an exploratory dataset. H_2 was detected repeatedly across several sites and campaigns, and its occurrence is spatially coherent with ultramafic lithologies, radiogenic helium anomalies and reducing subsurface conditions, factors that collectively support the plausibility of abiotic H_2 generation. At the same time, the low concentrations measured at the surface are consistent with microbial consumption, which is known to efficiently attenuate H_2 fluxes in shallow environments. Thus, while the existing dataset does not yet permit definitive source attribution, the combination of geochemical, isotopic and geological indicators strongly suggests that hydrogen generation processes may be active in West Macedonia. We trust that even without formal inferential statistics, the study provides a coherent, multi-line body of evidence:

1. High analytical precision and strict QA/QC confirm data reliability.
2. Temporal duplicate consistency supports the robustness of isotopic interpretations.
3. Isotope systematics (CH_4, He, water isotopes) align with recognised genetic pathways.
4. Geological context independently supports abiotic H_2 generation potential.

Future work will expand the dataset sufficiently to enable statistical modelling of spatial clustering, mixing processes and source–pathway relationships. At this early stage, however, the combination of geochemistry, isotopes and structural geology provides valid, defensible conclusions appropriate for an exploratory study.

Competing Interests: No competing interests were disclosed.

Reviewer Report 11 October 2025

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Review of Tyrologou et al., Manuscript
General Assessment
The manuscript presents an integrated study on gaseous systems (H_2, He, CH_4, and CO_2) in Western Macedonia, combining structural geology, sedimentology, and geochemistry. This multidisciplinary approach is a major strength of the paper, as it provides valuable insights into the mechanisms controlling gas generation, migration, and trapping. The authors provide an extensive new geochemical dataset (gas chromatography, inductively coupled plasma, and isotope analyses) from both water and gas samples collected at multiple hydrothermal sources, which constitutes a significant contribution to the understanding of natural hydrogen systems. However, despite the richness of the data, the manuscript lacks clarity and focus. The research question and logical thread of the paper are often difficult to follow due to structural issues, redundancies, and digressions into socio-economic and political topics that are not directly relevant to a scientific article. The paper would benefit from a thorough reorganization following a classical structure (Introduction, Geological Context, Methods, Results, Discussion, and Conclusion). The clarity, conciseness, and scientific rigor of each section should be enhanced to help the reader clearly understand the problem, the methodology, and the conclusions.
Major Comments
1. Introduction
The Introduction is overly long and fragmented, which prevents a clear understanding of the main research question. It should be condensed into a coherent scientific narrative that succinctly states the problem, its significance, and the context of natural hydrogen. Political and environmental aspects should be summarized in two or three lines at most.
The manuscript would benefit from a clearer definition of its scientific novelty. While it compiles valuable field and isotopic data, the research question remains vague. The authors should explicitly state their hypothesis and objectives at the end of the Introduction to help readers understand the study's purpose.
Some statements are unclear or inaccurate; for example, 'Natural hydrogen mechanisms are still under development' should be rephrased as 'The understanding of natural hydrogen generation mechanisms is still under investigation.' The section 'Current State of the Art' lacks a detailed discussion of hydrogen production mechanisms and diverges into unrelated topics. It should focus on generation processes relevant to the study area and cite up-to-date references.
2. Geological Context
The geological description is detailed and thorough, but overly extensive. Many details are not

directly relevant to the research question and tend to obscure the main message. The section should focus on key aspects such as source rocks, basin thickness, structural controls (faults) on gas migration, and potential reservoir rocks.
The authors should merge 'Previous research related to West Macedonia and neighbouring areas' with 'Geological setting' to avoid repetition. Maintain a logical flow—start with regional geology, then focus on specific study zones relevant to the presented data. Clarify whether the study concerns West Macedonia or Greece, as this point remains ambiguous.
Figures 1–5 include excessive descriptive geological information. Simplifying these or adding a conceptual schematic linking tectonic setting, source rocks (ophiolites, lignites, carbonates), and migration pathways would greatly improve readability.
3. Materials and Methods
The methodology is comprehensive but overly detailed. Only essential information should be provided—sample types, volumes, sampling conditions, and analytical tools. Avoid unnecessary procedural details such as 'The values of the latter were recorded in field books or digital devices.' Figures 6–8 showing sampling steps could be moved to Supplementary Material.
Simplify over-detailed methodological descriptions and remove non-scientific instructions (e.g., 'practice a lot before commencing the sampling process,' 'check under daylight for bubbles'). Provide details relevant to the analytical precision, such as GC column type, detection limits, and isotopic accuracy.
4. Results
The Results section is rich but should focus solely on presenting data without interpretation. Interpretations belong in the Discussion. Values should be expressed as ranges with appropriate units, and isotopic notation must follow standard conventions ($\delta^{13}C–CH_4$, $\delta D–CH_4$, $\delta D–H_2$, $\delta D–H_2O$, $\delta^{18}O–H_2O$).
The link between data and interpretation should be strengthened. Rather than listing numerous values, summarize and highlight key geochemical and isotopic trends (e.g., $\delta D–\delta^{18}O$ correlations, spatial variations in $CH_4/H_2/He$) and discuss their geological implications.
Remove irrelevant details such as water potability discussions. Ensure tables contain measurement units. Clarify the purpose of blind samples—specify whether they served as analytical blanks or matrix controls—and verify the consistency of repeated samples (e.g., 'Katakali-2' and 'Kivotos-2').
5. Discussion
The Discussion lacks structure and clarity. It should be reorganized into subsections addressing specific scientific questions such as gas sources, migration pathways, trapping mechanisms, and implications. The discussion should be hypothesis-driven, directly linking interpretations to results. Currently, some parts of the Discussion are embedded in the Results section. These should be consolidated. Address the noted inconsistency: the manuscript claims that 'low silica enhances H_2 generation' but also that 'low silica can be indicated by magnetite.' In ultramafic contexts, magnetite is an indicator of serpentinization and thus hydrogen generation. Clarify this contradiction.
6. Minor, Technical, and Specific Comments
6.1 General Technical Corrections
- Ensure isotopic notation ($\delta^{13}C–CH_4$, $\delta D–H_2O$, $\delta^{18}O–H_2O$) is consistently formatted using correct Greek symbols and superscripts (use 'O' not zero).
- Verify typographic consistency for isotopic symbols ($\delta^{13}C$, δD, $\delta^{18}O$).
- Include measurement units consistently across all tables, particularly for δ-values and gas concentrations.
- Correct typographical errors such as '1..22' in Table 5 (double dot under dissolved O_2).

- Condense figures and tables to highlight only essential data; move over-descriptive figures to Supplementary Material.
- Clarify the expression 'public indications' (Page 10).
- Use consistent terminology: distinguish between 'potential gas-bearing formations' and 'storage reservoirs.'
- Replace outdated or irrelevant references with more recent sources.
- Rephrase ambiguous or awkward sentences for clarity, e.g., 'Hydrogen is highly reactive... preservation throughout migration is a risk' → 'Hydrogen is highly reactive and susceptible to redox conditions, making long-term preservation during migration challenging.'
- Rephrase 'This is also making sure...' → 'This is also to make sure⋯'
- Replace 'deploying natural hydrogen in the EU' with 'establishing a natural hydrogen economy in the EU.'
- Figures 6–8 show overly detailed sampling equipment. Consider moving these to Supplementary Material to streamline the main text.
- Clarify whether 'Katakali-2' and 'Kivotos-2' samples are duplicates or replicate analyses.
- Explain the rationale for using 'blind samples'—was bottled water used as an analytical blank or matrix control?

6.2 Page-Specific Remarks

Page 4, paragraph 5: Rephrase 'Natural hydrogen mechanisms...' as 'The understanding of natural hydrogen generation mechanisms is still under development.'

Page 4, paragraph 6: Adjust preservation and EU hydrogen economy phrasing as above.

Page 5, paragraph 6: Clarify the contradiction regarding silica content and H_2 generation mechanisms.

Page 10, paragraph 4: Differentiate between CO_2 storage and natural H_2 leakage—surface leaks may indicate deeper accumulations.

Page 10, paragraph 4: Clarify what is meant by 'public indications.'

Page 22, paragraph 2: Discuss the robustness of measurements given the small He/H_2 concentrations and analytical uncertainty.

Tables 2–5: Ensure $\delta D–H_2O$ uses the letter O (not zero).

Overall Conclusion

Overall, the study provides valuable geochemical and isotopic data from a poorly documented region. However, its scientific impact would increase significantly if the manuscript were restructured around its core research questions, with redundant methodological details condensed. A stronger integration of geological, hydrogeochemical, and isotopic evidence would result in a clearer conceptual model of gas generation and migration in West Macedonia.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and does the work have academic merit?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Geochimie et production de l'hydrogène naturel, migration et exploration.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 05 Dec 2025

Pavlos Tyrologou

Response to report 1 Review of Tyrologou et al., Manuscript

General Assessment
The manuscript presents an integrated study on gaseous systems (H_2, He, CH_4, and CO_2) in Western Macedonia, combining structural geology, sedimentology, and geochemistry. This multidisciplinary approach is a major strength of the paper, as it provides valuable insights into the mechanisms controlling gas generation, migration, and trapping. The authors provide an extensive new geochemical dataset (gas chromatography, inductively coupled plasma, and isotope analyses) from both water and gas samples collected at multiple hydrothermal sources, which constitutes a significant contribution to the understanding of natural hydrogen systems.
Response: We thank the reviewers for their comments and feedback on the scientific concept of the manuscript. The authors truly appreciate the devoted volunteer time of the reviewer to support the rigorous process of scientific correction.

However, despite the richness of the data, the manuscript lacks clarity and focus. The research question and logical thread of the paper are often difficult to follow due to structural issues, redundancies, and digressions into socio-economic and political topics that are not directly relevant to a scientific article. The paper would benefit from a thorough reorganization following a classical structure (Introduction, Geological Context, Methods, Results, Discussion, and Conclusion). The clarity, conciseness, and scientific rigor of each section should be enhanced to help the reader clearly understand the problem, the methodology, and the conclusions.
Response: We thank the reviewers for their comments. The manuscript has now restructured along the suggested structure – Introduction, Geological Context, Methods, Results, Discussion and Conclusion.

Major Comments
1. Introduction
The Introduction is overly long and fragmented, which prevents a clear understanding

of the main research question. It should be condensed into a coherent scientific narrative that succinctly states the problem, its significance, and the context of natural hydrogen. Political and environmental aspects should be summarized in two or three lines at most.
The manuscript would benefit from a clearer definition of its scientific novelty. While it compiles valuable field and isotopic data, the research question remains vague. The authors should explicitly state their hypothesis and objectives at the end of the Introduction to help readers understand the study's purpose.
Some statements are unclear or inaccurate; for example, 'Natural hydrogen mechanisms are still under development' should be rephrased as 'The understanding of natural hydrogen generation mechanisms is still under investigation.' The section 'Current State of the Art' lacks a detailed discussion of hydrogen production mechanisms and diverges into unrelated topics. It should focus on generation processes relevant to the study area and cite up-to-date references.
Response: Many thanks for your comments. The introductions has now been simplified and significantly shortened about political and environmental aspects to three lines . The introduction and current state of the art of natural hydrogen have merged into one. 'Natural hydrogen mechanisms are still under development' was rephrased as 'The understanding of natural hydrogen generation mechanisms is still under investigation.' A hypothesis and objectives have been explicitly stated together with the objectives. On the interest of saving space and keeping introduction short as requested by the reviewers, only the mechanisms of natural hydrogen are provided with the relevant references to the reader's interest. Providing more detail on the natural mechanisms for hydrogen will render the manuscript onto the review domain. The references provided have been updated and include additional newest ones where it makes sense, however the original ones kept as are still exceptionally valid.

2. Geological Context
The geological description is detailed and thorough, but overly extensive. Many details are not directly relevant to the research question and tend to obscure the main message. The section should focus on key aspects such as source rocks, basin thickness, structural controls (faults) on gas migration, and potential reservoir rocks. The authors should merge 'Previous research related to West Macedonia and neighbouring areas' with 'Geological setting' to avoid repetition. Maintain a logical flow—start with regional geology, then focus on specific study zones relevant to the presented data.
Response: We thank the reviewers for their comments. The sections now have been merged, and repetitions have been identified and deleted accordingly. The sections are now restructured to reflect the flow from supra regional to regional to local geology based on the reviewer's recommendations. **Clarify whether the study concerns West Macedonia or Greece, as this point remains ambiguous. Response:** We thank the reviewers for their comments. A specific phrase has been added in the hypothesis section in the introduction clarifying that West Macedonia is the focus of the study.
Figures 1–5 include excessive descriptive geological information. Simplifying these or adding a conceptual schematic linking tectonic setting, source rocks (ophiolites, lignites, carbonates), and migration pathways would greatly improve readability.
Response: Many thanks for your comment. Figure 1 and 3 have been deleted; a conceptual

schematic is provided in figure 9 with the remaining figures **3. Materials and Methods**
The methodology is comprehensive but overly detailed. Only essential information should be provided—sample types, volumes, sampling conditions, and analytical tools. Avoid unnecessary procedural details such as 'The values of the latter were recorded in field books or digital devices.' Response: Many thanks for your comment. The section has been cleaned of overly detailed procedural details, while ensuring that methods are described in sufficient detail for others to repeat the research in accordance with the Open Research Europe requirements. **Figures 6–8 showing sampling steps could be moved to Supplementary Material.**

Simplify over-detailed methodological descriptions and remove non-scientific instructions (e.g., 'practice a lot before commencing the sampling process,' 'check under daylight for bubbles'). Provide details relevant to the analytical precision, such as GC column type, detection limits, and isotopic accuracy.
Response: Many thanks for your comments. The section has been cleaned of over-detailed procedural details, including non-scientific instructions. GC column type and detection limits for the gas analysis were added. We have kept Figures 6-8 to provide a reference for future readers who want to reproduce the methodology. We have provided and clarified the type of GC column, gas analysis detection limits and description of the isotopic precision (reproducibility) and accuracy (treacability) for all stable isotope measurements quantifying precision via one standard deviation values and ensuring accuracy through normalisation against international standards of SMOW, VSMOW2 and SLAP2 V-PDB. The analysis for He isotopes is also quantified, with its total analytical error, encompassing both precision and accuracy, stated as generally less than 1%. We understand isotopic accuracy on the basis of which international standard was used and which Certified Reference Materials (CRMs) were used for calibration with a statement on the final quantified uncertainty (± value) on the reported δ values. Regarding gas detection limits for H2 we have modified the text in various places accordingly.

4. Results
The Results section is rich but should focus solely on presenting data without interpretation. Interpretations belong in the Discussion. Values should be expressed as ranges with appropriate units, and isotopic notation must follow standard conventions ($\delta^{13}C–CH_4$, $\delta D–CH_4$, $\delta D–H_2$, $\delta D–H_2O$, $\delta^{18}O–H_2O$).
Response: We thank the reviewers for their comments**.** We agree that, according to conventional scientific structure, interpretations should be presented primarily in the Discussion. However, given the complexity of the geochemical and isotopic datasets, we retained minimal and factual commentary within the results section to help guide the reader and enhance clarity. This includes brief contextualisation but avoids extensive interpretation or mechanism discussion. Nevertheless, we have reviewed the section again and relocated more detailed interpretations to the Discussion, in line with the reviewer's suggestion, while preserving clarity and narrative flow. Isotopic notations follow standard conventions ($\delta^{13}C_{CH_4}$, δD_{CH_4}, δD_{H_2}, δD_{H_2O}, $\delta^{18}O_{H_2O}$) including Figure 9.

The link between data and interpretation should be strengthened. Rather than listing numerous values, summarize and highlight key geochemical and isotopic trends (e.g., $\delta D–\delta^{18}O$ correlations, spatial variations in CH_4/H_2/He) and discuss their geological

implications.
Response: We thank the reviewer for their comments. A methane genetic diagram has been added to visualise results for Katakali and Kivotos.

Remove irrelevant details such as water potability discussions. Ensure tables contain measurement units. Clarify the purpose of blind samples—specify whether they served as analytical blanks or matrix controls—and verify the consistency of repeated samples (e.g., 'Katakali-2' and 'Kivotos-2').
Response: Many thanks for your comments. Certain details have been omitted to facilitate further the flow of the text. Text has been added to clarify the purpose of blind samples. Tables have been improved and contain measurement units. We clarify that 'Katakali-2' and 'Kivotos-2' are not analytical duplicates but independent field duplicates, i.e., separately collected samples from the same spring location. These were analysed in parallel to assess site-level variability. $\delta^{13}C_{CH_4}$ values showed good agreement for Katakali (–69.5‰ vs –69.8‰), but $\delta^{2}H_{CH_4}$ differed by ~21‰. Kivotos showed greater variability (6.0‰ in $\delta^{13}C_{CH_4}$ and 15.0‰ in $\delta^{2}H_{CH_4}$), likely reflecting local heterogeneity rather than analytical error. A clarifying paragraph has been added before Table 8.

5. Discussion
The Discussion lacks structure and clarity. It should be reorganized into subsections addressing specific scientific questions such as gas sources, migration pathways, trapping mechanisms, and implications. The discussion should be hypothesis-driven, directly linking interpretations to results.
Response: We duly noted the reviewer's comments. These made us aware of the confusion, and we have now restructured the discussion section based on the scientific hypothesis, combined with the structure pointed out by the reviewer.

Currently, some parts of the Discussion are embedded in the Results section. These should be consolidated.
Response: Many thanks for your comment. Please see our answer above. The results section has been reorganised. Some minor interpretative text remains to facilitate the argument development as well as to help guide the reader and enhance clarity.

Address the noted inconsistency: the manuscript claims that 'low silica enhances H_2 generation' but also that 'low silica can be indicated by magnetite.' In ultramafic contexts, magnetite is an indicator of serpentinization and thus hydrogen generation. Clarify this contradiction.
Response: We thank the reviewer for their comments and support in the rigorous scientific peer-review correction process. We acknowledge that the sentence structure is confusing and have improved it for clarity and corrected it accordingly to reflect the proper H_2 generation conditions. The section has also been enhanced with the addition of scientific references.

6. Minor, Technical, and Specific Comments
6.1 General Technical Corrections
- Ensure isotopic notation ($\delta^{13}C–CH_4$, $\delta D–H_2O$, $\delta^{18}O–H_2O$) is consistently formatted

using correct Greek symbols and superscripts (use 'O' not zero). Response: Amended where needed
- Verify typographic consistency for isotopic symbols ($\delta^{13}C$, δD, $\delta^{18}O$). Response: Amended where needed **- Include measurement units consistently across all tables, particularly for δ-values and gas concentrations. Response:** Amended where needed **- Correct typographical errors such as '1..22' in Table 5 (double dot under dissolved O_2). Response:** Corrected

Condense figures and tables to highlight only essential data; move over-descriptive figures to Supplementary Material.
Response: Many thanks for your comments. We believe that the current figures are essential for guiding the reader through the key geochemical and isotopic interpretations presented in the main text. They provide critical visual support for understanding the spatial and isotopic relationships discussed, and removing them may hinder the clarity and coherence of the narrative.

Clarify the expression 'public indications' (Page 10).
Response: Phrase was omitted as it provides the same meaning as the b) anecdotal data **-**

Use consistent terminology: distinguish between 'potential gas-bearing formations' and 'storage reservoirs.'
Response: Many thanks for your comments. We acknowledge the difference between the engineered or naturally suitable formations to inject and store gas such as CO_2 and the geological formations that naturally generate or contain gas. The ambiguity arose as the text described how the research evolved from the Pilot Strategy, a carbon capture and storage project. The ambiguity is resolved with the reorganisation of the text and the provision of a definition in the introductory section

- **Replace outdated or irrelevant references with more recent sources.**
Response: Thanks for your comments. We acknowledge the importance of incorporating recent literature and have reviewed the reference list accordingly. However, several of the cited long established sources remain foundational in the field and continue to be widely referenced by recent publications. Their inclusion ensures a robust conceptual framework and historical context, particularly where newer studies build directly upon or reaffirm these earlier works. Where appropriate, we have also cross-checked and added complementary recent references to demonstrate continuity and relevance with current scientific understanding. -

Rephrase ambiguous or awkward sentences for clarity, e.g., 'Hydrogen is highly reactive… preservation throughout migration is a risk' → 'Hydrogen is highly reactive and susceptible to redox conditions, making long-term preservation during migration challenging.'
Response: The authors are obliged by the constructive support of the reviewer and his thorough reading of the manuscript. We have rephrased as it was pointed out.

- Rephrase 'This is also making sure…' → 'This is also to make sure⋯'
Response: Corrected

- Replace 'deploying natural hydrogen in the EU' with 'establishing a natural hydrogen economy in the EU.'
Response: Many thanks for your suggestion, the phrase was deleted as part of the section shortening.

- **Figures 6–8 show overly detailed sampling equipment. Consider moving these to Supplementary Material to streamline the main text.**
Response: Many thanks for your comments and we appreciate the reviewer's suggestion to streamline the manuscript by relocating Figures 6–8 to the Supplementary Material. We respectfully believe that retaining these figures in the main body adds value by clearly illustrating the field methodology, which is essential for transparency and reproducibility, even if this sampling equipment is standardised. These visuals also enhance accessibility for readers who are less familiar with the equipment or protocols used in gas and water sampling.

- Clarify whether 'Katakali-2' and 'Kivotos-2' samples are duplicates or replicate analyses. Explain the rationale for using 'blind samples'—was bottled water used as an analytical blank or matrix control?
Response: Many thanks for your comments. The question remark has been clarified in the text and has also been answered in an aforementioned remark. We used two independent samples per site, taken minutes apart, to assess spatial or micro-scale variability within the same system. These are not laboratory duplicates or analytical replicates. An explanatory text has been added in the manuscript.

6.2 Page-Specific Remarks
Page 4, paragraph 5: Rephrase 'Natural hydrogen mechanisms...' as 'The understanding of natural hydrogen generation mechanisms is still under development.'
Response: Many thanks for your comments., text has been ammended

Page 4, paragraph 6: Adjust preservation and EU hydrogen economy phrasing as above.
Response: We thank the reviewer for their comments , the phrase was deleted as part of the section shortening and restructuring.

Page 5, paragraph 6: Clarify the contradiction regarding silica content and H_2 generation mechanisms.
Response: We acknowledge that the sentence structure is confusing and have improved it for clarity and corrected it accordingly to reflect the proper H2 generation conditions. The section has also been enhanced with the addition of scientific references.

Page 10, paragraph 4: Differentiate between CO_2 storage and natural H_2 leakage—surface leaks may indicate deeper accumulations.
Response: We thank the reviewer for their comment, which has strengthened the manuscript's scientific credibility. We have differentiated between CO_2 storage and hydrogen mobility based on their solubility properties. Text has been added in the

corresponding paragraph for further clarification.

Page 10, paragraph 4: Clarify what is meant by 'public indications.'
Response: Phrase was omitted as it provides the same meaning as the b) anecdotal data

Page 22, paragraph 2: Discuss the robustness of measurements given the small He/H_2 concentrations and analytical uncertainty.
Response: We thank the reviewer for this important observation. Hydrogen and helium were analysed by gas chromatography (Perkin Elmer Clarus 500 equipped with a double Carboxen 1000 packed column system and TCD–FID detectors), using Ar as the carrier gas. The TCD detection limit for H_2 and He is 1–10 μmol/mol (ppmv). The H_2 values reported are near the lower end of this range, and we acknowledge that such low concentrations require cautious interpretation. We still believe that the H_2 hold value since they were reproducibly detected at the same locations across multiple campaigns apart from us and show spatial coherence with ultramafic lithologies, major fault systems, and independent geochemical indicators such as alkaline pH, elevated Li–B, and reducing Fe–Mn conditions. We have added a new paragraph in the Discussion clarifying these points and acknowledging the limitations while demonstrating why the H_2 signals remain valid for interpretation. We appreciate the reviewer's comment, which has improved the transparency of our dataset interpretation.

Tables 2–5: Ensure δD–H_2O uses the letter O (not zero).
Response: Ammended

Overall Conclusion
Overall, the study provides valuable geochemical and isotopic data from a poorly documented region. However, its scientific impact would increase significantly if the manuscript were restructured around its core research questions, with redundant methodological details condensed. A stronger integration of geological, hydrogeochemical, and isotopic evidence would result in a clearer conceptual model of gas generation and migration in West Macedonia.
Response: We thank the reviewer for the constructive feedback and his commitment to the scientific review process. The manuscript was revised and restructured accordingly.

Is the work clearly and accurately presented and does it cite the current literature? No – **Response:** Thanks for your comments and we partially agree on the work that needs enhancement and needs to be clearer as it was described previously. The manuscript benefits from a very comprehensive literature review, we have included more references and we remain open to any additional literature that we may have missed by not being aware. Foundational sources are retained because they remain central in the current scientific literature, and more recent studies are built directly upon them. While every effort has been made to engage with the most relevant and up-to-date scientific literature, it is important to acknowledge a structural limitation inherent to the current scholarly landscape. A significant proportion of research outputs remains behind paywalls, as many authors publish in subscription-based journals. Access to these works is therefore restricted and not uniformly available across institutions or research teams, creating an unavoidable disparity in information accessibility. This manuscript reflects the fullest integration of all

literature that was accessible through institutional subscriptions, open-access platforms, and publicly available databases at the time of writing. The authors strongly support the principles of open science, as unrestricted access to scientific publications and data is essential for ensuring equitable participation in research, fostering transparency, and accelerating scientific progress.

Competing Interests: No competing interests were disclosed.