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Subsurface advective flow unveils the architecture of Earth's crustal biosphere

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Perspective

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Abstract

The subsurface biosphere is one of Earth’s largest microbial reservoirs, yet its spatial extent remains poorly constrained due to limited direct access. Here we propose using the integrated environmental readouts encoded in deep subsurface fluids to shift from simply mapping where life can be detected to constraining where life can exist. Deeply-sourced advective fluids provide an integrated signal of subsurface microbial processes, recording the combined effects of heat flow, water–rock interaction, volatile inputs, and microbial activity along fluid pathways. By coupling surface measurements of fluid temperature, geochemistry, and volatile provenance embedded in diverse tectonic settings, we can define the maximum theoretical depth of the habitable crust and constrain the degree of surface overprint. This framework enables comparisons across geological settings and scales that are otherwise inaccessible, linking subsurface habitability to crustal structure and dynamics. It provides a quantitative basis to evaluate the extent of Earth’s subsurface biosphere from the surface, and to extend these principles to other planetary bodies.

Keywords: Subsurface biosphere, habitable zone, hot springs, advecting fluids, deeply-sourced seeps

Main Text

Earth’s subsurface hosts one of the largest reservoirs of microbial life, comprising an estimated 3×10^{29} and 6×10^{29} cells in the marine and terrestrial domains, respectively[1,2]. This biosphere spans a wide range of geological environments, including sediments, hydrothermal systems, aquifers, subduction zones, volcanic provinces, deep faults, and spreading centers, and sustains substantial phylogenetic and metabolic diversity[3,4]. Subsurface microorganisms drive key biogeochemical cycles, regulate redox transformations in the crust, and mediate exchanges between surface and deep Earth reservoirs[5–8]. These processes are directly relevant to energy storage, carbon sequestration, and the long-term evolution of planetary habitability[9–12].

Despite its significance, the physical extent of the subsurface biosphere—particularly its depth distribution across geological settings—remains poorly constrained. Current knowledge derives from a limited number of boreholes and mines, which provide high-resolution but spatially restricted observations[1,2,13]. As a result, the subsurface is sampled as isolated points rather than as a connected system, preventing a coherent description of the habitable volume of the crust. Deeply-sourced seeps provide an alternative mode of access to subsurface microbial habitats[14]. These systems transport fluids, gases, and microbial biomass from depth to the surface, integrating signals acquired along subsurface flow paths[14–17]. The chemical and biological properties of these fluids encode the combined effects of heat flow, water-rock interaction, volatile inputs, and microbial activity during ascent. For this reason, fluids sampled at the surface reflect the structure of the subsurface through which they have traveled rather than approximating discrete depth horizons. As such, we suggest that they become valuable indicators of the extent of biologically habitable space in Earth’s subsurface.

In spite of the information they provide, advecting fluids often reach the surface from unknown depths and follow complex flow paths. We propose that by linking surface measurements of deeply-sourced fluids to the geological context it is possible to infer the upper bound of subsurface habitability depth across diverse geological settings, effectively providing an estimate of the maximum theoretical depth for which the fluids provide biological information. This approach constitutes a scalable basis to resolve the depth and architecture of the subsurface biosphere, guide sampling design and compare systems that are otherwise inaccessible to direct observation.

Deeply-sourced seeps as a window to reconstruct subsurface habitats

Advective subsurface fluids provide indirect but scalable access to the deep biosphere by transporting dissolved compounds, volatiles, and microbial cells from depth to the surface[14,16–18]. Unlike boreholes and mines, which sample discrete points, these systems integrate material along subsurface flow paths, extending observations across spatial scales that are otherwise inaccessible[14]. Advective fluids have been proposed as windows into the subsurface biosphere since the early 1990s[16], and recent work has demonstrated their utility in resolving large-scale geosphere–biosphere interactions across diverse geological settings[19–25]. We collectively define advective fluid systems as "deeply-sourced seeps" *sensu* Giovannelli et al. (2022). This definition extends beyond the conventional focus on high-temperature hydrothermal systems as temperature alone does not capture the relevance of a seep to subsurface processes. Samples as low as 14°C have been demonstrated to be highly relevant subsurface fluids, based on mantle gas contributions and ^{14}C measurements[25]. In many cases, lower-temperature outlets provide more informative windows into subsurface circulation, as they reflect fluids that have cooled, mixed, and emerged under conditions compatible with sustained biological activity[17,18,20–23,26]. Restricting sampling to conspicuously hot systems introduces a systematic bias toward a limited thermal (and potentially shallow) subset of subsurface environments. Deeply-sourced seeps are therefore defined by their connection to subsurface circulation rather than by temperature, morphology, or visual characteristics. They provide a unifying framework for environments often treated separately, linked by their common role as conduits of subsurface-derived material to the surface[14].

The water and volatile phases in deeply-sourced seeps can have diverse and overlapping origins (Fig. 1). Fluids may derive from meteoric recharge or seawater recirculation, or be sourced directly from the subsurface through magmatic degassing, mineral dehydration or unconventional water formation[27] (Fig. 1C). Heat flow drives circulation, enabling fluids to penetrate to variable depths where they interact with crustal rocks, subducted materials, and mantle-derived volatiles (Fig. 1A–F). During this residence, fluid composition is progressively modified through water–rock interaction, buffering reactions, and volatile exchange, which together define pH, redox state, and chemical composition. As fluids ascend, these deep signatures are further modified by phase separation, degassing, water–rock interaction during ascent and mixing with shallow waters and atmospheric components (Fig. 1F–G). As a result, fluids emerging at the surface do not represent discrete depth horizons or single geochemical environments. They represent integrated signals of the pathways through which they have traveled. In this framing, deeply-sourced fluids do more than just provide access to subsurface samples, they encode it by integrating the thermal structure, fluid residence time, lithological interactions, volatile inputs, and microbial processes that define the architecture of the habitable crust.

This integrative nature is evident in both geochemical and biological observations. Geochemical tracers such as noble gas systematics, carbon isotopes, and fluid chemistry and water isotopes constrain the relative contributions of deep and shallow sources[28–30]. At the same time, microbial communities recovered from deeply-derived fluids frequently include metabolisms that would not co-occur within a single geochemical environment, such as obligate aerobes and anaerobes or simultaneous signatures of methanogenesis and methanotrophy[21,26,31,32]. These assemblages are more parsimoniously explained as the result of integration across spatially distinct niches rather than coexistence within a single habitat. The implication is that deeply-sourced seeps should not be interpreted as direct samples of subsurface environments as they existed *in situ*, but as structured outputs of integrated subsurface processes. The analytical objective is therefore to resolve the constraints imposed by the range of conditions encountered along fluid pathways.

Within this framework, deeply-sourced seeps become an observable vertical integration of the subsurface biosphere. Each seep provides access not to a specific depth, but to the architecture of the subsurface. By resolving the processes encoded in advecting fluids, it becomes possible to constrain the physical and thermodynamic boundaries within which life can be sustained, and to infer the first order upper bound of subsurface habitability as a function of geological setting.

Drivers and controls on the depth of the subsurface biosphere

The depth of the subsurface biosphere is not a fixed property of the crust, but an emergent constraint set by the interaction between thermal structure, fluid circulation, and rock properties. These factors define the physical and thermodynamic boundaries within which microbial life can be sustained. Temperature provides a first-order limit[4]. The geothermal gradient, controlled by regional heat flow and geological setting, defines the depth at which temperatures exceed the currently observed upper bounds for life[2]. Microbial growth has been demonstrated up to 122 °C[4,33], providing a practical constraint on the maximum habitable temperature. However, this boundary is not uniform with depth. Variations in heat flow across tectonic settings produce large differences in the depths at which this thermal limit is reached, from shallow systems in high heat flow regions to substantially deeper habitable zones in cooler crust[34]. In addition, thermal structure in permeable crust is rarely purely conductive. Advective circulation redistributes heat along preferential flow paths, generating strong local departures from regional conductive gradients[35,36]. As a result, conductive heat flow models provide a useful first-order approximation of habitable depth at regional scale, but may substantially underestimate biologically accessible space at the local scale, particularly in hydrothermal, volcanic, and fault-controlled systems where convective circulation dominates heat transport.

Physical structure imposes a second constraint. With increasing depth, compaction and mineral precipitation reduce porosity and permeability, limiting the availability of connected pore space required for fluid circulation and nutrient transport[37,38]. The subsurface biosphere is therefore restricted not only by temperature, but by the persistence of a connected fluid network that enables metabolic exchange. This constraint is inherently geological, depending on lithology, deformation, and fluid pressure regimes. Fluid circulation links these constraints by coupling thermal structure, permeability, and energy availability. Advective transport redistributes heat, solutes, and electron donors and acceptors, effectively coupling thermal and chemical structure along permeable pathways, producing habitable environments that may deviate substantially from conductive thermal predictions alone. As a result, habitability is not solely a function of depth, but of connectivity within the flow network. Regions of the crust that are thermally permissive for life but are hydraulically isolated may remain uninhabitable, while deeper zones may remain viable where fluid flow maintains connectivity and energy supply.

These constraints are organized by geological setting. Convergent margins, volcanic systems, rifts, and fault-dominated regions differ systematically in heat flow, volatile sources, permeability structure, and fluid residence time (Fig. 2). Along subduction systems, for example, variations in slab-derived inputs, mantle contributions, and crustal interaction generate strong gradients in temperature, pH, and redox conditions from forearc to arc settings [20–23]. Volcanic systems exhibit contrasting regimes between conduit-proximal environments, where heat flow is high and habitable space is compressed, and flanks or calderas, where cooler conditions and longer residence times allow for expanded habitable zones [24,32,39,40]. In contrast, deep faults and off-axis ridge systems may sustain lower temperatures but maintain deep circulation pathways, extending the potential depth of habitable environments and with that, the variety of niches to be accessed[17,20,41,42].

Within this framework, the depth of the subsurface biosphere is best understood as a first order upper bound defined by the intersection of thermal limits, permeability structure, and fluid accessibility. This bound is not directly observable, but can be predicted from observables related to the geological setting (Fig. 2) and constrained through the integrated signals carried by advecting fluids[14]. Surface measurements of temperature, fluid composition, and volatile provenance reflect the conditions encountered along flow paths, and therefore encode information about the full depth and range of environments accessed by the circulating system.

The problem is therefore not to measure the depth of life directly, but to constrain the environmental limits within which it can exist. By linking fluid-derived observables to geological controls, it becomes possible to estimate the depth of the habitable subsurface as a function of geological setting, and to compare these limits across diverse systems that are otherwise inaccessible to direct sampling.

Predicting the extent of the subsurface biosphere

The framework outlined above can be used to constrain the extent of the subsurface biosphere by linking surface measurements of advecting fluids to the geological and thermodynamic controls that define habitability. The objective is not to reconstruct specific subsurface environments, but to estimate the upper bound of habitable space accessible within a given system, guiding experimental design, sampling efforts and data interpretation.

This requires combining independent observables that encode complementary aspects of subsurface structure. A priori and in the field assessment of basic parameters can provide substantial information on the maximum depth of the subsurface biosphere and expected microbial groups and functions. Volatile provenance (from existing literature and from predictions based on nearby measurements), type of sampled feature (fumarole, mud pot, spring, shallow-vent, etc…) and geological setting can be assessed at the planning stage. Fluid temperature, aqueous geochemistry, including pH, redox-sensitive species, major ion composition and gas composition, can be obtained in the field or in the lab to complete the assessment and provide stronger constraints

The Italian peninsula provides an ideal natural laboratory to apply this approach because it combines strong tectonic heterogeneity, abundant deeply-sourced yet accessible seeps, and extensive independent subsurface observations within a relatively compact area (Fig. 3). Italy encompasses convergent margin systems, active volcanic provinces, extensional fault networks, foreland basins, and localized high heat flow regions, producing strong lateral gradients in thermal structure, volatile provenance, and fluid circulation depth across the peninsula. At regional scale, geological structure and conductive heat flow datasets already provide a first-order prediction of the expected depth and architecture of the subsurface biosphere (Fig. 3A–B). High heat flow volcanic and extensional provinces are expected to host thermally compressed habitable zones, whereas cooler foreland and peripheral regions may sustain substantially deeper habitable domains. Similarly, tectonically active fault systems are predicted to enhance deep fluid accessibility by maintaining crustal permeability and advective circulation pathways.

The distribution of seep systems and deep wells across Italy (Fig. 3C) offers a unique opportunity to compare inferred habitable structure against independently accessible subsurface environments. Surface measurements of fluid temperature, volatile provenance, aqueous geochemistry, and microbial composition can therefore be interpreted within a predictive geological framework to estimate the maximum extent of biologically accessible crustal space across different tectonic settings. For example, deeply-sourced seeps located with areas of high heatflow and high temperature activity (Figure 3), such as the Campi Flegrei volcano and the Aeolian Island of Vulcano, represent locations where the subsurface habitable space is compressed toward the surface (Fig. 2, see volcanoes, closed vent crater, slopes and calderas). In spite of the deep nature of the volatiles in these locations (confirmed by mantle influenced helium isotopes and deep CO_2 sources)[43,44], the microbial communities will be dominated by high temperature facultative anaerobic and microaerophilic species, often linked to thermophilic acidophiles archaea when in proximity of magmatic sources[20,24,32,39,45,46]. Sampling at these locations thus provides limited information regarding the subsurface microbial community simply because the maximum habitable depth is between a few to tens of meters. On the contrary, deeply-sourced seeps located in areas of low heat flow and away from active volcanoes (like for example the Central Appenine area or the Colli Euganei in Veneto region) represent locations where the subsurface habitable space might extend to high depth (hundreds of meters to a few km)(Fig. 3). Subsurface microbial communities in the fluids of similar locations are represented by mesophilic, anaerobic taxa commonly associated with deep subsurface communities accessed through deep drilling[17,20,22,23,41]. Similar constraints can be drawn for other locations globally where the microbial communities have been investigated in advective fluids, like Yellowstone[39,45], Argentina[47], Costa Rica[20,21,23], Peru[22], New Zealand[48] and can be applied to any location globally to guide sampling design and inform subsurface biosphere analysis.

Conclusion

Deeply-sourced seeps provide a scalable observational framework to constrain the architecture of Earth's subsurface biosphere from the surface. The maximum depth of the habitable crust sampled by seeps emerges from the geological structure. Heat flow, permeability, volatile transport, and fluid circulation define the volume of crust that remains thermally and hydraulically accessible to life. Different tectonic settings therefore impose fundamentally different architectures of habitability. Within this framework, geological priors such as heat flow, crustal structure, tectonic regime, and seep type provide first-order constraints on the expected depth and extent of the biosphere before direct sampling. This approach allows subsurface habitability to be treated as a predictable geological property of the crust. Surface observables can therefore be linked to the expected structure and extent of habitable space across regions and tectonic settings that remain inaccessible to direct observation, on Earth and potentially on other planetary bodies. A priori predictions of habitable volume and circulation depth may ultimately guide sampling strategies and analytical approaches designed to link accessible surface observations to inaccessible subsurface processes.

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Contributions

DG conceived the idea. Both authors equally contributed to the manuscript.

Ethics declarations

The authors declare no competing interest.

Tables and Figures

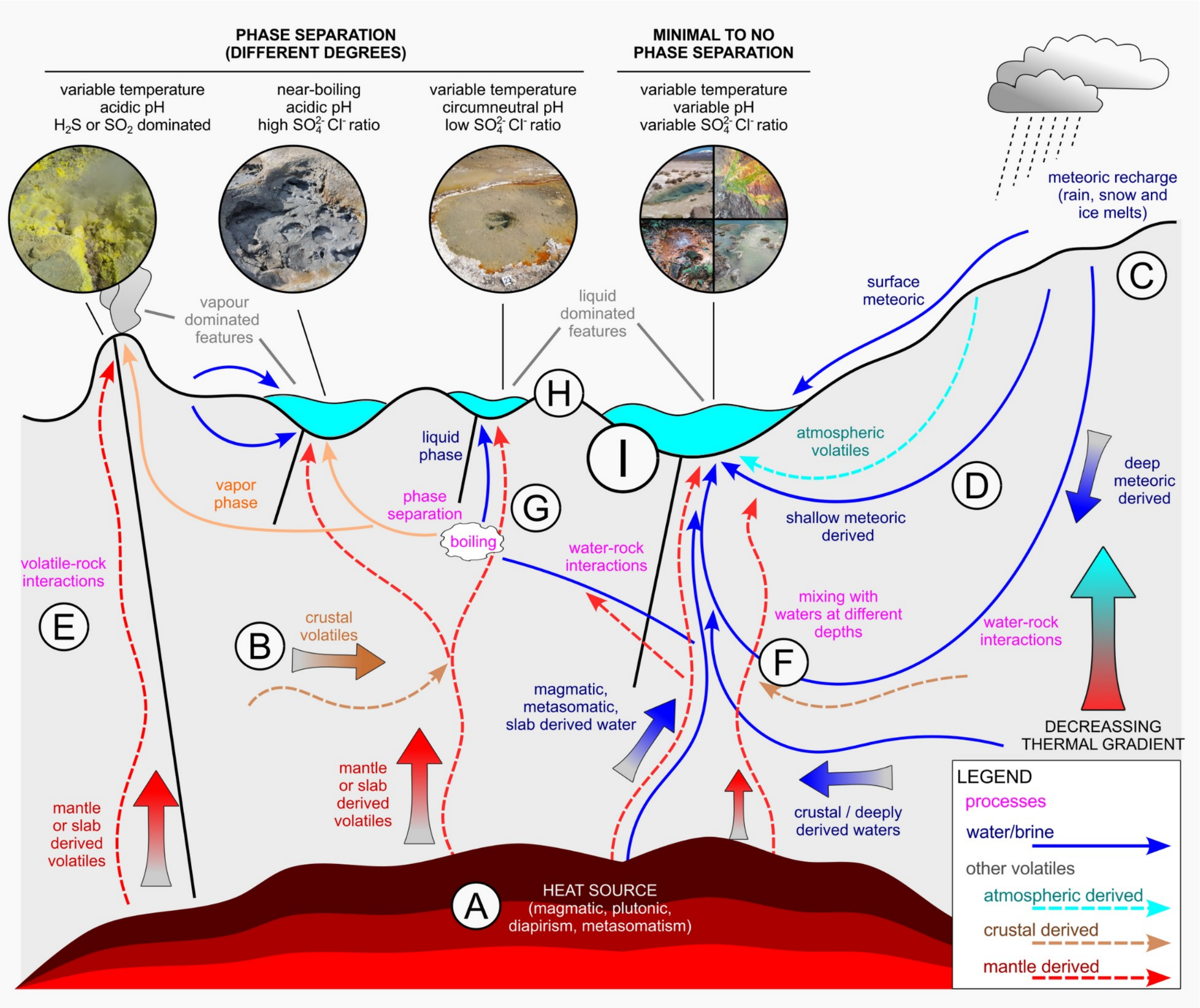

Fig. 1. Microbially-relevant geological processes shaping deeply-sourced fluids. (A) Heat sources associated with different tectonic settings establish the thermal gradients that drive subsurface fluid circulation. These also influence the type and contribution of deeply-derived volatiles entering the system. (B) Deep volatiles, including water and gas phases, may originate from mantle, crustal, or subducted sources and are often mixed during ascent. (C) Recharge fluids may derive from meteoric inputs (rain, snow, ice melt) or seawater, and infiltrate the subsurface to variable depths. (D) The depth of penetration, residence time, and interaction with host rocks determine the extent of chemical equilibration and the contribution of different volatile sources. (E) Water–rock interactions progressively modify fluid composition, including pH, redox state, and dissolved species. (F) Fluids originating from different sources may intersect and mix at multiple depths, generating complex compositional gradients along the flow path. (G) Phase

separation during ascent, driven by pressure decrease, further modifies fluid composition through boiling and gas loss. (H) Near-surface mixing with shallow waters and atmospheric inputs overprints deeper signatures. (I) Microbial activity both responds to and modifies these physicochemical conditions, contributing to the transformation of fluid composition in both subsurface and surface environments. Together, these processes produce the diversity of deeply-sourced seeps and define the integrated signal observed at discharge. Rather than representing discrete environments, these fluids encode the cumulative effects of processes operating along subsurface flow paths.

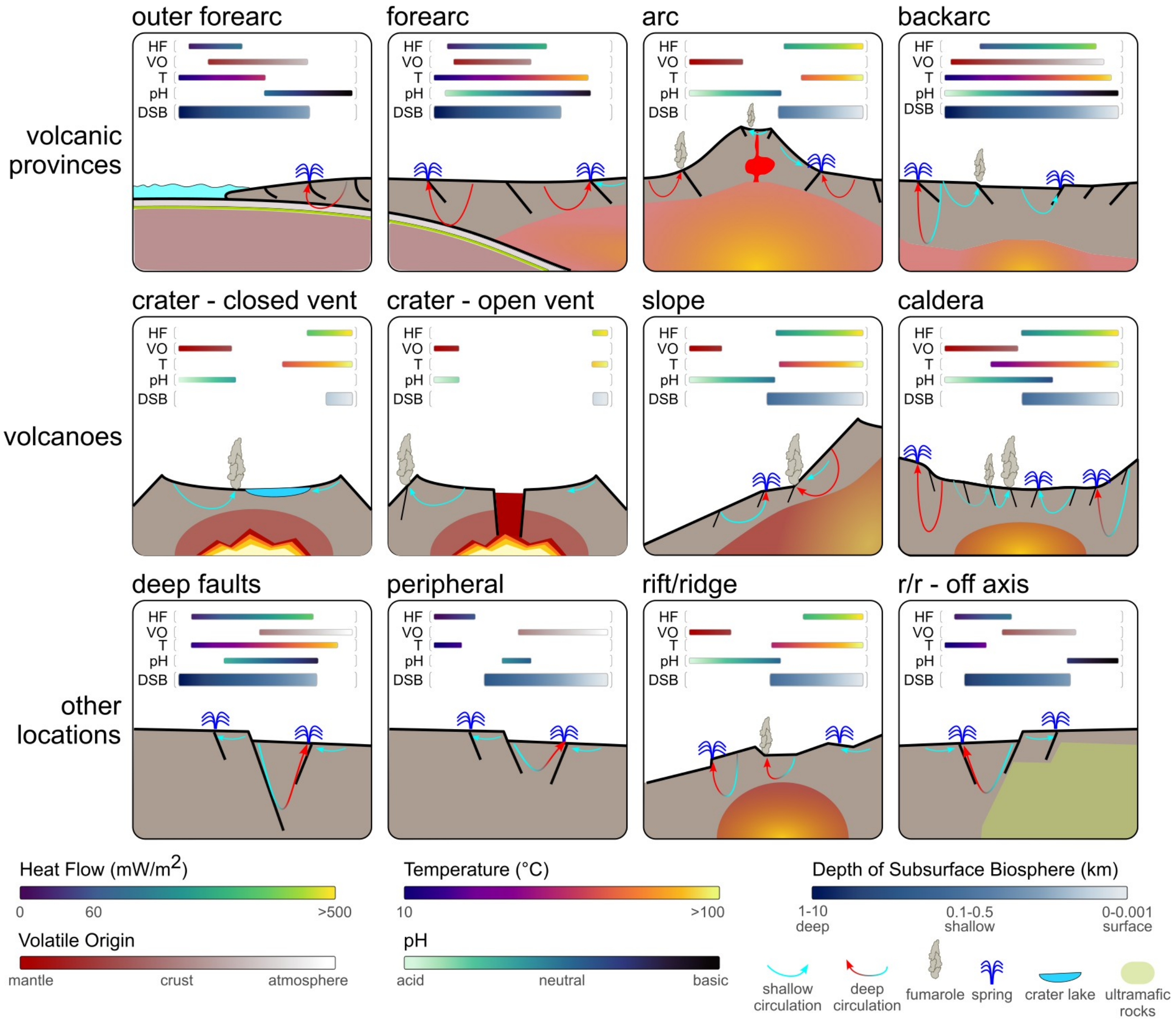

Figure 2. Geological controls on the inferred upper bound of subsurface habitability. The panels illustrate representative geological settings and the combinations of heat flow (HF), volatile sources, and fluid circulation that constrain subsurface habitability. Surface-measurable properties of deeply-sourced seeps, including temperature (T), pH, and volatile origin (VO), reflect these underlying controls and provide the basis to infer the upper bound of habitable space. This bound is not directly observed, but emerges from the interaction between thermal structure, permeability, and fluid accessibility. **Top row**: Convergent margin settings (outer forearc, forearc, arc, and backarc), characterized by systematic variations in slab-derived inputs, mantle contributions, and thermal structure. These gradients produce distinct constraints on fluid composition and circulation depth, resulting in laterally variable upper bounds of habitability. **Middle row**: Volcanic systems, including closed-vent and open-vent volcanoes, volcanic flanks,

and calderas. High heat flow near conduits compresses the habitable domain toward shallow depths, whereas flanks and caldera systems, with lower thermal gradients and longer fluid residence times, allow for a broader and potentially deeper habitable space. **Bottom row**: Fault-dominated and extensional systems, including deep fault zones, peripheral springs, and mid-ocean ridge settings (on- and off-axis). These environments are typically characterized by lower heat flow but sustained permeability and deep fluid circulation, which can extend the accessible habitable domain to greater depths. Across all settings, the depth of the subsurface biosphere is expressed as an inferred upper bound defined by the intersection of thermal limits and fluid-accessible space, rather than as a directly measurable property.

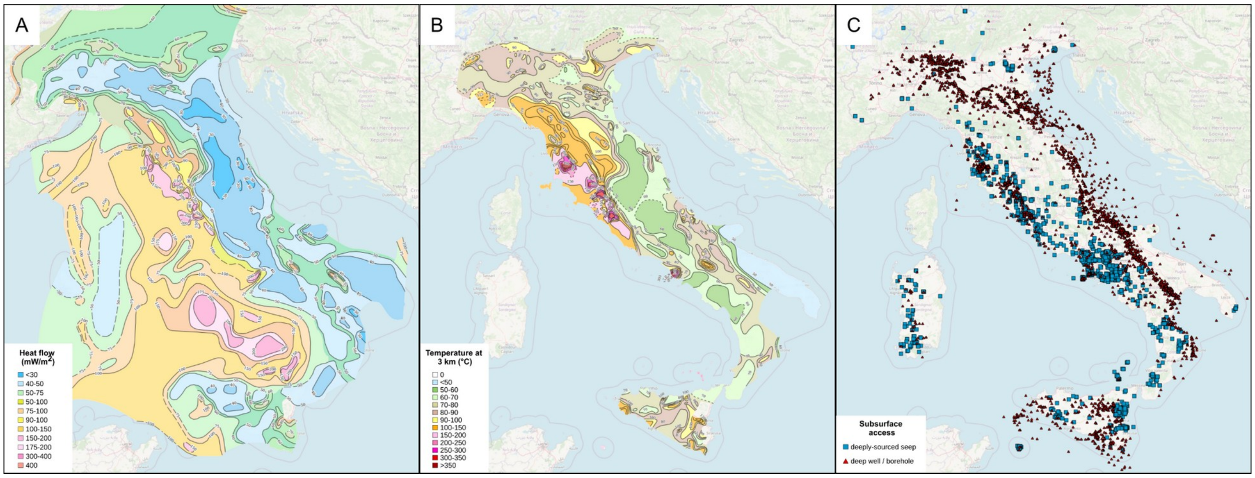

Figure 3. Example of constraining subsurface habitability from surface observables. (A) Spatial distribution of heat flow across Italy (in mW/m^2). (B) Temperature at depth (°C at 3 km) and isotherm distribution derived from geothermal data. (C) Locations of deeply-sourced seeps (blue) and deep wells (red). The co-occurrence of surface expressions and independently sampled subsurface data provides a natural test of the framework. Surface measurements of fluid temperature and composition can be used to infer the depth and extent of habitable conditions, which can then be evaluated against direct observations from wells. This approach illustrates how advecting fluids provide quantitative constraints on subsurface habitability at regional scale. Data was obtained from the GeoThopica 2.0 database (Istituto di Geoscienze e Georisorse - CNR, 2023, https://geothopica.igg.cnr.it).