

**Reply to RC1: 'Comment on egosphere-2026-1255', Anonymous Referee #1, 22 May 2026**

We sincerely thank the Referee for the valuable and constructive comments. We respond point by point below.

*This manuscript showed comparable results between four rapid approaches including aggregate stability (measured with SLAKES), soil respiration (measured with portable CO<sub>2</sub> analyzer), microbial biomass carbon (measured with microBIOMETER®) and enzyme activities (measured with Soil Enzymatic Activity Reader, SEAR), against standard laboratory measurements. Using 132 soil samples from long-term experimental multi-species pastures in Spain across 11 sampling campaigns, the author concludes that while these field tools show comparable overall trends, indicating them as complementary options in applications such as informing soil health assessments and management decisions, they exhibit weakness in capturing the relationship in soil respiration and the fungal-to-bacterial ratio. This study addresses a critical bottleneck (e.g., the time lag and cost of laboratory-based biological analyses) in practical soil health monitoring.*

*The manuscript is well-organized, relevant for practical management and within the scope of the journal. However, the reviews have comments below for authors' considerations before this manuscript can be accepted for publication.*

*1) In the introduction, the author explains the ecological relevance for each selected indicator. Could the author add the rationale for choosing these four as a representative set of soil health metrics?*

**ANSWER:** The rationale for selecting these four indicators was to evaluate a set of complementary soil health measurements that collectively capture different dimensions of soil functioning. Specifically, we included aggregate stability as an indicator integrating physical and biological processes, soil respiration and microbial biomass as indicators of biological activity and microbial abundance, and enzyme activities as indicators of soil biochemical functioning and nutrient cycling. An important criterion for selection was the availability of rapid methodologies that can be applied easily in the field or with minimal infrastructure requirements, without the need for specialized laboratory facilities and with the capacity to provide results within a short timeframe.

*2) In lines 41-42, the authors introduce in-situ respiration. Because field respiration varies based on immediate soil moisture and temperature on the day of testing, while lab methods standardize these variables through controlled incubation, please expand the Discussion to address this inherent decoupling. Do the authors recommend a specific environmental 'window' (e.g., specific moisture range or time of day) for land managers to use these rapid tools to ensure data is comparable?*

**ANSWER:** We thank the Referee for this important point. We will expand the Discussion to address the inherent decoupling between in-situ and laboratory respiration measurements. We will explicitly acknowledge that field respiration reflects actual microbial activity under prevailing conditions, and therefore cannot be directly compared to laboratory-standardized values in absolute terms. However, we argue that in-situ

measurements retain strong value as relative indicators of soil biological activity when collected under consistent conditions.

Based on established literature, we will recommend conducting in-situ respiration measurements within a defined environmental window: soil moisture around 60% of field capacity (Linn and Doran, 1984, <https://doi.org/10.2136/sssaj1984.03615995004800060013x>), soil temperature between 10–20°C (avoiding seasonal extremes; Lloyd and Taylor, 1994, <https://doi.org/10.2307/2389824>), and outside of 48–72 hours following intense rainfall events to avoid the Birch effect. Recording soil temperature and moisture at the time of measurement with low-cost sensors is strongly encouraged to enable post-hoc correction using established Q10 or moisture-response functions.

*3) Rapid tools are sensitive to environmental factors such as soil type, climate, land use, management history, PH and organic matter. The study demonstrates comparability within one long-term grassland experiment; however, the broader applicability of these rapid tools remains unclear. Could the author discuss the need for regional or soil-type-specific calibration datasets and reference ranges before these methods can be generalized across various climate and land use conditions?*

**ANSWER:** We fully agree that the performance of rapid soil health assessment tools may be influenced by environmental and edaphic factors. As correctly noted by the Referee, our study was conducted within a single long-term grassland experiment, and therefore the conclusions should be interpreted within the context of the conditions tested. The objective of this study was to provide an initial evaluation of the agreement between rapid and laboratory-based methods under a well-characterized experimental setting. We agree that broader validation across contrasting soils, climates, and land-use systems will be necessary before these approaches can be generalized. In particular, the development of regional or soil-type-specific calibration datasets and reference ranges may be required for some indicators to ensure robust interpretation across different environmental contexts. To acknowledge this limitation, we will expand the Conclusions section.

*4) In Lines 148–149, the authors state that higher aggregate stability was associated with deeper soil layers (20–50 cm). This finding is counterintuitive and seems to contradict the biological framework established in the Introduction (Lines 37–40), which notes that aggregates rely on biologically derived binding agents (which are concentrated in the 0–20 cm layer). Finding higher stability in the subsoil suggests that either: a) the SLAKES and wet-sieving methods are responding heavily to inherent subsoil mineralogy (e.g., high clay or calcium carbonate accumulation) rather than biological health, or b) there is an issue with data orientation. Could the authors expand the discussion to ecologically explain why aggregate stability increased with depth despite a significant decline in microbial biomass carbon and activity?*

**ANSWER:** Following this suggestion, we have further examined additional physicochemical soil properties not explicitly discussed in the original manuscript. These analyses indicate that clay content is a likely key driver of the observed increase in aggregate stability with depth. In particular, clay content shows a statistically significant

increase from 31.9% in the 0–20 cm layer to 37.7% in the 20–50 cm layer. Given the well-established role of clay particles in aggregate stabilization through physical protection and organo-mineral interactions, it provides a plausible explanation for the higher aggregate stability observed in subsoil layers, despite lower microbial biomass and activity. We will include this interpretation in the revised Discussion.

5) In Line 174 of the Conclusions, the authors state that rapid methods represent a complementary or alternative, to laboratory analyses. Given that two out of the four biological metrics tested (insitu respiration and F:B ratio) showed weak or statistically non-significant relationships with laboratory reference standards, using the word 'alternative' may be overstating their readiness. The review suggests softening this phrasing to emphasize that they are “valuable tools for dynamic trend tracking” rather than direct alternatives to standard laboratory quantifications.

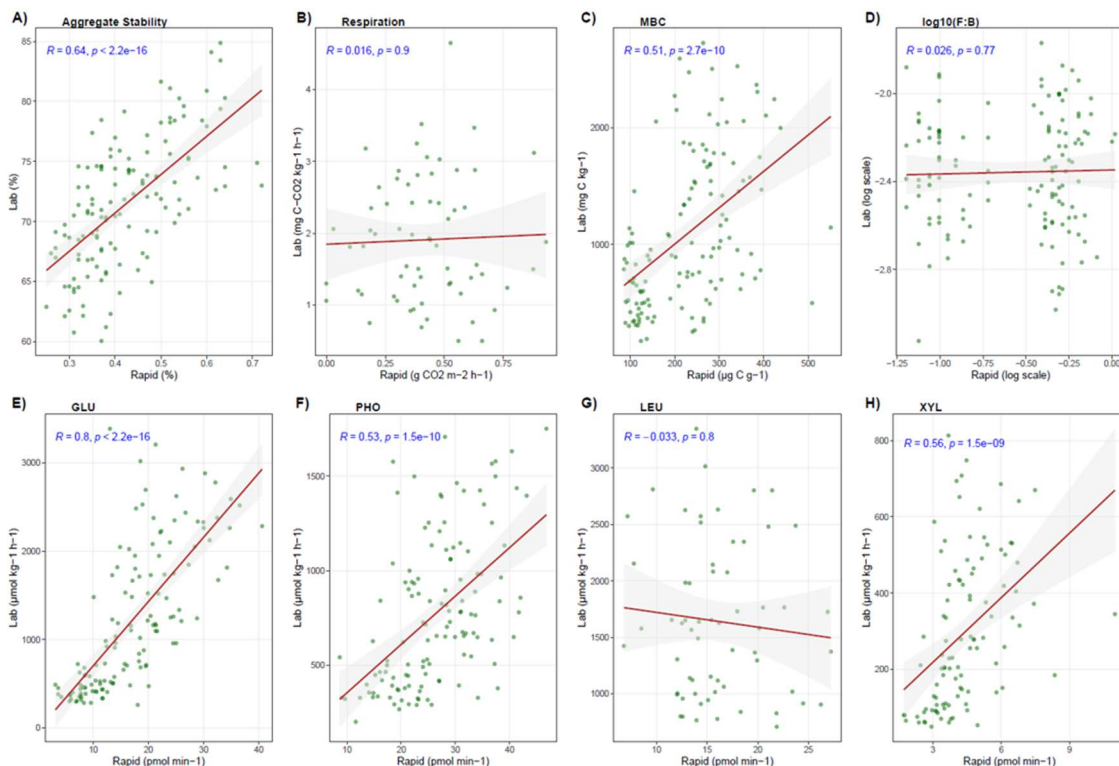
**ANSWER:** We agree with this comment and we will revise the text accordingly.

6) In line 74, please add the unit for variables.

**ANSWER:** We are not sure which variable the Referee is referring to. In the corresponding sentence, soil organic matter is already reported with its unit (%), whereas pH is a dimensionless parameter.

7) In figure 1, please put the x-label in inverse position.

**ANSWER:** We will rotate Figure 1 by 90 degrees to improve readability and visualization of the data.



*8) Regarding the FAIR principles for open science, the review recommends that the raw data can be archived in a dedicated, public data repository (such as Zenodo, Figshare, or the European Soil Data Centre - ESDAC) to ensure a permanent DOI and easier machine-readability. Furthermore, since the multivariate statistics (RDA and Procrustes analysis using the vegan package) are core to the paper's conclusions, the authors could make their R script publicly available (e.g., via a GitHub repository linked to Zenodo). This would allow other researchers to replicate the exact statistical workflows for validating these rapid methods in their own local regions.*

*In Supplement A, the authors should ensure that a comprehensive metadata codebook is included. Specifically, for the rapid tools (SLAKES, microBIOMETER®, and SEAR), please ensure the exact raw units, software version numbers (e.g., the specific version of the SLAKES mobile application used in 2024), and smartphone operating system or camera hardware details are explicitly noted. As rapid methodologies rely heavily on software algorithms that update over time, documenting these technical parameters is vital for the long-term reusability of the dataset by the global soil health community.*

**ANSWER:** We thank the Referee for this valuable suggestion. We have requested the deposition of the raw dataset (<https://doi.org/10.5281/zenodo.20525860>) in the Zenodo repository of the AI4SoilHealth project.

We will also include the R scripts, software versions and hardware specifications used. Specifically, SLAKES measurements were performed using version 1.3 of the application, while microbial measurements were obtained using the microBIOMETER® Classic Starter Kit and version 3.8 of the microBIOMETER® app. All image-based measurements were acquired using a Samsung Galaxy A33 5G smartphone equipped with a 48 MP rear camera. For SEAR, we will clarify that the device used in this study was a pre-commercial prototype undergoing field validation.