SOIL, 12, 1187–1194, 2026
https://doi.org/10.5194/soil-12-1187-2026
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Beyond the laboratory: rank concordance of rapid methodologies for soil health assessment

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Received: 6 March 2026 – Discussion started: 13 March 2026
Revised: 6 November 2026 – Accepted: 9 November 2026 – Published: 16 November 2026

Abstract. Soil health assessment increasingly relies on biological indicators because of their sensitivity and direct links to ecosystem functioning. However, conventional laboratory methods are time-consuming, require specialized infrastructure, and are often incompatible with rapid decision-making in applied contexts. Several rapid or field-deployable tools have recently been developed to address this limitation, but their ability to reproduce the relative ordering of samples obtained with laboratory methods remains insufficiently evaluated. Here, we compared four rapid approaches with their corresponding laboratory methods in a long-term grassland experiment: aggregate stability (SLAKES), soil respiration (portable CO_2 analyzer), microbial biomass carbon and fungal-to-bacterial ratio (microBIOMETER®), and enzyme activities (Soil Enzymatic Activity Reader, SEAR). Rank concordance between methods was assessed using Spearman correlations, partial redundancy analyses and Procrustes analysis. Aggregate stability showed strong rank concordance between rapid and laboratory measurements (median $\rho = 0.65$; range: 0.44–0.79), whereas soil respiration exhibited very weak rank concordance, likely reflecting that in situ and laboratory approaches capture different aspects of respiratory activity. Microbial biomass carbon displayed strong rank concordance between methods (median $\rho = 0.68$; range: 0.54–0.76), while fungal-to-bacterial ratios did not. Enzyme activities measured with SEAR generally reproduced the patterns observed with laboratory assays. Multivariate analyses indicated that, overall, rapid methods captured patterns of variation associated with sampling depth that were similar to those revealed by laboratory protocols. These findings indicate that selected rapid tools can reproduce relative patterns among samples and treatments and may therefore provide useful information when laboratory facilities are unavailable or timely soil health information is required to inform management decisions.

1 Introduction

Soil health is fundamental to the maintenance of ecosystem functions, including primary productivity, nutrient cycling, water regulation, and climate mitigation (Díaz de Otálora et al., 2021; Vogel et al., 2019). Despite its importance, there is ongoing debate regarding how to operationalize soil health and which indicators most appropriately capture it, reflecting its inherently multidimensional nature (Lehmann et al., 2020). Among the different types of indicators, biological indicators are increasingly recognized as particularly informative due to their high sensitivity to management and disturbance and their direct links to soil multifunctionality (Creamer et al., 2022).

In many applied contexts, however, soil health assessment is needed rapidly to support management decisions. Traditional laboratory-based analyses often require specialized facilities and can involve substantial delays, ranging from several days to weeks between sampling and results. This limits their practical utility for on-farm decision-making or adap-

tive management. Consequently, there is growing interest in the development of low-cost, easy-to-use, non-invasive and rapid assessment tools that can be applied directly in the field (Ameer et al., 2024), even if this comes at the expense of analytical precision.

Soil biological measurements have lacked such rapid, field-deployable approaches, but recent years have seen the emergence of several tools addressing this gap. Aggregate stability, while sometimes classified as a physical property, can be considered a hybrid indicator because the persistence of aggregates largely depends on biologically derived binding agents, such as glomalin, microbial exudates and mucilages, fungal hyphal networks, plant roots, and the activity of soil macrofauna (Six et al., 2004). Recent tools such as SLAKES allow for rapid assessment of aggregate stability (Fajardo et al., 2016; Fajardo et al., 2025). Similarly, soil respiration can be measured in situ using portable CO_2 analyzers (e.g., Biau et al., 2013), providing an immediate proxy for overall biological activity. More recently, commercial kits such as microBIOMETER® (mB) (2026) have been developed to estimate microbial biomass and the fungal-to-bacterial ratio, the latter being an ecologically relevant indicator linked to sustainable soil systems (Fierer et al., 2021). Finally, portable systems such as the Soil Enzymatic Activity Reader (SEAR) enable standardized measurements of soil enzyme activities (Fetzer et al., 2025), which are closely associated with organic matter turnover and nutrient acquisition.

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, without the need for specialized laboratory facilities and with the capacity to provide results within a short timeframe.

Despite their increasing availability and potential utility, the ability of these rapid assessment tools to reproduce patterns captured by established laboratory methods remains insufficiently evaluated. Comparing their ability to consistently rank samples or treatment pairs can provide relevant information on whether rapid tools capture similar relative patterns of soil health variation. However, such comparisons should not necessarily assume that established laboratory methods represent an unconditional gold standard, as their widespread use may reflect historical adoption and methodological convention rather than demonstrated superiority in sensitivity or ecological relevance. The objective of this study was therefore to compare four rapid, field-based biological soil health measurements (i.e., aggregate stability measured with SLAKES, soil respiration measured with EGM-5, microbial biomass and fungal-to-bacterial ratio measured with mB, and enzyme activities measured with SEAR) with their corresponding laboratory-based methods, focusing on the concordance in the relative ordering of treatment pairs produced by methods targeting the same soil health indicator rather than on numerical agreement or equivalence between analytically interchangeable procedures. We further examined the extent to which rapid and laboratory methods captured similar patterns of variation across sampling depth and grazing management.

2 Materials and methods

2.1 Experimental design, soil sampling and characterization

Soil sampling was conducted in the experimental multispecies pastures of NEIKER in Arkaute (42°51′11.41″ N, 2°37′27.20″ W; Basque Country, Spain) from April to November 2024, with sampling performed every three weeks, resulting in a total of 11 sampling campaigns. During this period, the mean air temperature was 15.2 °C (mean maximum 21.4 °C and mean minimum 9.1 °C), and cumulative precipitation amounted to 492 mm. The experimental fields host a long-term experiment established in 2013, eleven years prior to the present sampling. The experiment follows a completely randomized block design. The grazing area comprises 4.5 ha of semipermanent pasture divided into three plots, which are further subdivided into two grazing management treatments: rotational and conventional grazing (for details on the experimental design, see Díaz de Otálora et al., 2021).

Within each of the six resulting subplots, a random sampling point was selected and repeatedly sampled at soil depths of 0–20 and 20–50 cm following the standardized protocol of the LUCAS network. Briefly, five subsamples (one collected at the central point and four additional subsamples collected 2 m away in the cardinal directions) were composited to obtain a single representative sample (Ballabio et al., 2016). In total, 132 soil samples were collected. One representative fraction of each sample was kept unsieved for aggregate stability determination, a second fraction was sieved fresh to < 2 mm for the determination of soil biological properties and stored at 4 °C until analysis, and a third fraction was air-dried to constant weight for physicochemical characterization. All determinations were performed within 10 d of sample collection. The hands-on time and total time required to obtain results for each rapid and laboratory-based method are summarized in Table 1.

In addition, soil pH was measured following MAPA (1994). Soil organic matter content was estimated from organic carbon content, determined according to DIN 19539 (2016) using a SoliTOC® Cube analyzer (Elementar, Langenselbold, DE), and converted using a factor of 1.72. Particle size distribution was determined by laser diffraction

Table 1. Comparison of hands-on time and total time required to obtain results using rapid and laboratory-based methods.

	Rapid method		Laboratory method	
	Hands-on time	Total time	Hands-on time	Total time
Aggregate stability	∼ 5 min per sample	24 h (drying)[a]	∼ 15 min per sample	24 h (drying)[a]
Respiration	∼ 10 min per point	Immediate	∼ 15 h per 24 samples[b]	∼ 3 d
Microbial biomass carbon	∼ 10 min per sample[d]	∼ 10 min per sample[d]	∼ 10 h per 24 samples[b]	∼ 3 d
Fungal to bacteria ratio	∼ 10 min per sample[d]	∼ 10 min per sample[d]	∼ 11 h per 24 samples[b]	∼ 3 d
Enzyme activities	∼ 10 min per sample	∼ 45 min per sample	∼ 8 h per 18 samples[c]	∼ 3 d

[a] For aggregate stability, both methods required 24 h of drying. [b] For these laboratory analyses, the reported hands-on time refers to processing a batch of 24 samples. [c] For laboratory enzyme activity assays, processing 18 samples required approximately 8 h of hands-on work; this time does not decrease proportionally with fewer samples. [d] The microBIOMETER® method provides MBC and F : B ratio estimates simultaneously.

(Mastersizer 3000 Hydro LV, Malvern Panalytical, Malvern, UK) in accordance with ISO 13320 (2009). On average (± standard deviation), soils exhibited a pH of 8.17 ± 0.18, an organic matter content of 4.42 ± 1.69 %, and a clay loam texture.

2.2 Aggregate stability

A low-cost assessment of aggregate stability was performed using SLAKES, a free smartphone application developed by the University of Sydney (Australia) and promoted by the Soil Health Institute (USA). The methodology underpinning the SLAKES software is described in Fajardo et al. (2016). All measurements were conducted following the step-by-step instructions provided within the version 1.3 of the application. The image-based measurements for this and the following methods were acquired using a Samsung Galaxy A33 5G smartphone equipped with a 48 MP rear camera. We acknowledge that SLAKES was originally validated using soil aggregates collected from 0–6 cm depth; however, subsequent studies have applied the method to deeper soil layers (e.g., Adetsu et al., 2024; Flynn et al., 2020), suggesting its potential applicability beyond the depth range of the original validation.

In the laboratory, soil aggregate stability was determined using the wet sieving apparatus of Eijkelkamp (Giesbeek, The Netherlands). A known mass of air-dried soil aggregates within a size range comparable to that used for SLAKES was subjected to vertical oscillation in a water column through a 2 mm sieve. After a 3 min immersion period, the mass of soil remaining on the sieve was recorded and used to calculate the percentage of water-stable aggregates. Measurements were performed in triplicate.

2.3 Respiration

In situ soil respiration was measured using a portable CO_2 infrared gas analyzer (IRGA) (EGM-5, PP Systems, Amesbury, MA, USA) connected to a cylindrical soil respiration chamber (SRC-2, PP Systems), following the procedure described by Biau et al. (2013). Laboratory basal soil respiration measurements were conducted in accordance with ISO 16072 (2002). Briefly, soil was incubated in an airtight glass jar with a vial containing a NaOH solution, which traps the CO_2 released during soil respiration. After incubation, the remaining NaOH was titrated with HCl to quantify the CO_2 evolved. For this analysis, the comparison between in situ and laboratory measurements was restricted to the 0–20 cm soil layer, comprising a total of 66 samples.

2.4 Microbial biomass carbon and fungal to bacteria ratio

Microbial biomass carbon (MBC) and fungal : bacterial (F : B) ratio were assessed using the commercial mB Classic Starter Kit (Everett, WA, USA) following the procedure provided by the manufacturer and using version 3.8 of the mB app. The MBC estimate provided by mB was compared with the laboratory-based MBC determined using the chloroform fumigation–extraction method described by Vance et al. (1987). The F : B ratio estimated by microBIOMETER® was compared with an F : B ratio derived from qPCR-based quantification of bacterial and fungal abundances. Soil DNA was extracted from 0.25 g dry-weight soil using the DNeasy PowerSoil™Pro Kit (Qiagen, Venlo, NL). Quantification of bacterial (16S rRNA gene) and fungal (18S rRNA gene) abundances was performed by qPCR using the primers, reaction mixtures, and cycling conditions described in Epelde et al. (2014).

2.5 Enzyme activities

Finally, β-glucosidase (GLU), phosphomonoesterase (PHO), L-leucine aminopeptidase (LEU), and β-xylosidase (XYL) activities were measured on site using the laboratory-independent SEAR (Fetzer et al., 2025); the device used in this study was a pre-commercial prototype undergoing field validation. Corresponding laboratory measurements of these enzyme activities were conducted following ISO 22939 (2010) using fluorogenic substrates in 96-well microplates, as described by Anza et al. (2019).

2.6 Statistical analysis

Spearman's rank correlation was employed as the primary metric to assess the relationship between methods. To account for the nested experimental design and avoid pseudoreplication, correlation analyses were performed at the subplot level ($n = 6$). Non-parametric Spearman rank correlation coefficients (ρ) were first calculated independently within each subplot across time points and depths ($n = 22$ observations per subplot; $n = 11$ for soil respiration, measured only at 0–20 cm). To summarize the central tendency and variability of these within-subplot ρ values across the six subplots, we report the median Spearman correlation coefficient (median ρ), its range (minimum–maximum), and the associated 95 % bootstrap confidence interval. All correlation analyses were performed in R. For descriptive interpretation, median ρ values were classified as very weak ($\rho < 0.20$), weak ($0.20 \leq \rho < 0.40$), moderate ($0.40 \leq \rho < 0.60$), strong ($0.60 \leq \rho < 0.80$) or very strong ($\rho \geq 0.80$).

Partial redundancy analyses (pRDA) were applied separately to the datasets derived from the rapid assessment tools and from the laboratory protocols to examine the patterns of variation associated with the experimental factors and to assess their consistency across the two measurement approaches. Measurements of soil respiration were excluded from the RDA due to the lack of data for the 20–50 cm depth in the rapid method. For enzyme activity values below the limit of quantification, the lowest quantified value was substituted prior to analysis. To account for the nested experimental structure and temporal autocorrelation, sampling time was included as an adjusted covariate, and permutation tests were conducted using a restricted permutation scheme that accounted for subplot structure and temporal dependence. All pRDA models were performed using the *vegan* package (Oksanen et al., 2025) in R. Complementarily, to evaluate multivariate consistency between the rapid field-based suite of measurements and the laboratory methods, a Procrustes analysis was performed on the unconstrained ordination configurations (PCA) using the *protest* function of the *vegan* package (Oksanen et al., 2025), applying the same stratified permutation scheme.

3 Results and discussion

Figure 1 summarizes the Spearman rank correlations between rapid field-based measurements and their corresponding laboratory methods, and all raw data are provided in Table S1 in the Supplement. Overall, the strength and consistency of rank concordance varied markedly among indicators. Aggregate stability showed strong rank concordance between methods (Fig. 1A), indicating that SLAKES generally reproduced the relative ordering observed with the laboratory method. This result is consistent with previous studies evaluating SLAKES (Flynn et al., 2020) and further supports its use in the growing body of studies that have applied this approach (see examples in Fajardo et al., 2025). In contrast, the relationship between in situ and laboratory measurements of soil respiration was very weak, indicating poor rank concordance between the two approaches (Fig. 1B). For the mB outputs, MBC exhibited strong rank concordance with the laboratory-based method (Fig. 1C), whereas the F ratio showed very weak rank concordance (Fig. 1D). Similarly, Laine et al. (2025) reported that mB did not match PLFA-based estimates when determining the F ratio. The performance of SEAR varied among enzymes, with strong rank concordance for GLU (Fig. 1E) and XYL (Fig. 1H), moderate rank concordance for PHO (Fig. 1F), and very weak rank concordance for LEU (Fig. 1G). The one-sample Wilcoxon signed-rank test indicated consistently positive correlations across all six subplots for those indicators showing a minimum p value of 0.016. However, given the small number of subplots, this test has limited discriminatory power and should be interpreted cautiously. For LEU, the device prototype tested was not optimized, as this enzyme requires a different wavelength; this limitation will be addressed in future versions of the device, which will incorporate additional wavelengths.

These results must be interpreted in light of methodological differences between rapid and laboratory measurements. In particular, soil respiration and enzyme activities measured with rapid tools reflect the temperature and moisture conditions at the time of sampling, whereas laboratory assays are performed under standardized and near-optimal conditions of temperature, moisture, and, in the case of enzyme activities, pH. Moreover, soil disturbance, drying and rewetting, incubation conditions, and differences in the sources of CO_2 measured may further contribute to differences between field- and laboratory-based respiration measurements (Ananyeva et al., 2020; Patel et al., 2022; Li et al., 2025). Consequently, the two approaches may capture different components of soil respiration and are not expected to yield numerically equivalent results. This interpretation is supported by the high correlation reported by Haney et al. (2008) between respiration measured by titration and by an IRGA when both methods were applied under laboratory conditions, suggesting that the lack of correlation observed in this study may be related to the use of this equipment under field conditions. The relative value of each approach therefore depends on the study objective: rapid measurements capture the in situ functional status of the soil at a given moment, while laboratory measurements are generally conducted under standardized conditions and may therefore provide estimates of potential activity (Nannipieri et al., 2018), facilitating more standardized measurements across samples and studies. For field applications, measurements should ideally be conducted under sufficiently consistent environmental conditions; as a practical guideline, soil moisture around 60 % of field capacity and moderate soil temperatures (approximately 10–20 °C) have been suggested as suitable conditions for respiration mea-

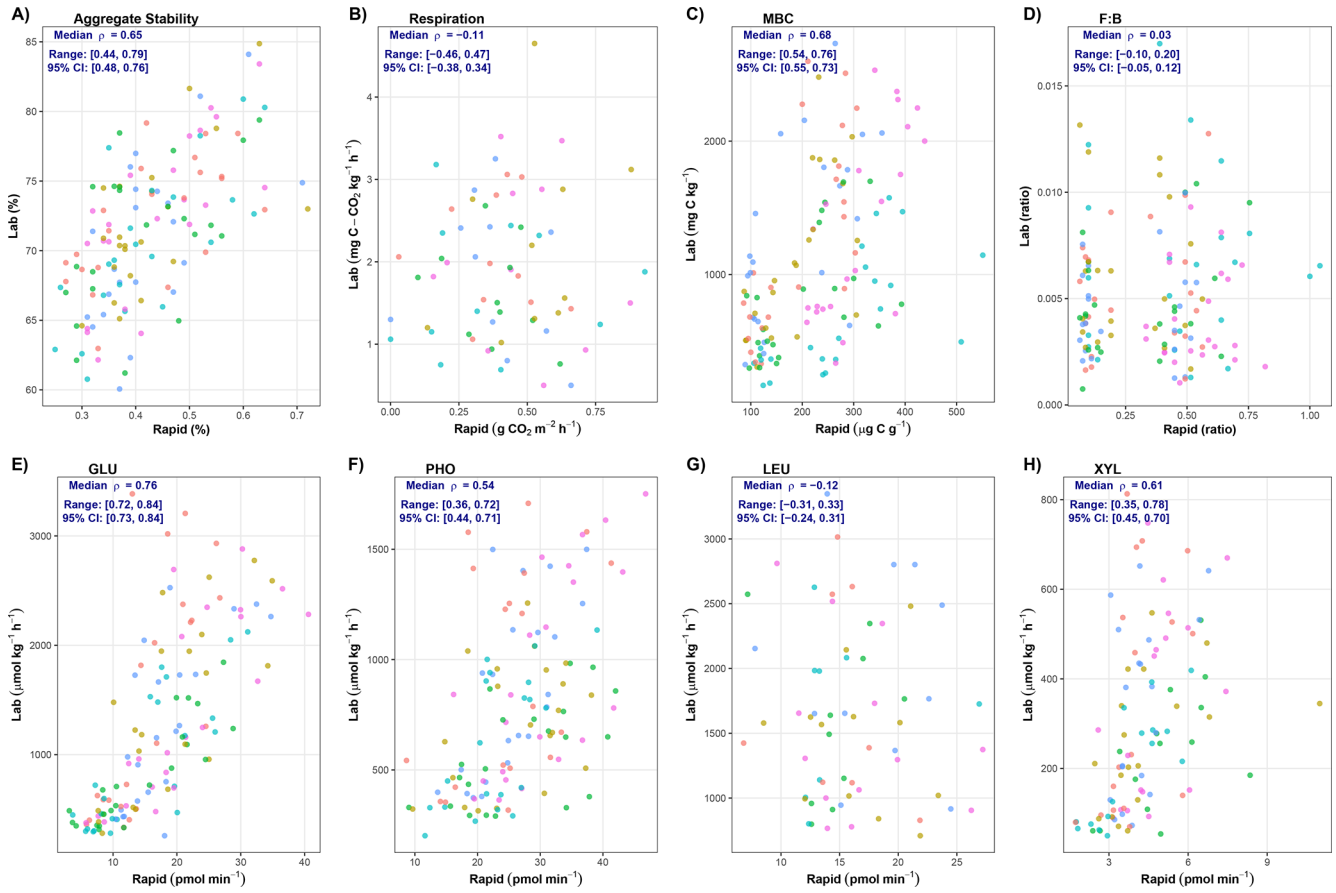


Figure 1. The relationship between rapid metrics (x axis) and laboratory protocols (y axis) for: **(A)** aggregate stability, **(B)** soil respiration, **(C)** microbial biomass carbon (MBC), (D) fungal to bacterial (F : B) ratio, and **(E–H)** activities of β-glucosidase (GLU), phosphomonoesterase (PHO), L-leucine aminopeptidase (LEU), and β-xylosidase (XYL). Data points are color-coded by subplot ($n = 6$). Blue text indicates the median Spearman rank correlation coefficient (Median ρ) calculated across independent subplots, its range (minimum–maximum) and the corresponding 95 % bootstrap confidence interval.

surements (Linn and Doran, 1984; Lloyd and Taylor, 1994). Recording soil temperature and moisture at the time of measurement can further facilitate interpretation and comparison among measurements.

The Procrustes analysis revealed a statistically significant concordance between the unconstrained multivariate ordination configurations (PCA) of the rapid assessment tools and the laboratory methods ($R = 0.382$; $p = 0.010$), indicating a moderate but meaningful similarity in the multivariate configuration of samples. Consistent with this finding, partial redundancy analyses showed that the suite of rapid indicators captured similar overall ecological patterns of variation to those identified by the laboratory methods (Fig. 2). Sampling depth emerged as the dominant factor structuring the data along the first ordination axis (RDA1), explaining 39 % of the total variance in the rapid assessment dataset (Fig. 2A) and 59 % in the laboratory dataset (Fig. 2B). Higher aggregate stability was associated with deeper soil layers (20–50 cm). This pattern may be related to differences in soil physicochemical properties, particularly clay content, which increased from 31.9 % in the 0–20 cm layer to 37.7 % in the 20–50 cm layer (data not shown). The higher clay content in the subsoil may contribute to greater aggregate stabilization through physical protection and organo-mineral interactions. In contrast, higher values of microbial biomass and activity were predominantly linked to surface soils (0–20 cm), consistent with established vertical patterns in soil biological functioning (Taylor et al., 2002). The second ordination axis (RDA2) reflected the effect of grazing management, although its contribution to the overall explained variance was very limited, accounting for only 0.3 % in the rapid assessment data and 0.8 % in the laboratory data.

Examination of correlations among response variables within the RDAs provided additional information on the relationships among indicators within each measurement approach. In particular, LEU activity measured with SEAR showed a poor representation and low correlation with the explanatory variables in the selected dimensions. Also, the

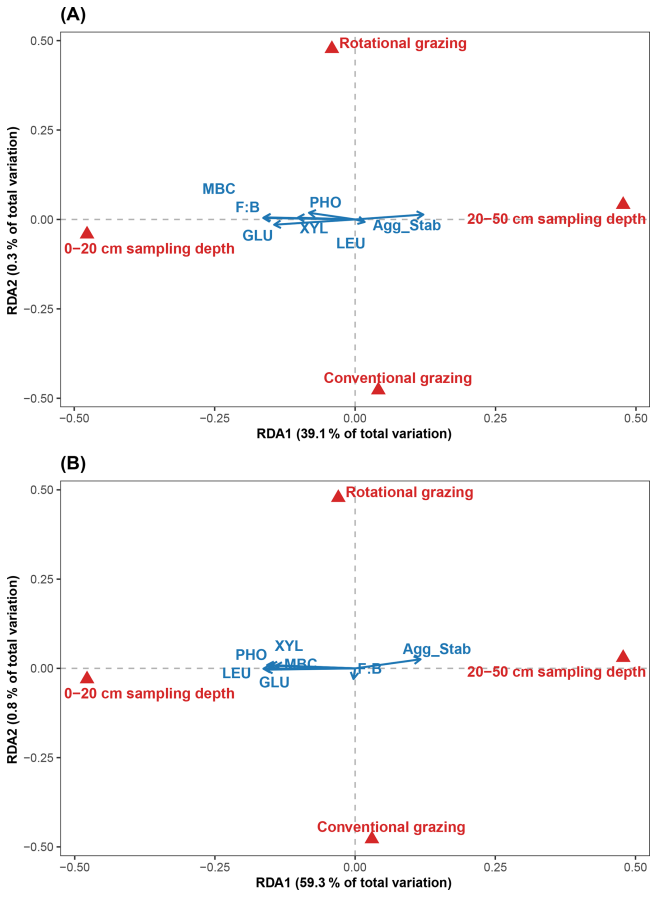


Figure 2. Biplots of the partial redundancy analyses (pRDA) performed using **(A)** rapid assessment metrics (Pseudo-F = 23.5, p = 0.002) and **(B)** laboratory protocol data (Pseudo-F = 78.4, p = 0.002) as response variables (blue), with experimental factors included as explanatory variables (red) and sampling time as covariate. Agg_Stab: aggregate stability; F : B: fungal to bacterial ratio; GLU: β-glucosidase; LEU: L-leucine aminopeptidase; MBC: microbial biomass carbon; PHO: phosphomonoesterase; XYL: β-xylosidase.

F : B ratio derived from the rapid method was strongly correlated with the MBC value obtained from the same kit. Meanwhile, the laboratory-based F : B ratio was weakly associated with management.

An important limitation of this study is that the assessment was conducted within a single long-term grassland experiment, and the observed rank concordance between rapid and laboratory methods should therefore be interpreted within the environmental and experimental conditions represented here. Soil health measurements may be influenced by soil properties, climate, land use, and management history (Norris et al., 2020), which can affect both the magnitude and relative variability of biological indicators. Assessing whether the observed patterns are transferable across contrasting environmental contexts will require studies involving independent sites and a broader range of soil types, climates, and land-use systems. Such studies could also help determine whether regional or soil-type-specific calibration datasets and reference ranges are needed for the robust interpretation of particular indicators. For example, Culman et al. (2012) evaluated a soil health indicator using both within-site and cross-site analyses, illustrating the value of assessing indicator behaviour across multiple environmental contexts.

4 Conclusions

Overall, under the conditions tested, the performance of the rapid methods varied among indicators. SLAKES provided results that showed strong rank concordance with laboratory measurements, supporting its use for aggregate stability assessment, whereas soil respiration measured in situ showed weak rank concordance, likely reflecting that the two approaches capture different aspects of respiratory activity. The mB showed consistent ranking with laboratory-based MBC measurements, but weak rank concordance for the F : B ratios. Enzyme activities measured with SEAR generally reproduced the relative patterns observed with laboratory methods. Despite these differences at the individual indicator level, multivariate analyses demonstrated that the suite of rapid measurements captured overall patterns of variation broadly similar to those identified by laboratory protocols, particularly in relation to sampling depth. Overall, these results indicate that selected rapid methods can provide meaningful and timely insights into soil biological functioning and represent valuable tools for dynamic trend tracking, complementing laboratory analyses when rapid decision-making or limited analytical infrastructure is a constraint.

Data availability. The raw data are available in Zenodo (https://doi.org/10.5281/zenodo.20525860, Anza et al., 2026).

Supplement. The supplement related to this article is available online at https://doi.org/10.5194/soil-12-1187-2026-supplement.

Author contributions. LE: Conceptualization, funding acquisition, writing – original draft. MA: Formal analysis, writing – review and editing. JF: Writing – review and editing. KJS: Project administration, writing – review and editing. JH: Project administration, writing – review and editing. NM: Funding acquisition, writing – review and editing.

Competing interests. Author JF was employed by Digit Soil. The remaining authors declare that they have no conflict of interest.

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Acknowledgements. The authors thank Aitor Anitua for technical support.

Financial support. This research has been supported by the HORIZON EUROPE Food, Bioeconomy, Natural Resources, Agriculture and Environment, HORIZON EUROPE Global Challenges and European Industrial Competitiveness (grant no. 101086179).

Review statement. This paper was edited by Maria de Mar Alguacil and reviewed by five anonymous referees.

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