https://doi.org/10.5194/egusphere-2026-1255
Preprint. Discussion started: 13 March 2026

Beyond the laboratory: performance and agreement of rapid methodologies for soil health assessment

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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 comparability with standard laboratory methods remains insufficiently evaluated. Here, we compared four rapid approaches with their corresponding laboratory reference 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). Agreement between methods was assessed using Spearman correlations, redundancy analyses and Procrustes analysis. Aggregate stability showed strong correspondence between rapid and laboratory measurements ($R = 0.64$), whereas soil respiration exhibited weak agreement, likely reflecting that in situ and laboratory approaches capture different aspects of respiratory activity. Microbial biomass carbon displayed moderate comparability between methods ($R = 0.51$), while fungal-to-bacterial ratios did not. Enzyme activities measured with SEAR were generally consistent with laboratory assays. Multivariate analyses indicated that overall, rapid methods captured ecological patterns similar to those revealed by laboratory protocols. These findings support the use of selected rapid tools as complementary or alternative options 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 (Diaz de Otalora 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

https://doi.org/10.5194/egusphere-2026-1255
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recognized as particularly informative due to their high sensitivity to management and disturbance and their direct links to soil multifunctionality (Creamer et al., 2022; Garbisu et al., 2011).

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 adaptive 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 field-based 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.

Despite their increasing availability and potential utility, the performance of these rapid assessment tools relative to established laboratory methods remains insufficiently evaluated. Assessing their comparability is essential to determine whether they can reliably inform soil health assessments and management decisions. 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 reference methods, to evaluate their agreement and to identify potential sources of divergence where discrepancies occur.

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, ten years prior to the present sampling. The experiment follows a completely randomized block design.

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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 cm 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 analysis of soil biological properties, and a third fraction was air-dried to constant weight for physicochemical characterization. Soil pH was measured following MAPA et al. (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 (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 application.

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 (comparable to the amount 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.

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 soil respiration measurements were conducted in accordance with ISO 16072 (2002). 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.

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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 system (Everett, WA, USA) following the procedure provided by the manufacturer. In parallel, laboratory-based MBC was determined using the chloroform fumigation–extraction method described by Vance et al. (1987). 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). 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. Before this analysis, data for the F:B ratio were log-transformed to reduce heteroscedasticity and improve data symmetry. All correlation analyses were performed in R.

Redundancy analyses (RDA) were applied separately to the datasets derived from the rapid assessment tools and from the standard laboratory protocols to evaluate whether both approaches detected the effects of the experimental factors. 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. Redundancy analyses were performed using Canoco 5 (ter Braak and Šmilauer, 2012). Complementarily, to evaluate multivariate consistency between the rapid field-based suite of measurements and the laboratory reference methods, a Procrustes analysis was performed using the *protest* function of the *vegan* package (Oksanen et al., 2025).

3 Results and discussion

Figure 1 summarizes the Spearman rank correlations between rapid field-based measurements and their corresponding laboratory reference methods, and all raw data are provided in Supplement A. Overall, the strength and significance of the relationships varied markedly among indicators. Aggregate stability showed a strong and significant correlation between methods (Fig. 1a), supporting the validity of SLAKES as a rapid assessment tool. This result is consistent with previous validation studies of the application (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

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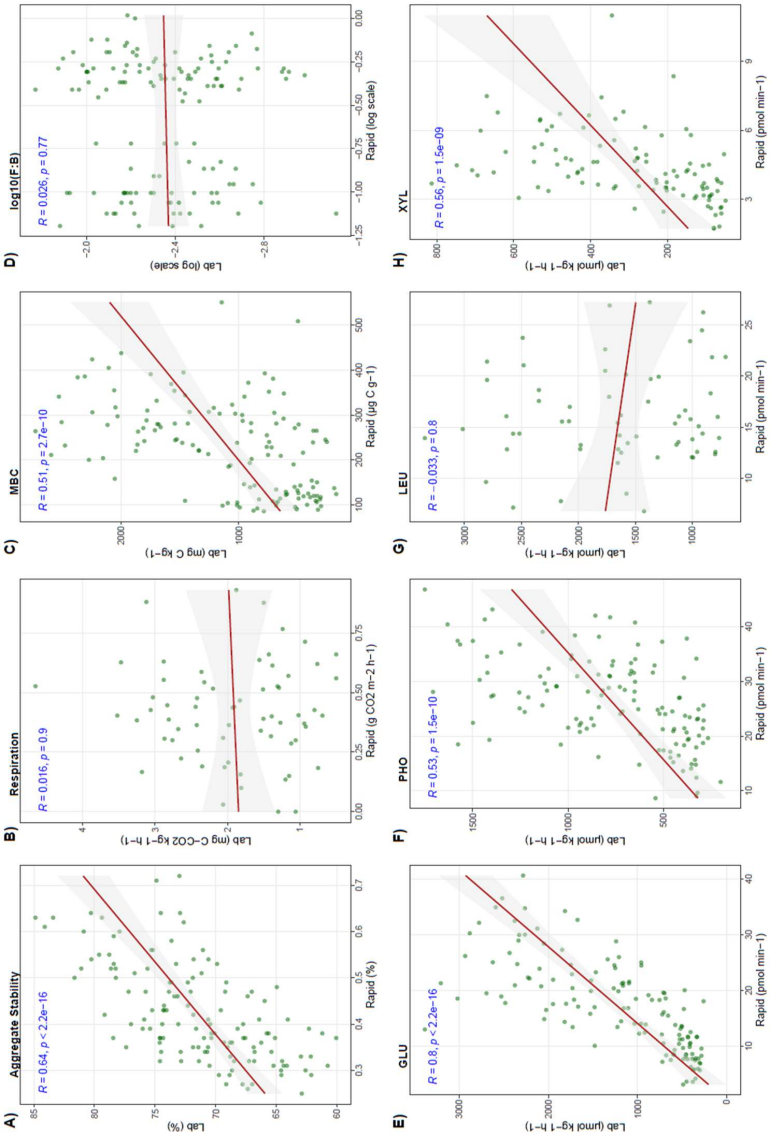

Figure 1: The relationship between rapid metrics (x axis) and standard laboratory protocols (y axis) for: (A) aggregate stability, (B) soil respiration, (C) microbial biomass carbon (MBC), (D) fungal to bacterial (F:B) ratio, and (E-H) activities of β-glucosidase (GLU), phosphomonoesterase (PHO), L-leucine aminopeptidase (LEU), and β-xylosidase (XYL). Solid red lines represent the linear regression fit, with light gray shaded areas indicating the 95% confidence interval. Blue text denotes Spearman's rank correlation coefficient and associated p-values.

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measurements of soil respiration was weak and not statistically significant (Fig. 1b), indicating limited agreement between the two approaches. For the mB outputs, MBC exhibited a moderate and significant correlation with the laboratory-based method (Fig. 1c), whereas the F:B ratio showed a weak relationship (Fig. 1d), suggesting lower comparability between methods for this parameter. Similarly, Laine et al. (2025) reported that mB did not match PLFA-based estimates when determining the F.B ratio. The performance of the SEAR varied among enzymes. Correlations were very strong and significant for GLU (Fig. 1e), moderate and significant for PHO (Fig. 1f) and XYL (Fig. 1h), and weak and non-significant for LEU (Fig. 1g). 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. 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 more faithfully capture the in situ functional status of the soil at a given moment, while laboratory measurements estimate potential activity (Nannipieri et al., 2018) and offer greater comparability across samples and studies.

The Procrustes analysis revealed a statistically significant concordance between the rapid assessment tools and the laboratory standard methods ($R = 0.473$; $P = 0.011$), indicating a moderate but meaningful similarity in the multivariate configuration of samples. Consistent with this finding, redundancy analyses showed that the suite of rapid indicators captured the same overall ecological patterns as the laboratory standards (Fig. 2). Sampling depth emerged as the dominant factor structuring the data along the first ordination axis (RDA1), explaining 33% of the total variance in the rapid assessment dataset (Fig. 2a) and 46% in the laboratory dataset (Fig. 2b). Higher aggregate stability was associated with deeper soil layers (20–50 cm), whereas 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) captured the effects of sampling time and grazing management, although these factors explained a substantially smaller proportion of the total variance, accounting for 2% in the rapid assessment data and 5% in the laboratory data. Despite this lower explanatory power, both datasets showed qualitatively similar trends along this axis.

Examination of correlations among response variables within the RDAs further supported the patterns observed in the Spearman analyses. Rapid indicators that showed weak agreement with laboratory methods displayed distinct ordination behavior. In particular, LEU activity measured with SEAR showed a poor representation and low correlation with the explanatory variables in the selected dimensions. Also, the 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 primarily associated with

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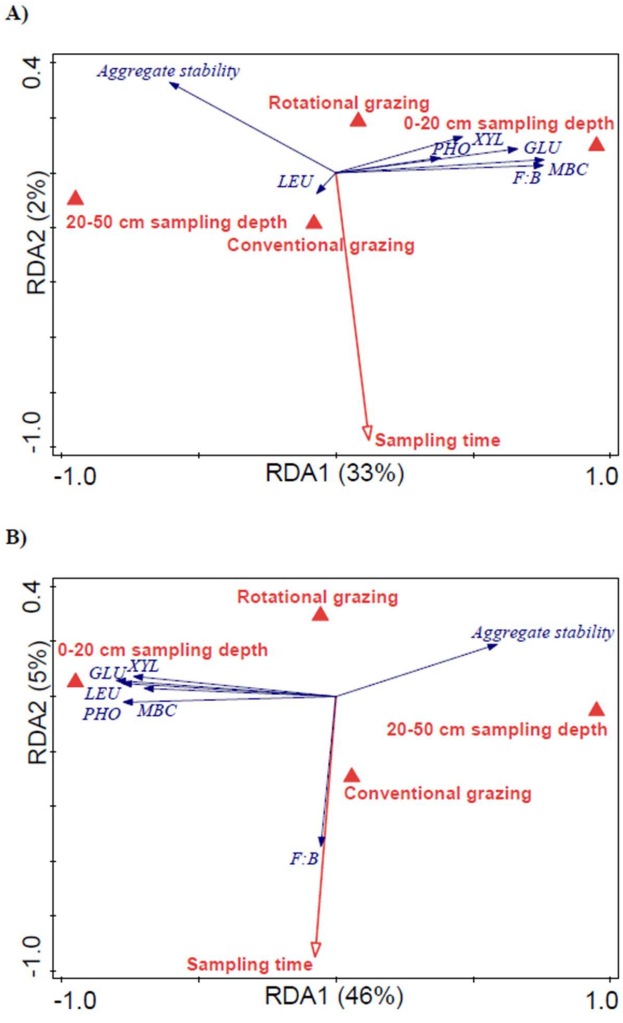
159 sampling time, suggesting a strong temporal signal that may reflect batch effects or a higher sensitivity to short-term
160 environmental variability.



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162 **Figure 2: Biplots of the redundancy analyses (RDA) performed using (A) rapid assessment metrics (Pseudo-F=23.4, P=0.002) and**
163 **(B) standard laboratory protocol data (Pseudo-F=45.2, P=0.002) as response variables (blue), with experimental factors and**
164 **sampling time included as explanatory variables (red). F:B: fungal to bacterial ratio; GLU: β-glucosidase; LEU: L-leucine**
165 **aminopeptidase; MBC: microbial biomass carbon; PHO: phosphomonoesterase; XYL: β-xylosidase.**

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4 Conclusions

Overall, under the conditions tested, the performance of the rapid methods varied among indicators. SLAKES provided results that closely matched laboratory measurements, supporting its use for aggregate stability assessment, whereas soil respiration measured in situ showed poor agreement with laboratory assays, likely reflecting that the two approaches capture different aspects of respiratory activity. The mB yielded comparable estimates of MBC but did not reliably reproduce laboratory-based F:B ratios. Enzyme activities measured with SEAR were generally consistent with laboratory methods. Despite these differences at the individual indicator level, multivariate analyses demonstrated that the suite of rapid measurements captured ecological patterns similar to those identified by laboratory protocols. Overall, these results indicate that selected rapid methods can provide meaningful and timely insights into soil biological functioning and represent a valuable complement, or alternative, to laboratory analyses when rapid decision-making or limited analytical infrastructure is a constraint.

Author contributions

LE: Conceptualization, funding acquisition, writing - original draft. MA: Formal analysis, writing - review & editing. JF: Writing - review & editing. KJS: Project administration, writing - review & editing. JH: Project administration, writing - review & editing. NM: Funding acquisition, writing - review & editing.

Competing interests

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

Acknowledgements

The authors thank Aitor Anitua for technical support.

Financial support

This research has been financially supported by the European Union's Horizon Europe research and innovation programme under grant agreement No. 101086179 (AI4SoilHealth).

References

Ameer, S., Ibrahim, H., Kulsoom, F. N. U., Ameer, G., and Sher, M.: Real-time detection and measurements of nitrogen, phosphorus & potassium from soil samples: a comprehensive review, J. Soils Sediments, 24, 2565–2583, https://doi.org/10.1007/s11368-024-03827-5, 2024.

https://doi.org/10.5194/egusphere-2026-1255
Preprint. Discussion started: 13 March 2026

Anza, M., Salazar, O., Epelde, L., Becerril, J. M., Alkorta, I., and Garbisu, C.: Remediation of organically contaminated soil through the combination of assisted phytoremediation and bioaugmentation, Appl. Sci., 9, 47–57, 2019.

Ballabio, C., Panagos, P., and Montanarella, L.: Mapping topsoil physical properties at European scale using the LUCAS database, Geoderma, 261, 110–123, https://doi.org/10.1016/j.geoderma.2015.07.006, 2016.

Biau, A., Santiveri, F., Mijangos, I., and Lloveras, J.: The impact of organic and mineral fertilizers on soil quality parameters and the productivity of irrigated maize crops in semiarid regions, Eur. J. Soil Biol., 53, 56–61, 2013.

Creamer, R. E., Barel, J. M., Bongiorno, G., and Zwetsloot, M. J.: The life of soils: Integrating the who and how of multifunctionality, Soil Biol. Biochem., 166, 108561, https://doi.org/10.1016/j.soilbio.2022.108561, 2022.

Díaz De Otálora, X., Epelde, L., Arranz, J., Garbisu, C., Ruiz, R., and Mandaluniz, N.: Regenerative rotational grazing management of dairy sheep increases springtime grass production and topsoil carbon storage, Ecol. Indic., 125, 107484, https://doi.org/10.1016/j.ecolind.2021.107484, 2021.

DIN 19539:2016-08: Investigation of solids - Temperature-dependent differentiation of total carbon (TOC400, ROC, TIC900), Beuth Verlag, Berlin, Germany, 2016.

Epelde, L., Burges, A., Mijangos, I., and Garbisu, C.: Microbial properties and attributes of ecological relevance for soil quality monitoring during a chemical stabilization field study, Appl. Soil Ecol., 75, 1–12, 2014.

Fajardo, M., McBratney, A. B., Field, D. J., and Minasny, B.: Soil slaking assessment using image recognition, Soil Till. Res., 163, 119–129, https://doi.org/10.1016/j.still.2016.05.018, 2016.

Fajardo, M., Pino, V., Jones, E., Morgan, C., Stevenson, B., Saby, N., Lacoste, M., and McBratney, A.: Measuring soil aggregate stability with mobile phones, lessons, challenges, and future work, Sci. Rep., 15, 24536, https://doi.org/10.1038/s41598-025-09925-9, 2025.

Fetzer, J., Meller, S., Iven, H., Baur, D., Rivera, P. G., Meller, A., and Luster, J.: Novel, laboratory-independent device to measure extracellular enzymatic activity in soils, Front. Environ. Sci., 13, 1663635, https://doi.org/10.3389/fenvs.2025.1663635, 2025.

Fierer, N., Wood, S. A., and de Mesquita, C. P. B.: How microbes can, and cannot, be used to assess soil health, Soil Biol. Biochem., 153, 108111, 2021.

Flynn, K. D., Bagnall, D. K., and Morgan, C. L. S.: Evaluation of SLAKES, a smartphone application for quantifying aggregate stability, in high-clay soils, Soil Sci. Soc. Am. J., 84, 1152–1162, https://doi.org/10.1002/saj2.20078, 2020.

Garbisu, C., Alkorta, I., and Epelde, L.: Assessment of soil quality using microbial properties and attributes of ecological relevance, Appl. Soil Ecol., 49, 1–4, https://doi.org/10.1016/j.apsoil.2011.04.018, 2011.

Haney, R. L., Brinton, W. F., y Evans, E.: Soil CO_2 respiration: Comparison of chemical titration, CO_2 IRGA analysis and the Solvita gel system, Renew. Agr. Food Syst., 23, 171–176, https://doi.org/10.1017/S174217050800224X, 2008.

ISO 16072: Soil Quality: laboratory methods for determination of microbial soil respiration, International Organization for Standardization, 2002.

ISO 13320: Particle size analysis: Laser diffraction methods, International Organization for Standardization, 2009.

https://doi.org/10.5194/egusphere-2026-1255
Preprint. Discussion started: 13 March 2026

ISO 22939: Soil quality: measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates, International Organization for Standardization, 2010.

Laine, M. B., Pekkinen, K., Taipale, S. J., and Tiirola, M.: Comparison of methods for assessing fungi-to-bacteria ratio of soil, Biol. Fertil. Soils, 61, 941–954, https://doi.org/10.1007/s00374-025-01911-7, 2025.

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., and Rillig, M. C.: The concept and future prospects of soil health, Nat. Rev. Earth Environ., 1, 544–553, https://doi.org/10.1038/s43017-020-0080-8, 2020.

MAPA: Métodos oficiales de análisis de suelos y aguas para riego, in: Métodos Oficiales de Análisis, Vol. III, editado por: Horwitz, W., Ministerio de Agricultura, Pesca y Alimentación, Madrid, España, 1994.

microBIOMETER®: Soil Test, available at: https://microbiometer.com/our-test/ (last access: 14 February 2026), 2026.

Nannipieri, P., Trasar-Cepeda, C., and Dick, R. P.: Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis, Biol. Fertil. Soils, 54, 11–19, https://doi.org/10.1007/s00374-017-1245-6, 2018.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Borman, T., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., Martino, C., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., and Weedon, J.: vegan: Community Ecology Package, R package version 2.8-0 [code], https://github.com/vegandevs/vegan, 2025.

Six, J., Bossuyt, H., Degryze, S., and Denef, K.: A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics, Soil Till. Res., 79, 7–31, https://doi.org/10.1016/j.still.2004.03.008, 2004.

Taylor, J. P., Wilson, B., Mills, M. S., and Burns, R. G.: Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques, Soil Biol. Biochem., 34, 387–401, https://doi.org/10.1016/S0038-0717(01)00199-7, 2002.

ter Braak, C. J. F. and Šmilauer, P.: Canoco reference manual and user's guide: software for ordination (version 5.0), Microcomputer Power, Ithaca, USA, 2012.

Vance, E., Brookes, P., and Jenkinson, D.: An extraction method for measuring soil microbial biomass C, Soil Biol. Biochem., 19, 703–707, 1987.

Vogel, H. J., Wollschläger, U., Helming, K., Heinrich, U., Willms, M., Wiesmeier, M., Russell, D., and Franko, U.: Assessment of soil functions affected by soil management, in: Atlas of Ecosystem Services, edited by: Schröter, M., Bonn, A., Klotz, S., Seppelt, R., and Baessler, C., Springer, Berlin, Germany, 77–82, 2019.