Reply to RC3: 'Comment on egusphere-2026-1255', Anonymous Referee #3, 05 Aug 2026

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

Epelde et al. compare several rapid soil health measurements with conventional laboratory indicators. The manuscript is generally well written and easy to follow. However, I have several concerns regarding the experimental design, statistical analyses, and interpretation that should be addressed before the manuscript can be considered for publication.

My first general comment concerns the experimental design. The study is based on 132 soil samples collected from a single grassland experiment (L67). However, these samples are not independent because they follow a nested sampling design, with multiple sampling times and two soil depths. As a result, the subsequent correlation analyses treat pseudoreplicates as independent observations (Fig. 1), which may bias the reported relationships. This limitation should be explicitly acknowledged, and the authors should consider revising the analyses to account for the hierarchical structure of the data.

ANSWER: Following this comment and a similar concern raised by Referee 2, we will revise the statistical analyses to explicitly account for the sampling structure. Specifically, for the Spearman correlations, we will calculate within-subplot rank correlations between the rapid and laboratory methods across sampling times and depths, and assess the consistency of the resulting correlation coefficients across the six subplots, rather than treating all 132 observations as independent. For the RDA, we will include sampling time as an adjusted covariate to account for temporal variation and constrain permutations by subplot. We will revise the corresponding Results and Discussion accordingly.

Related to this point, the reported correlations are valuable for understanding relationships within this experiment but should not be interpreted as broadly representative across soil types or management systems. Validation across independent sites would still be necessary before drawing more general conclusions. I encourage the authors to acknowledge this limitation. The authors could refer to Culman et al. (2012) for an example of using both within-site and cross-site analyses to evaluate the performance of a new indicator of soil health.

ANSWER: As also noted in our response to Referee 1, we agree that the present study should be considered an initial assessment conducted within a single long-term grassland experiment. We will explicitly acknowledge this limitation in the revised manuscript and emphasize that validation across independent sites and environmental conditions, ideally supported by broader calibration datasets and reference ranges, will be necessary before the performance of these rapid methods can be generalized. We also thank the Referee for the useful suggestion of Culman et al. (2012).

My second comment concerns the comparison between field-based and laboratory-based soil respiration measurements. In my view, these measurements represent fundamentally different processes. Field measurements quantify soil CO_2 flux, which is strongly influenced by environmental conditions such as temperature, soil moisture, and substrate availability. In contrast, the laboratory assay appears to quantify the potentially mineralizable carbon pool under controlled conditions. I therefore question the value of this comparison and would recommend either removing it or substantially revising the discussion.

ANSWER: A similar concern was also raised by Referee 2. We agree that these approaches reflect different operational expressions of soil respiratory activity. Our intention was not to imply that these are equivalent methods measuring exactly the same process under comparable conditions, but rather to evaluate whether they provide consistent relative patterns. We will substantially revise the Discussion to make these methodological and conceptual differences explicit and to avoid overinterpreting the correlation. If the Referee considers that these differences make the comparison inappropriate even with this clarification, we would be willing to remove the respiration comparison from the manuscript.

Finally, I am not convinced that the redundancy analysis (RDA) provides more insight than the correlation analyses. An alternative and potentially more informative comparison would be to evaluate whether the rapid indicators and conventional laboratory measurements respond similarly to management or temporal variation. For example, comparing the effect sizes of the grazing treatments or temporal trends would provide a more direct assessment of whether the rapid methods capture the same ecological responses as the conventional indicators. See Culman et al. (2012) for an example.

ANSWER: We will revise the multivariate analysis and its interpretation to place greater emphasis on the consistency of the responses to the experimental factors, rather than presenting the RDA as evidence of method comparability. We agree that this distinction should be made clearer in the manuscript. We will also consider the approach of Culman et al. (2012), as suggested by the Referee.

Specific comments:

L21: The phrase "ecological patterns" is too vague. Please describe more specifically what patterns are being investigated.

ANSWER: We will revise the sentence to clarify that the methods captured patterns of variation in relation to sampling depth, sampling time and grazing management.

Materials and Methods: The Introduction emphasizes the time-saving advantages of the rapid methods. It would therefore be helpful to briefly report the approximate time required for the rapid methods compared with the conventional laboratory analyses.

ANSWER: We thank Referee 3 for this useful suggestion. We will incorporate this information into the revised Materials and Methods section.

L87: Please provide a brief description of the ISO procedure used for the respiration measurements. If I understand correctly, the standard introduces the methods for basal respiration and substrate-induced respiration. A concise description would improve reproducibility without requiring readers to consult the standard.

ANSWER: We will add a brief description of the ISO procedure to improve reproducibility. Specifically, the laboratory measurement used in this study corresponds to basal soil respiration. Soil was incubated in an airtight glass jar together with a vial containing a sodium hydroxide (NaOH) solution, which traps the CO_2 released during soil respiration. After the specified incubation period, the remaining NaOH was titrated with hydrochloric acid (HCl) to quantify the CO_2 evolved.

L103: Please justify the use of Spearman's rank correlation rather than alternative correlation methods.

ANSWER: Our objective was to assess whether the rapid and laboratory methods consistently ranked samples in a similar order. Spearman's correlation is appropriate for this purpose because it quantifies the strength of a monotonic association without requiring a linear relationship or normally distributed variables. This was particularly relevant given the different analytical principles underlying some of the paired methods. Following the comments of Referee 2, we will also clarify throughout the manuscript that Spearman's correlation assesses rank concordance rather than method agreement or interchangeability.

Culman, S. W., Snapp, S. S., Freeman, M. A., Schipanski, M. E., Beniston, J., Lal, R., Drinkwater, L. E., Franzluebbers, A. J., Glover, J. D., Grandy, A. S., Lee, J., Six, J., Maul, J. E., Mirksy, S. B., Spargo, J. T., & Wander, M. M. (2012). Permanganate Oxidizable Carbon Reflects a Processed Soil Fraction that is Sensitive to Management. Soil Science Society of America Journal, 76(2), 494–504. https://doi.org/10.2136/sssaj2011.0286