

Short Communication

Using mid-infrared spectroscopy to estimate soil microbial properties at the continental scale

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ABSTRACT

Understanding microbial community properties is critical to improving the predictions of biogeochemical processes that govern soil carbon sequestration. In this observational study, mid-infrared (MIR) spectroscopy and partial least squares regression were used to estimate soil microbial and chemical properties from diverse ecosystems across the continental USA. Random validation technique suggested the estimation potential for soil microbial properties using MIR spectra, with the strongest estimations for microbial respiration, followed by microbial biomass nitrogen, β -glucosidase activity and microbial biomass carbon, as well as soil chemical properties including organic carbon and organic nitrogen. Microbial properties were mainly positively correlated to spectral regions associated with aliphatic C-H groups and C=O stretches of polysaccharides and negatively correlated to quartz and silicate-associated regions. Overall, this work suggests that MIR spectroscopy can characterize soil microbial properties and be useful for the improvement of continental scale soil carbon modeling and prediction programs.

Improving the understanding of soil mineralogical, physicochemical, and microbiological influences on soil organic matter (SOM) stabilization and turnover is critical for predicting the future fate of carbon (C) and remains a grand challenge. This challenge is partially attributable to the limited availability of microbial functional data to calibrate biogeochemical models, whose predictive power can be enhanced by the inclusion of microbial explicit parameters (e.g., CORPSE, MIMICS: Sulman et al., 2018; Wieder et al., 2015). These data are mainly generated through time- and cost-intensive laboratory analyses, limiting analysis to relatively few samples (Luo et al., 2016). Where large-scale data do exist, they are often collected using varying methodologies, making direct comparisons challenging. Therefore, there is a necessity to develop cost-effective tools for quickly estimating soil and microbial properties from many samples collected across space and time to support conceptual understanding and next-generation biogeochemical models

of soil C dynamics.

Mid-infrared (MIR) spectroscopy is a cost- and time-efficient method of soil analysis (Saxton and McDougal, 2021; Wehrle et al., 2021) and has been used to predict soil microbial parameters such as respiration, biomass C (MBC), and biomass nitrogen (MBN) at the local and regional scales (Ludwig et al., 2015; Meyer et al., 2018; Zhang et al., 2022). Earlier studies have shown the necessity to develop models specific to the site or soil characteristics for broader applications such as continental scales (Meyer et al., 2018; Reeves et al., 2000) or include a diverse dataset to enhance predictive accuracy (Rasche et al., 2013). Here, we hypothesize that MIR spectra together with partial least squares regression (PLSR) modeling can estimate the variations in select microbial functional properties across diverse ecosystems at the continental scale that were sampled and analyzed following consistent methodology (Bowman et al., 2023a). Critically, our hypothesized

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relationship between MIR spectra and microbial properties is not dependent on the direct detection of soil microbes but arises as a result from the association between various soil properties (e.g., organic compounds) and MIR spectral signals that are connected with microbial community composition and functions (Zhang et al., 2022).

Specifically, improving estimations of microbial functional properties such as β -glucosidase (BG) activity potential, respiration, MBC and MBN, microbial metabolic quotient (qCO_2), and soil chemical properties such as water extractable organic C (OC) and water extractable organic nitrogen (ON) hold potential to improve the understanding and modeling of soil C dynamics at the continental scale. BG is crucial in decaying cellulose to glucose, providing energy for microbial metabolism (Cohen et al., 2005; Dick et al., 2013; Gates, 2018). Microbial respiration makes up the second largest C flux on terrestrial landscapes (Meyer et al., 2018); minor alterations in its rate can have substantial influence on atmospheric CO_2 concentrations. Carbon use efficiency (CUE) is central to soil C dynamics and represents C allocation towards microbial growth versus C consumed, which is related to soil C at the global scale (Tao et al., 2023). qCO_2 represents microbial efficiency in converting soil organic C (SOC) into microbial biomass. Its higher value suggests lower potential CUE and greater C loss through respiration or exudation, and lower value suggests more efficient C assimilation into biomass (Xu et al., 2017). MBC and MBN provide quantitative estimations of the living microbes in a system, from which soil C dynamics is often an emergent property (Scow, 1997). Lastly, soil properties like OC and ON mediate energy and nutrient availability for microbial growth and can govern microbial activity and SOM decomposition (Wu et al., 2024). New tools to estimate these properties in a more time- and cost-efficient manner hold the potential to enhance our understanding of factors determining C storage and turnover dynamics and will improve more accurate SOC modeling.

To assess the effectiveness of MIR in estimating select microbial properties across diverse ecosystems at the continental scale in our observational study, we obtained soils from 67 sites (Fig. 1A) as part of the Environmental Molecular Sciences Laboratory's (EMSL) 1000 Soils Molecular Observation Network (MONet) Project (Bowman et al., 2023a; further detail in Supplementary Material). A comprehensive soil sampling protocol is accessible at protocols.io (Bowman et al., 2023b). Soil samples were taken from two depths (0–10 cm and 20–30 cm), and the fresh samples were shipped on ice, stored at 4 °C upon receipt to the lab ($N = 126$ samples; Table 1), and analyzed within 48 h. of sample

collection by MONet staff. Microbial (respiration, BG, MBC, MBN) and soil chemical (OC, ON) properties were generated from EMSL's 1000 Soils MONet Project (Fig. 1B). MBC and MBN were measured via chloroform fumigation, and BG and respiration rates were measured using colorimetric assays and CO_2 burst, respectively (Bowman et al., 2023a). qCO_2 was estimated using the following equation (Ashraf et al., 2022).

$$qCO_2 = \frac{\text{Microbial respiration rate}}{\text{Microbial biomass carbon}} \quad (1)$$

MIR spectra were generated from air-dried and finely-ground (<180 μm) soil samples as described in Zhang et al. (2022; Supplementary Material) and sequential scanning of the samples was performed using an Alpha II benchtop spectrometer (Bruker Optics Inc.). Spectral absorbance data were collected from 4050 to 550 cm^{-1} , at a resolution of 2 cm^{-1} , however, the spectral region of 4000 to 600 cm^{-1} was selected for analysis to avoid a low signal-to-noise ratio. Preprocessing of the spectra was carried out by applying Savitzky-Golay filter, followed by spectral resampling at a resolution of 10 cm^{-1} and standard normal variate transformation (SNV). The absorption features associated with organic functional groups including carboxylic acids, amides, aromatics, aliphatics, methyls, alkyls, phenolics, carbohydrates, and minerals including quartz, and clay minerals were identified (Fig. 2; Table S1; Margenot et al., 2015; Matamala et al., 2019; Meyer et al., 2018; Rumpel et al., 2001; Zhang et al., 2022). PLSR models were used to associate microbial and soil properties with MIR spectra. To test our hypothesis, random and leave-one-out cross-validation (LOOCV- based on ecoclimatic domains and soil orders, Supplementary Material) methods were implemented. Additionally, Pearson correlation was used to estimate the relationship between MIR wavenumbers and measured soil properties.

Through random sampling of calibration (70 %) and validation (30 %) datasets, MIR spectra suggested a moderate estimation accuracy of soil microbial properties at the continental scale. A moderate estimation of microbial respiration and BG activity potential at the continental scale was observed (validation $r^2 = 0.56$ and 0.4 , respectively; Table 2) in our study. At regional scales, a high estimation accuracy, validation $r^2 = 0.83$ for both respiration and enzyme activity using MIR has been observed previously (Gates, 2018; Meyer et al., 2018). This observation for soil enzyme activities and microbial respiration at regional scales may be due to their strong relationship with soil substrate availability (Moorhead et al., 2013) and the use of lab incubation-based methods.

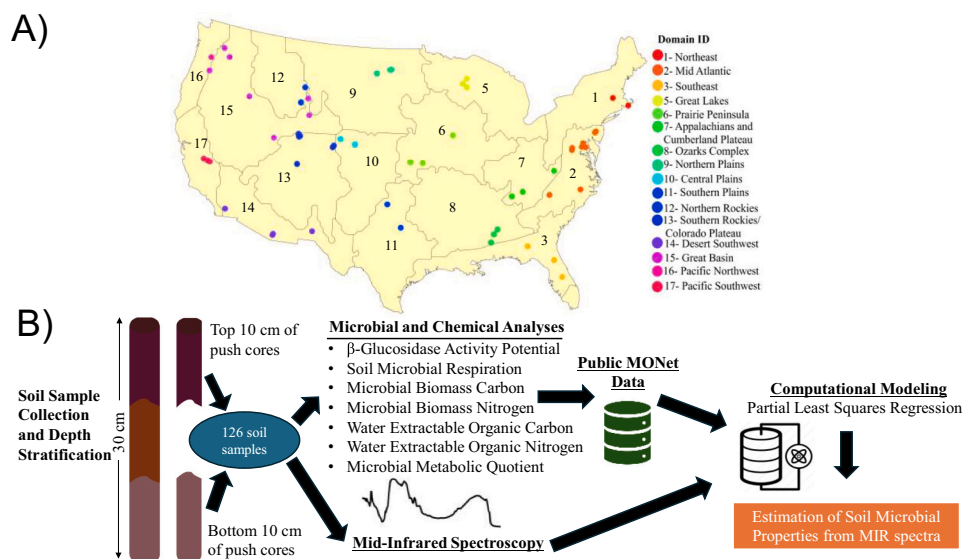


Fig. 1. Locations of field sites from where soil samples were received by Molecular Observation Network for the 1000 Soils Pilot Project that are included in this study, projected on National Ecological Observatory Network ecoclimatic domains (Panel A). Soil sampling and analysis workflow, based on (Bowman et al., 2023a; Panel B).

Table 1
Domain and soil order specific samples allocation in PLSR models for specific soil microbial properties.

Sample Category	β -glucosidase potential activity		Microbial respiration, water extractable organic C, water extractable organic N		Microbial metabolic quotient, microbial biomass C, microbial biomass N		Soil orders ⁺	Elevation range (m)
	0–10 cm	20–30 cm	0–10 cm	20–30 cm	0–10 cm	20–30 cm		
NEON domain ID*								
1- Northeast	1	–	2	2	1	–	Spodosols, Inceptisols	1–338
2- Mid Atlantic	3	3	12	12	12	12	Alfisols, Ultisols, Inceptisols	20–312
3- Southeast	3	1	3	3	3	3	Spodosols, Ultisols, Entisols	24–50
5- Great Lakes	3	3	3	3	3	3	Spodosols	457–513
6- Prairie Peninsula	5	5	5	5	5	5	Mollisols	231–394
7- Appalachians and Cumberland Plateau	3	3	3	3	3	3	Inceptisols, Aridisols, Ultisols	310–601
8- Ozarks Complex	3	3	3	3	3	3	Ultisols, Inceptisols	28–131
9- Northern Plains	3	2	3	2	3	2	Mollisols	569–580
10- Central Plains	2	–	2	–	2	–	Mollisols	1366–1641
11- Southern Plains	2	2	2	2	2	2	Alfisols, Inceptisols	274
12- Northern Rockies	3	3	3	3	3	3	Inceptisols, Mollisols	2274–2865
13- Southern Rockies/ Colorado Plateau	7	5	7	5	6	5	Aridisols, Inceptisols	1764–3277
14- Desert Southwest	4	4	4	4	4	4	Entisols, Aridisols	191–1326
15- Great Basin	3	2	11	9	7	6	Mollisols, Aridisols, Andisols, Inceptisols	300–2639
16- Pacific Northwest	1	–	1	–	1	–	Andisols	587
17- Pacific Southwest	3	3	3	3	3	3	Alfisols, Entisols	383–1169
<i>Total</i>	<i>49</i>	<i>39</i>	<i>67</i>	<i>59</i>	<i>61</i>	<i>54</i>		
Soil order								
Spodosols	5	3	5	4	5	4		
Inceptisols	7	7	12	13	11	11		
Alfisols	5	5	7	7	7	7		
Ultisols	5	4	7	7	7	7		
Entisols	4	4	4	4	4	4		
Mollisols	11	8	13	10	12	9		
Aridisols	6	5	9	8	9	8		
Andisols	1	–	4	2	1	–		
<i>Total</i>	<i>44</i>	<i>36</i>	<i>61</i>	<i>55</i>	<i>56</i>	<i>50</i>		

⁺ Soil orders determined with: <https://websoilsurvey.nrcs.usda.gov/app/>

^{*} NEON domains accessed from: <https://www.neonscience.org/field-sites/about-field-sites>

These methods assess activity potential, minimizing the influence of potential unmeasured confounding factors like local environmental conditions thus supporting. MBC and MBN, in our study were estimated with a validation r^2 of 0.38 and 0.50, respectively, while OC and ON were both estimated with an r^2 of 0.38 (Table 2). Recent work at the regional scale showed that MBC increased as OC increased (Liddle et al., 2020) and was well-estimated using MIR ($r^2 = 0.92$) (Rasche et al., 2013). The relatively low estimation accuracy of MBC in our study might be because of its subtle influence on soil spectra as it represents 5 % or less of the total SOC (Fan and Liang, 2015). It is also likely that the variance in quality and quantity of soil macronutrient availability, associated to MBC and not studied in our study, might have limited the applicability of the PLSR model for MBC estimation at large spatial scales (Soriano-Disla et al., 2014). Apparent effects from MBC and OC may also be obscured by the contribution of peaks from soil minerals, i. e., quartz, or other constituents that have overlaps in similar spectral regions (Soriano-Disla et al., 2014). The difficulty in estimating ON may be due to its low concentrations in soils and its dynamic nature (Gates, 2018). The relatively low validation r^2 of qCO_2 (0.35, Table 2) is likely because it is highly temporally variable and has greater sensitivity to the local unmeasured environmental conditions and physiological status of microbial community (a characteristic unlikely to affect the spectra significantly enough to be detected) compared to other microbial properties estimated here (Kane et al., 2023; Schnecker et al., 2023; Xu et al., 2017). Since the sampling time was carefully selected to ensure consistency and comparability across eco-climatic domains aligning with peak vegetation growth periods and avoiding freezing conditions, lab-

based, controlled soil microbial respiration measurements based on a one-time field sampling campaign is representative of microbial processes at that sampling time. This is distinct from season-dependent, dynamic properties such as soil CO_2 emission flux measured in the field chambers. Therefore, MIR models built on soil microbial measurements should have their values in estimating soil microbial properties at the selected sampling time.

All microbial properties were positively correlated to spectral regions representing aliphatic C-H groups and C=O stretches of polysaccharides, likely representing thermodynamically labile C (Figs. 2 and 3; Matamala et al., 2019; Meyer et al., 2018). Current conceptual frameworks support this association which argue that when nitrogen and phosphorus are sufficient, there would be a strong incentive for soil microbes to invest in the acquisition of easily degradable C substrates to mitigate C limitation, stimulating respiration and production of enzymes such as BG (Allison and Vitousek, 2005; Simon, 2005). Conversely, microbial properties were negatively correlated to spectral regions associated with quartz and silicates with an additional negative correlation of respiration with lignin and aromatic-related regions. Both soil mineralogy and lignin/aromatic abundance are known to have substantial consequences on microbial communities (Figs. 2 and 3; Meyer et al., 2018; Whitman et al., 2018). The negative correlation for OC can also be attributed to the presence of carbonate signals from aridic soils (Figs. 2 and 3), as carbonates can interfere with the OC quantification (McCarty et al., 2002).

The LOOCV, both across eco-climatic domains and soil orders (Table 1, Figs. S2, S3 and S4), resulted poorer estimations than simple

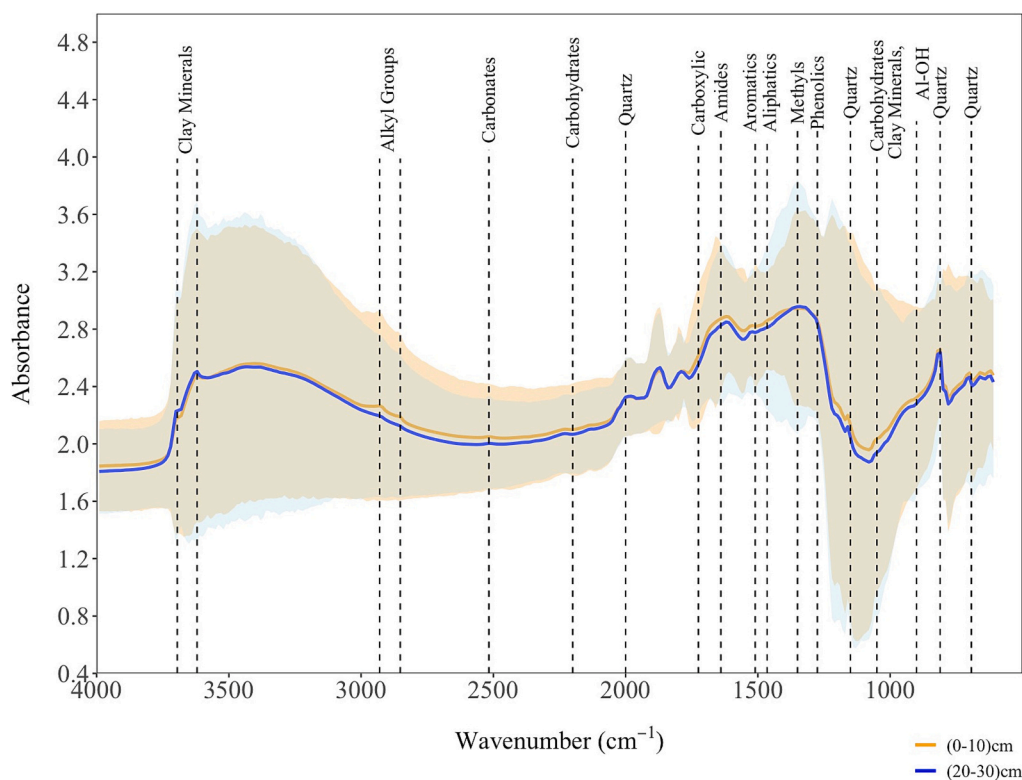


Fig. 2. Distribution of the mean and range of the mid-infrared spectra at 0–10 cm and 20–30 cm soil depth across all the sites, along with the identification of significant absorption features. The two distinct darker lines represent the mean and the shaded lighter areas represent the range for the respective soil depths.

Table 2

The mean calibration and validation outcomes using PLSR to estimate soil microbial properties from soil mid-infrared (MIR) spectra with simple random sampling.

Soil microbial property	Calibration				Validation			
	r^2	RMSE ^a	ME ^b	RPIQ ^c	r^2	RMSE	ME	RPIQ
β -glucosidase potential activity	0.63	0.81	0.00	2.25	0.40	1.12	-0.07	1.62
Microbial respiration	0.71	0.62	0.00	2.58	0.56	0.83	0.04	1.93
Microbial metabolic quotient (qCO ₂)	0.68	0.58	0.00	4.35	0.35	0.92	0.01	1.62
Microbial biomass carbon (MBC)	0.57	0.59	0.00	1.98	0.38	0.74	-0.01	1.54
Microbial biomass nitrogen (MBN)	0.63	0.59	0.00	2.44	0.50	0.72	-0.01	1.94
Water extractable organic carbon (OC)	0.54	0.65	0.00	1.98	0.38	0.79	-0.03	1.64
Water extractable organic nitrogen (ON)	0.55	0.63	0.00	2.41	0.38	0.80	-0.04	1.94

RMSE^a - Root Mean Square Error; ME^b - Mean Error; RPIQ^c - Ratio of Performance to Inter-Quartile Distance.

random validation (Fig. S1), which suggests a limitation in the model transferability across diverse geographical scales and soil types, as has been noted previously (Brown et al., 2005; Rasche et al., 2013; Zhang et al., 2022). Hence, spectral models should only be applied to the samples that fall within the range of training dataset, as models developed for a given location may not be applicable to other locations (Zhang et al., 2022). Accounting for the moderate model accuracy observed in this study, conventional methods need to be prioritized while analyzing soil microbial properties when high precision is needed and soil spectroscopic approaches when involving extensive datasets for large-scale studies (e.g., mapping, modeling) (Meyer et al., 2018; Rinnan and Rinnan, 2007).

This study demonstrated that combining MIR with PLSR is a promising approach for the rapid estimation of soil microbial properties across diverse spatial and temporal scales at the continental scale. While the continental scale nature of this study makes it unique among the body of work conducted at the regional scales aiming to estimate soil microbial properties from MIR spectra, the small sample size of our study may have contributed to the lower estimation accuracy than what has been reported in the literature at local scales. This can limit the

model's transferability across broader spatial and temporal scales, pointing out critical challenges in the continental scale estimations beyond the sampled regions, soil types, and mineralogy or under different abiotic conditions. In this way, future studies with larger sample sizes across diverse environments and soils are needed for developing and parameterizing the models with higher predictive power and transferability. Microbial functional estimations should integrate stable-isotope tracing methods, time-series soil sampling, and continuous soil sensor monitoring in order to better estimate key microbial properties like qCO₂ and CUE to capture their fine-scale soil variability (Geyer et al., 2019; Kane et al., 2023). Combining soil biogeochemical data with seasonally fluctuating parameters (e.g., nitrate, moisture) through in-situ sensing methods (Chen et al., 2024) can also enhance the practical usefulness of MIR for understanding soil biological processes. These challenges, if accounted will improve our understanding of intricate soil-microbe-C relationship and our ability of leveraging soil microbial communities for ecosystem sustainability and soil C forecasting.

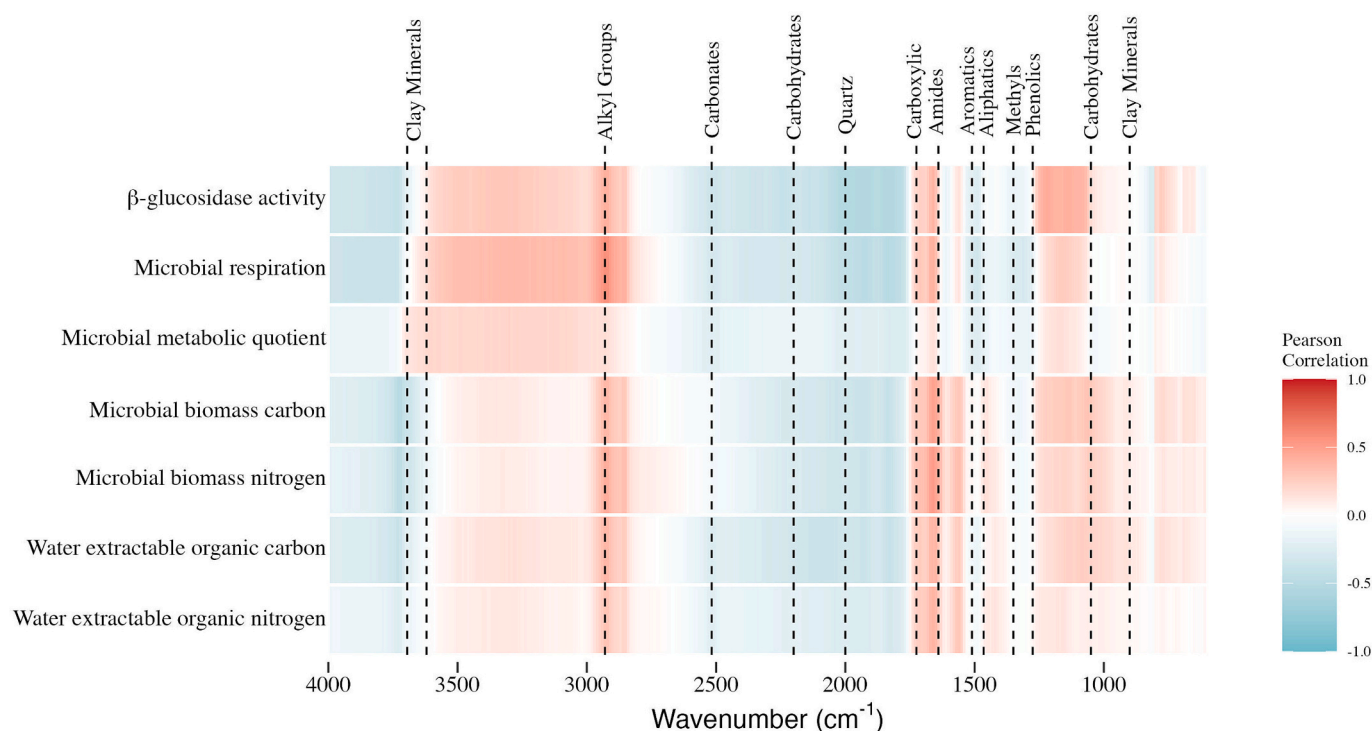


Fig. 3. Heatmap representing the Pearson correlation analysis between natural 'In-transformed' soil microbial and chemical properties, and preprocessed mid-infrared (MIR) spectra by wavenumber for all samples, along with the representation of significant absorption features. Each tile represents the correlation between the specific property to the corresponding wavenumbers. The β -glucosidase activity potential (BG), and microbial respiration demonstrated a moderate negative correlation with MIR spectra within the spectral ranges of (1820 cm^{-1} -2040 cm^{-1}) and (1780 cm^{-1} -2040 cm^{-1}) respectively. Conversely, BG and microbial respiration exhibited a moderate positive correlation at (1130 cm^{-1} -1150 cm^{-1} , 1200 cm^{-1} -1230 cm^{-1} and 2920 cm^{-1} -2930 cm^{-1}) and (2850 cm^{-1} -2970 cm^{-1}) respectively. Microbial metabolic quotient exhibited a weak positive correlation at (3250 cm^{-1} -3350 cm^{-1}) and a weak negative correlation at (1480 cm^{-1} -1510 cm^{-1} , 1770 cm^{-1} -2020 cm^{-1} and 2500 cm^{-1} -2540 cm^{-1}). Microbial biomass carbon and microbial biomass nitrogen exhibited a negative and positive correlation at (1810 cm^{-1} -1850 cm^{-1} , 1910 cm^{-1} -1960 cm^{-1} , and 3700 cm^{-1} -3740 cm^{-1}) and (1630 cm^{-1} -1680 cm^{-1} and 2910 cm^{-1} -2940 cm^{-1}) respectively. Water extractable organic carbon and water extractable organic nitrogen displayed a positive correlation at (1650 cm^{-1} -1670 cm^{-1} and 2920 cm^{-1} -2930 cm^{-1}). While water extractable organic carbon displayed negative correlations at (1810 cm^{-1} -1840 cm^{-1} , 1920 cm^{-1} -1930 cm^{-1} , 2040 cm^{-1} -2130 cm^{-1} and 3730 cm^{-1} -3740 cm^{-1}), water extractable organic nitrogen displayed the same at (1790 cm^{-1} -1850 cm^{-1} and 1910 cm^{-1} -1960 cm^{-1}).

CRediT authorship contribution statement

Soni Ghimire: Writing – original draft, Formal analysis. **Yakun Zhang:** Writing – review & editing, Formal analysis. **Jingyi Huang:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Erica L.-W. Majumder:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Alfred E. Hartemink:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Emily B. Graham:** Writing – review & editing, Data curation. **Odetta Qafoku:** Writing – review & editing, Data curation. **Joseph Andrews:** Methodology, Funding acquisition, Conceptualization. **Zachary B. Freedman:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2025.106110>.

Data availability

Data will be made available on request.

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