In-situ Decomposition Sensor Output Correlates with Soil Health Indicators

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ABSTRACT

Monitoring of microbiological processes in soil is important for understanding and improving soil health and agricultural productivity. Despite its significance, microbiological measurements are currently difficult to make in-situ and in real time. Evaluation of microbially-mediated soil processes usually involves manual sampling followed by laboratory analysis, which can be costly, time consuming, physically intensive, non-continuous, and reduces capacity for measuring changes at high temporal and spatial resolution, thereby limiting the ability to make prompt informed land management decisions. Low-cost soil sensors manufactured using printing techniques offer a potential scalable solution to these issues, allowing for wide distribution of sensors that collect time-series data for decomposition rates. Here, we tested the use of novel sensors for the evaluation of soil microbial processes. This comprises the first large-scale field deployment of these sensors, which use a biodegradable composite conductor that transduces microbial decomposition of substrates to a change in electrical resistance. Sensors were installed for 50 days across 44 experimental plots of a long-term grassland biodiversity restoration experiment, including treatments with additions of synthetic fertilizer, manure, mixed seed, and red clover, causing significant differences in soil microbial activity. Soil biological properties commonly used as soil health indicators, including microbial biomass and enzymatic activities related to carbon, nitrogen and phosphorus cycling, were measured using standard laboratory methods. Sensor responses were compared to these conventional soil health measures to better understand their potential utility by considering the signals of sensors within a set decomposition window and individual sensor degradation rates across various timescales, and correlating changes in signal variability with soil measured properties. All statistical approaches found positive correlations between the sensor signal and laboratory measurements of microbial biomass carbon and soil organic carbon, and some approaches found correlations with enzymatic measurements. These findings demonstrate the potential for the proxy measurement of soil microbial processes in-situ using readily distributable printed decomposition sensors, thereby supporting their potential for low-cost, high-resolution temporal and spatial monitoring of complex soil biological parameters and their ability to provide new insights into soil health.

KEYWORDS

environmental monitoring, printed electronics, continuous monitoring, in-situ sensing, soil biological activity, soil microbiome, soil sensing, degradable electronics

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INTRODUCTION

High spatial and temporal resolution monitoring of soils is required to accurately manage soil resources,[1] but currently we have limited capability beyond the monitoring of soil water, temperature, and some chemical parameters. Continuous monitoring of soil microbiological activity has, until now, been primarily restricted to measuring soil CO_2 concentrations[2] and controlled laboratory or small-scale applications of prototype sensors.[3] Real-time monitoring of soil biological activity would enable precise and sustainable management of this key component of soil. This is particularly important given the fundamental importance of the soil microbiome for maintaining soil health and ecosystem functioning.[4,5] These functions include nutrient cycling, decomposition, plant growth, and mediation of soil carbon fluxes due to respiration and sequestration.[6–8] Microorganisms in the soil affect the fate and transport of carbon in the largest terrestrial carbon pool on the planet.[9] In addition, they contribute to critical ecosystem services from the provisioning of food and safe drinking water to the regulation of pathogenic organisms.[10]

Soil health refers to a broad set of metrics and methodologies that relate to a soil's ability to provide ecosystem services.[11] Soil health indicators are usually broken into three categories: physical, chemical, and biological,[12,13] with biological indicators receiving relatively less attention in soil health assessments.[14,15] This is due, at least in part, to difficulties in both the measurement and integration of biological parameters into soil health models,[16] although there is broad agreement that more effective soil health models would place greater weight on biological measurements.[17,18] Traditional methods for assessing soil biological properties that are key to soil health are typically carried out away from the field. This means that they can be expensive and time consuming, do not provide the benefits of continuous, in-situ monitoring,[19] and often do not differentiate between currently active microbes and relic materials from the microbial necromass.[20] As such, there is a need for better ways to evaluate the biological components of soil health at scale, including the implementation of continuous monitoring to evaluate changing trajectories.

Emerging technologies have shown promise in supporting programs to restore soil health, including remote sensing, mobile applications, and in-situ sensors.[21] However, monitoring systems have challenges to overcome, including energy efficiency constraints, cost, response time, accuracy, and ease of use.[22] Due to their heterogeneity, soils can be particularly hard to meaningfully monitor without high spatial coverage, with soil microbial communities and soil properties showing high spatial and temporal variability.[20,23] Print-based fabrication of electronic devices offers a potential solution to several of these limitations, as the low-cost of manufacturing allows for large-scale deployment with high sensor counts, enabling high spatial and temporal resolution for soil monitoring. Others have demonstrated the utility of a variety of printed electronic sensors as applied in the continuous monitoring of soil physical and chemical properties, including moisture,[24–26] relative humidity,[27] pH,[28–30] nitrate,[31–34] and chloride.[35,36] We

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have previously demonstrated printed electronic sensors which transduce microbial decomposition activity into electrical resistance changes as soil microbes degrade a poly(hydroxybutyrate-co-valerate) (PHBV)/carbon composite conductor.[3] In this approach, the PHBV biopolymer (which is degraded in both aerobic and anaerobic conditions[37]), is blended with carbon flake to form a printable ink. Using a stencil-printing process the ink is deposited onto a surface and dried to create a resistor which is responsive to microbial activity. As the resistor is decomposed through biotic activity in the soil, it swells more effectively, leading to an increase in resistance over time with a gradient which dynamically corresponds to the level of microbial activity. The resistance changes that are provided by the sensor are trivially simple to measure using conventional low-cost electronics, thereby supporting high-density deployments that provide time-series data whilst keeping instrumentation costs low.

In this report, the performance of and methods for use and analysis of these sensors is evaluated in a first large-scale field deployment within a long-term grassland biodiversity restoration experiment in northern England. This site includes experimental treatments of different agricultural management practices, which have led to significant differences in soil properties and plant productivity over the site. We compared signals from the sensors and related them to standard measures of soil microbial biomass and function across 44 experimental plots over 50 days during the summer of 2023. Previous studies at this deployment site have demonstrated significant shifts in microbial communities, including their biomass and activity in response to management treatments.[38] As such, this experiment provided an excellent basis for examining the function of these novel sensors and the relationship between their response and traditional laboratory soil measurements

MATERIALS AND METHODS

Sensor Fabrication

The deployed sensors were prepared according to previous reports.[3] a single loop microbe responsive resistor (width 3 mm, length 75 mm) composed of a PHBV/carbon flake composite was printed using a laser-cut tape stencil on to a glass substrate. A conductive epoxy (*MG Chemicals 8331D*) was used to connect two leads at either side of the trace, followed by dipping in a weatherizing adhesive (*FlexSeal*) to waterproof the connections. This left the conductive polymer trace exposed (Figure 1b). Each sensor has a waterproof connector at the end of a signal wire that is roughly 15 cm in length. Sensor readout was achieved using custom made data loggers, where each logger supports up to six channels though which individual sensors can be read. Loggers were produced using low-cost electronics, including an SD-card enabled microcontroller (*Arduino, Inc.*), a lithium polymer battery, a watertight enclosure, and a timing circuit which provided power to the microcontroller every 30 minutes.

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Deployment Site and Approach

The study took place in Colt Park Meadows, located in the Ingleborough National Nature Reserve (Latitude 54°12'N, Longitude 2°21'W, 350 m.a.s.l.) in the United Kingdom. The test site is part of a long-term grassland biodiversity restoration experiment established in 1989 on agriculturally improved, species-poor mesotrophic grasslands.[39] It has provided a variety of management-relevant experimental restoration treatments on a field scale. The soil is characterized by moderate to high residual fertility in a shallow brown-earth profile. These grasslands have been targeted by various environmental land management schemes aimed at enhancing botanical diversity and soil health.[40,41] The experiment includes four main treatments with their respective controls, using a full factorial and split-plot design to provide 16 different management treatment combinations. These treatments include periodic applications of inorganic fertilizer (nitrogen:phosphorus:potassium = 20:10:10) at 25 kg nitrogen ha^{-1} y^{-1}; starting in 1990. Another involves the annual addition of farmyard manure at 12 t ha^{-1}, beginning in 1998. Efforts to enhance plant diversity began in 1990 with the addition of mixed seeds from 19 species. Furthermore, the promotion of the nitrogen-fixing leguminous herb, *Trifolium pratense* (red clover), began with seed additions in 2004, with a repeat in 2011. Each treatment combination is replicated across three blocks, resulting in a total of 48 sampling plots (each 3 × 3 m). These interventions have been shown to improve multiple soil health indicators, such as microbial biomass, enzymatic activity, and soil carbon storage.[38,42–45] As a result of these historical treatments, the Colt Park site now comprises plots that differ in plant community composition and soil microbiomes, creating a useful diverse test bed for evaluating sensor performance under different microbial conditions.

Sensor deployment occurred between June 19th and August 7th, 2023, coinciding with the end of a national drought in the UK. Sensors were installed within 44 plots, across the southern portions of all three blocks. Sensors were installed near the center of each instrumented plot, with signal lines running out to loggers (Figure 1). Within each plot, sensors were installed by cutting a slit in the soil surface, excavating a small cubic hole at the top of the slit to house the wider epoxied housing, and inserting the sensing surface containing the printed resistor taking care to minimize disturbance. Soil was then loosely packed back into the excavated space and allowed to rebound, closing the slit around the sensor.

In total 44 sensors were placed under the soil in the various experimental plots. The state of the sensors was continuously monitored over the course of 50 days using custom waterproof data loggers each capable of interrogating up to 6 individual sensors through wired connections, as depicted in Figure 1.

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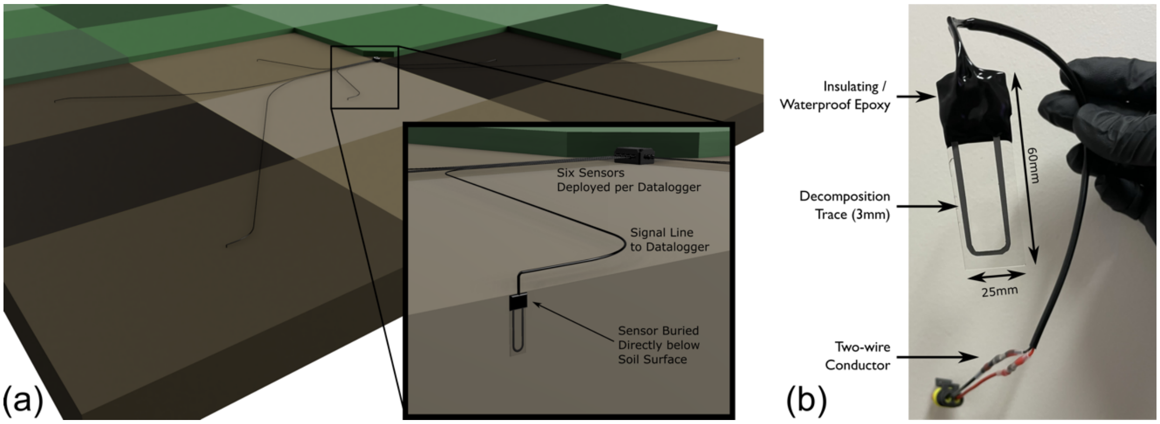

Figure 1. (a) Diagram showing the placement of printed decomposition sensors and loggers in the field. (b) Image of a single decomposition sensor.

Soil Sampling and Measurements

Within each plot, soil sampling methods were used to obtain laboratory measurements for a variety of chemical and biological parameters near the mid-point of the deployment period, in mid-July. The lab measurements included soil organic content (SOC), determined by an elemental analyzer (Elementar Vario EL CN analyzer) as no detectable inorganic carbon was found in the samples. Additionally, three extracellular enzyme activities were measured by standard colorimetric analysis:[46] glucosidase (GLC), glucosaminidase (NAG), and phosphatase (PHO), which represent carbon, nitrogen, and phosphorus decomposition or acquisition enzymes, respectively. Microbial biomass carbon (MBC) was obtained using the fumigation-extraction method.[47]

Sensor Data Analysis Methods

The soil decomposition sensors generally show a consistent increasing resistance over time in response to microbial activity in the soil (Supplemental Figures S1, S2). At some point (typically weeks after installation for this particular materials set and sensor design), the resistor material is sufficiently damaged by microbial activity and the resistance rapidly increases indicating that the device has reached the end of its useful lifetime. The data recorded, after an initial settling time and prior to this failure point, defines a decomposition window in which useful data can be extracted. In some cases, likely due to variability related to the fabrication process or issues which occur during installation, a sensor will either be non-responsive or will fail very rapidly in comparison to other devices. These devices are easily identified, and as such a first step in the data analysis pipeline is to use a polynomial fitting algorithm to remove these anomalous signals. In particular, devices with a low correlation coefficient ($R^2 < 0.5$) from a second-order polynomial regression were removed from the analysis, along with one additional sensor which had a spuriously high initial resistance. This left 31 sensors for inclusion in the analysis. With the remaining data set, three analysis approaches (Figure 2) were then evaluated: a single

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decomposition window approach (Analysis 1), an approach that uses individual sensor fits with two varying decomposition windows (Analysis 2), and a sensor signal changepoint analysis (Analysis 3), to understand how these different analytical approaches impact correlation to other field measurements.

Analysis 1 evaluated all sensor responses using a single decomposition window. To define a decomposition window for analysis, all filtered sensor signals were averaged and changepoint detection was used to define the point at which the average sensor signal changes significantly (Supplemental Figure S3). For the sensor fleet as a whole, this defined the end of the decomposition window and the beginning of the sensor failure phase. The signal following this sensor failure time was removed from the dataset. Next, a second changepoint detection was applied to the remaining data to define the beginning of the decomposition window following sensor settling in the soil. A short buffer of three days was applied at the beginning and end of the window in order to isolate the region in which sensor responses are well-defined (Supplemental Figure S4). Each sensor's slope within the decomposition window was taken using a second-order linear regression, and we took the linear coefficient to be the slope. On a per-sensor basis, sensor slopes were then compared to a range of measured attributes within the plots.

Analysis 2 differs from Analysis 1 in two primary ways: first, we considered individual decomposition windows on a per-sensor basis, varying the beginning and end of the period included in the analysis by sensor signal. Second, visual examination of the sensor signals suggested two distinct phases of decomposition, with the slope increasing more rapidly in a phase prior to total sensor failure, so we aimed to investigate the relationship between the rate of decomposition and the soil parameter measurements in two phases. In this analysis, changepoint detection was applied to each individual sensor signal iteratively. First, a changepoint analysis, assuming changes in mean and variance, was applied to each individual sensor signal to identify the time of sensor failure. The time of failure is defined as the changepoint where the ratio of variance before and after the changepoint reaches the maximum. A minimum decomposing time of 10 days was used to avoid the identification of unrealistic early time of failure due to the division of a moderate variance by a very small variance in the segment before the changepoint. The signal beyond this point was removed from the analysis. A second changepoint detection, assuming there is at most one change in the slope, was used to differentiate phase 1 and phase 2 of the remaining decomposition signal. Finally, a first-order linear regression model was applied to each phase. The estimated slope parameters from the two phases were correlated to soil parameter measurements respectively (Supplementary Figure S5).

The third analysis method focused on the number of significant changepoints observed in a given sensor's signal, rather than the rate of sensor decomposition. This analysis does not require the definition of any specific decomposition window, looking instead at the total number of changepoints detected in a particular sensor's signal during deployment prior to sensor failure .

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For this approach the failure time of each sensor signal is detected in the same way as described for analysis 2. Whereas the previous two analyses considered a polynomial fit or regression model for the remaining data, we instead continued with the changepoint approach and allowed the number of changepoints to be estimated (Supplementary Figure S6). The changepoint model includes both linear and quadratic terms to model more local features and is fit using the optimal pruned exact linear time (PELT) changepoint search algorithm[48] via the EnyCpt R package.[49] The number of detected changes was then correlated to soil parameter measurements (Figure 2, Analysis 3).

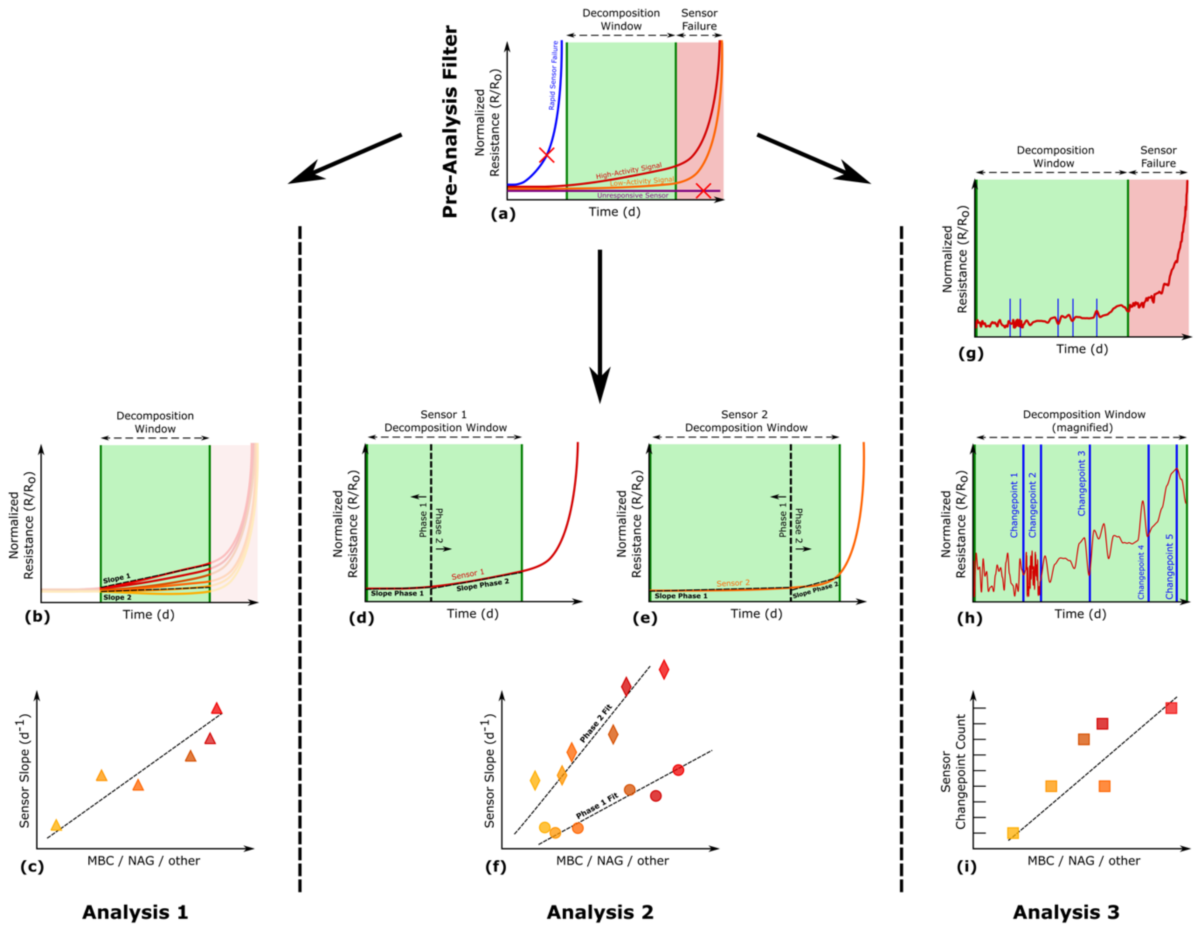

Figure 2. Illustration of the data extraction procedure for the three analysis approaches. For analysis approach 1 slopes within the decomposition window are extracted and are correlated to laboratory measurements. The second analysis broke each sensor signal into two sensor-specific decomposition windows (d, e). On a per-sensor basis, correlations were then taken between the measured parameter and slopes within each phase (f). The third approach used the number of decomposition function changepoints as a proxy for microbial activity and stability, breaking each sensor signal into sensible changepoints (g, h). On a per-sensor basis, the number of changepoints was then correlated to each measured parameter (i).

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The goal of this work was to determine whether the sensor signals mirrored more conventional measurements from the plots. To this end, we carried out a series of regression analyses of sensor signals against measured soil attributes and present these analyses and the associated R^2 and p values. It should be noted that based on the focus of the study, sufficient sensors were not available in order to replicate a standard randomized design for evaluation of treatment differences. All data analysis was carried out with R version 4.3.2. utilizing the changepoint detection package,[50].

RESULTS AND DISCUSSION

Soil indicators

As expected, due to differing soil conditions originating from combinations of historical management treatments, lab measurements of soil parameters show a wide range of values across the experimental plots. These indicators are used in current soil health measurements,[14,51–53] and are commonly known as key factors controlling soil organic matter decomposition rates. SOC is one of the most widely used soil health indicators,[14] given its role in enhancing soil physical stability, improving water retention, and carbon sequestration. The SOC content ranges from 6.65% to 10.58% across all plots, indicating generally high but variable substrate availability for microbial decomposition. MBC represents a small yet active carbon pool in this study, comprising approximately 2.1% of the total SOC on average, consistent with findings from other studies.[54] It has been suggested as dominant factor that controls the overall decomposition rate of soil organic matter.[55] Extracellular enzymes in the soil, mainly produced by microbes, are directly involved in the breakdown of certain organic materials and nutrient cycling (e.g., carbon, nitrogen, phosphorus cycling), thereby directly affecting plant growth and soil fertility,[56] and can be used to indicate substrate-specific decomposition processes. The enzymes measured here also exhibit high variability (Figure 3), highlighting their sensitivity to management differences. Together, these indicators provide a comprehensive picture of soil health and the soil decomposition process. Monitoring these parameters helps in understanding the impacts of ecosystem management practices and environmental changes on soil health and guides sustainable soil management strategies, and for the purposes of this study demonstrates that a wide range of soil conditions are available at this test site.

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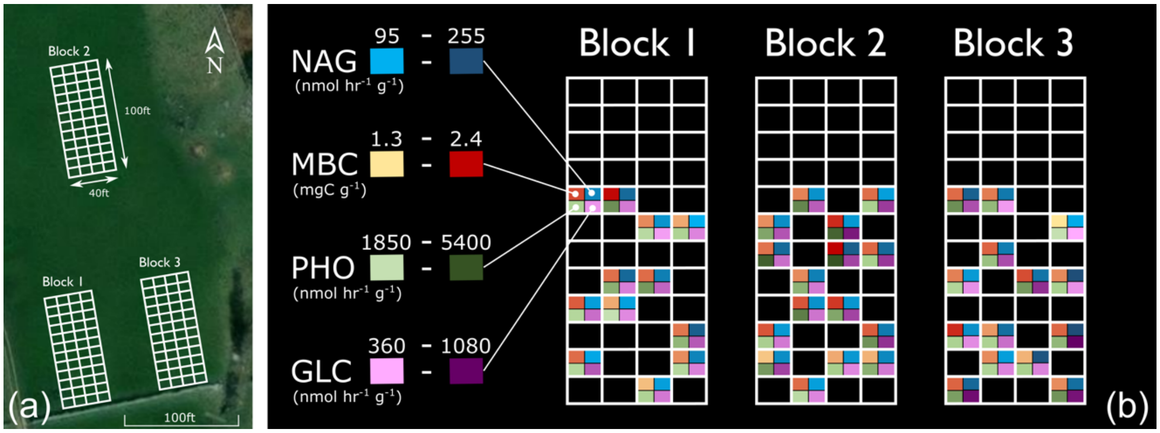

Figure 3. (a) Satellite image of Colt Park Meadows, showing the positions of the 3 replicate experimental blocks, each of which contain 48 plots (*EarthSat image courtesy of the U.S. Geological Survey).* (b) Results from biological soil property measurements taken from within each plot that was instrumented with a decomposition sensor.

Sensor Data Analysis

Analysis 1: Single Decomposition Window

The results of Analysis 1 indicate that the rate of resistance change recorded by the sensor is correlated with conventional measures of microbial biomass and activity, with significant variability due to the hardware constraints of the deployed sensors. A positive correlation was observed with MBC ($R^2 = 0.53$, $p = 1.2\times10^{-5}$) and a negative correlation with the ratio GLC:NAG ($R^2 = 0.21$, $p = 0.014$), which has been considered as an index for early chitin decomposition processes.[57] A significant but weaker correlation is also observed with SOC ($R^2 = 0.16$, $p = 0.035$) (Figure 4).

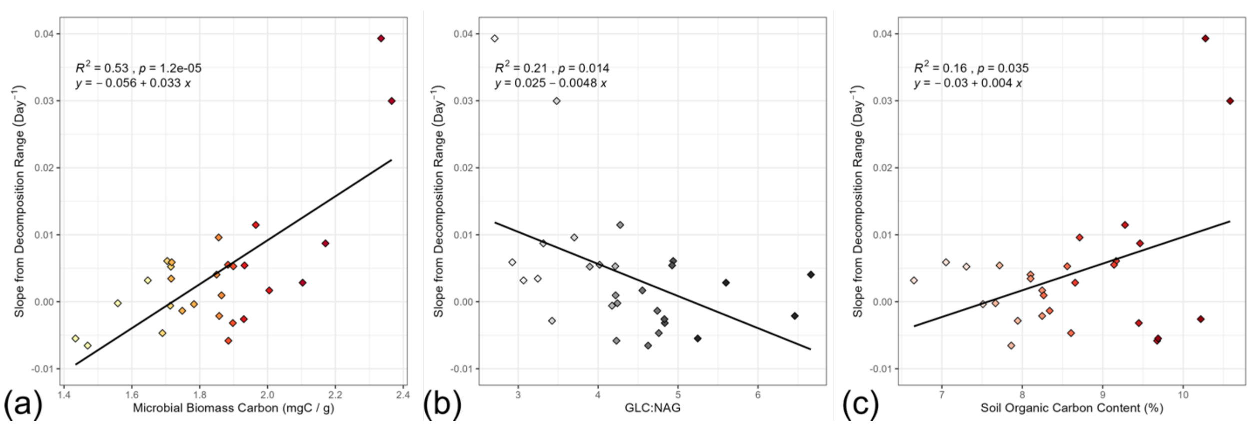

Figure 4. Result of a single decomposition window analysis approach, comparing sensor response to (a) microbial biomass carbon (b) GLC:NAG ratio, and (c) soil organic carbon content.

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Analysis 2.

Considering two sensor-specific decomposition windows determined by changepoint analysis, Phase 1 and Phase 2 slopes positively correlated to MBC ($R^2 = 0.52$, $p = 0.003$, and $R^2 = 0.56$, $p = 0.001$ respectively). Weaker, yet still-significant correlations were found with SOC ($R^2 = 0.37$, $p = 0.043$, and $R^2 = 0.44$, $p = 0.015$ respectively). This suggests that there is utility to both Analysis 1 and Analysis 2, with useful biological information extractable from the fleet as a whole or from individual sensors, even at this early stage of sensor development.

Analysis 3

This approach found correlations between the number of significant changepoints in the time series data and laboratory measurements within plots. Statistically significant correlations were found with a number of measurements (SOC $R^2 = 0.53$, $p = 0.001$; PHO $R^2 = 0.46$, $p = 0.006$; MBC $R^2 = 0.37$, $p = 0.033$, GLC $R^2 = 0.36$, $p = 0.038$). It is worth noting that in the sites with higher numbers of detected change points, we see a high number of detectable events over a relatively short deployment period, suggesting that useful information can be gleaned by monitoring at this relatively high frequency. It is also interesting that the more static carbon-related measurement, SOC, correlates more strongly with the sensor signal in this approach than MBC does.

All of the data analysis approaches described here resulted in statistically significant correlations with MBC and SOC indicating a robust correlation to these soil properties. All statistically significant results ($p < 0.05$) are shown below (Table 1).

Table 1. Decomposition sensor signal correlations to laboratory measurements, all significant at p<0.05, n.s. indicates no statistically significant correlation was detected.

	Soil health parameter					
Method	MBC	GLC	NAG	PHO	GLC:NAG	SOC
Analysis 1	0.53	n.s.	n.s.	n.s.	-0.21	0.16
Analysis 2, Phase 1	0.52	n.s.	n.s.	n.s.	n.s.	0.37
Analysis 2, Phase 2	0.56	n.s.	n.s.	n.s.	n.s.	0.44
Analysis 3	0.37	0.36	n.s.	0.46	n.s.	0.53

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Potential Environmental Application

The sensor described here shows promise in enabling wide-scale, in-situ, time-series data collection that relates to widely used soil biological properties related to soil health. Low-cost printed microbial sensors could support efforts to track and verify the rehabilitation of degraded land and contribute to food security-related environmental monitoring. We have shown here that several statistical approaches suggest a robust relationship between the sensor signal and microbial biomass carbon and soil organic carbon, important indicators of soil health. Additionally, a binning and averaging approach resulted in a negative correlation between sensor response and the GLC:NAG ratio. With improvements to the fabrication of these decomposition sensors, large-scale deployments could help overcome current limitations in soil biological health monitoring programs.

Prominently, all three analysis approaches found significant correlations between the sensor signal and measured MBC and SOC values in instrumented plots. Prior studies have shown a positive correlation between MBC and organic matter mineralization,[58,59] and have, in fact, posited it as a more useful indicator when compared with other forms of SOC, including particulate organic carbon (POC) and mineral associated organic carbon (MAOC).[60] MBC is also often used as a biological indicator of soil health, showing promise as an early indicator of ecological change following land use change and changes in agricultural practices.[61–64] A low-cost and easily distributable solution for continuous MBC-proxy tracking would offer a new high-spatiotemporal resolution window into these dynamics.

A potential application for this sensing approach is to continuously monitor the soil microbiome's metabolism and activity immediately following environmental perturbations; this could yield useful insights into the resilience of the microbial community in the face of shocks including droughts or agricultural practices like pest fumigation. These sensors could determine whether critical nutrient cycling capacity is impacted by such events and help to characterize the resilience of the microbiome by monitoring changes in nutrient cycling responses over time.

A primary challenge in interpreting this sensor data and making the results useful is the stochastic response of individual sensors, both initially on installation and as decomposition rates increase and sensors begin to fail. Upon insertion, the resistor can be damaged, rendering the sensor useless; even with great care taken during installation, one sensor's initial resistance in this study indicated that physical damage had immediately occurred. Furthermore, if sensor failure is rapid the useful life of the sensor can be meaningfully reduced. It is worth noting that time to failure was not found to have a statistically significant correlation to measured soil parameters. The rate of decomposition in analytically useful windows, on the other hand, does. To address these issues ongoing experiments are examining approaches to control the degradation process through material and hardware modifications, in order to improve reliability of and reduce sensor-to-sensor variability.

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A promising aspect of this research is the apparent specificity of this particular printed composite conductor with regard to soil processes. Correlations with some traditional measures were strong, where other parameters of interest correlated weakly or not at all with the sensor signal. In particular, the more static measurement, SOC, correlated less significantly than MBC, which is a more dynamic measurement. Furthermore, several approaches found varying correlations with different sets of laboratory measurements. Significant opportunities might lie in the exploration of other conductor formulations, applied to a similar deployment platform, but targeting other decomposition pathways by selecting alternate binders. Another valuable aspect of the sensor response is that the signal should be related only to living microbes, avoiding issues associated with relic DNA, further enhancing the sensor's specificity.

CONCLUSION

This report describes the first field deployment of a novel soil sensor designed to determine microbial decomposition activity through a unique transduction mechanism based on a soil degradable resistor. These low-cost sensors, manufactured from readily available materials using printing approaches, and addressable by simple electronics could enable spatially dense, time-series data collection of parameters related to soil health. This study provides an initial step towards providing useful environmental data collection from this sensing approach by deploying 44 sensors over 50 days at an experimental field site with widely varying soil microbial properties. Three approaches for extracting data from the sensor signal were evaluated, with the principal finding being that all analyses demonstrated a robust correlation to microbial biomass carbon, and soil organic carbon. The results described here suggest powerful potential applications for these in-situ transient decomposition sensors to help characterize soil health trajectories at high resolution, aligning with the need for larger spatial and temporal scales of soil microbiome monitoring.

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