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RESEARCH LETTER

Which indicators are most effective at detecting rapid shifts in soil health?

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Abstract

Farmers are showing a growing interest in soil health. Therefore, it is necessary to understand how and when indicators respond to changes in land management. Measurements of soil carbon (C) and nitrogen can take up to a decade to shift. However, it is unknown how other biotic markers of soil health (i.e., nematode communities) react. Here we use the long-term agroecosystem research trial at the W. K. Kellogg Biological Station to determine which biotic indicators respond first to the implementation of sustainable agricultural management. High-diversity perennial forage and native prairie had higher nematode abundances compared to monoculture crops with perennial forage also being compositionally distinct. Additionally, nematode abundance was higher in the aspirational corn (*Zea mays*) treatment than conventionally managed corn ($p < 0.05$). Mineralizable carbon (minC) was marginally greater in high plant diversity treatments ($p < 0.1$). Overall, nematode abundance, in combination with incremental changes in labile C, are indicators of rapid shifts in soil health.

Plain Language Summary

Soil health is a major concern for farmers. However, it can be challenging to make sustainable management choices when tangible benefits can take a decade to materialize. The goal of this study is to determine if we can detect changes in soil health parameters only one growing season after a management shift. To address this goal, we used the newly implemented aspirational cropping system experiment, which had three treatments: (1) a five-crop rotation with cover crops, no-till, and other sustainable management; (2) a two-crop rotation with conventional management; and (3) a native prairie system. We focused on four soil health parameters related to soil carbon, nitrogen, and nematodes. Our results show that nematodes were more numerous in

Abbreviations: ACSE, aspirational cropping system experiment; ASP, aspirational; BAU, business-as-usual; KBS, W. K. Kellogg Biological Station; LTAR, Long-Term Agroecosystems Research Trial; minC, mineralizable carbon; PERMANOVA, permutational multivariate analysis of variance; POXC, permanganate oxidizable carbon.

certain crops relative to perennial systems when using sustainable rather than conventional management. These findings offer farmers and researchers an effective means of rapidly evaluating soil health after implementing a new management strategy.

1 | INTRODUCTION

Farmers continue to be interested in soil health because of its association with long-term sustainability and capacity to inform future management decisions (O'Neill et al., 2021; Singh et al., 2024). One criticism of the soil health framework is that there are too many indicators making soil health tests expensive and confusing to farmers (Thomsen et al., 2019). This has motivated scientists to identify which soil health indicators are most effective and useful (Singh et al., 2024). Several scientists argue that the most effective soil health indicators are responsive to recent changes in management (Morrow et al., 2016). Yet, it remains unclear which indicators can detect such responses. This is especially important because the tangible benefits of regenerative agriculture practices often take years to materialize (Cusser et al., 2020). If soil health indicators can serve as early predictors of ecosystem services, they could provide farmers with timely insights into whether their management decisions are in alignment with their sustainability goals.

Recently biological soil health indicators have gained attention from scientists and farmers because of their responsiveness to management (Lehmann et al., 2020; Singh et al., 2024). These indicators include permanganate oxidizable carbon (POXC) (Culman et al., 2012; Weil et al., 2003) and mineralizable C (minC) (Franzluebbers et al., 2022; Hurisso et al., 2016), which represent two different pools of soil C (Sprunger & Martin, 2023), and soil protein, which reflects bioavailable nitrogen (N) (Gillespie et al., 2011; Naasko et al., 2024). Additionally, nematodes have been consistently linked to soil health and more frequently used as indicators within agricultural systems (Martin et al., 2022; Neher, 2001). Through trophic interactions nematodes play a role in several nutrient cycles, including C and N (Bonaglia et al., 2014; Creamer et al., 2016). Nematodes can also be preserved and identified via microscopy, which can provide an accessible and more affordable biotic indicator compared to other soil microorganisms.

While it takes a minimum of 3–5 years after a management shift to detect changes in most soil health indicators (Angers & Eriksen-Hamel, 2008; Stewart et al., 2018), it is not yet clear as to what rate nematode communities respond. There is evidence that both plant parasitic and free-living nematodes can respond rapidly to stimuli (Anderson & Coleman, 1981; Curtis, 2008; Pline & Dusenbery, 1987; Rasmann et al., 2012). However, previous works have largely focused on the long-term effect of management decisions on nematode communities (Henneron et al., 2015; Li et al., 2010; Martin & Sprunger, 2022). Evidence of short-term changes in nematode communities often only focuses on one major disturbance or management shift (e.g., soil amendments or drought) (Homet et al., 2023; Stefanovska et al., 2022).

To determine if nematode communities can be used as indicators of substantive and rapid shifts in soil health because of management decisions, we utilized the newly implemented Long-Term Agroecosystems Research Trial (LTAR) at the W. K. Kellogg Biological Station (KBS) (Figure 1). The LTAR was developed with farmer input to evaluate the influence of management strategies on soil health, water quality, greenhouse gas mitigation, and biodiversity conservation (Robertson et al., 2024). Specifically, the experiment works to compare business-as-usual (BAU) management to an aspirational cropping system (ASP) that incorporates rotational diversity, cover crops, no-till, perennial forages, and manure management. The goal of this study is to determine which indicators respond most rapidly to the aspirational cropping system experiment (ACSE). We hypothesize that the nematode communities will respond more quickly relative to other soil health indicators that reflect biological activity. We expect this shift to be observed at the trophic scale and reflect potential functional changes in nematode community composition.

2 | MATERIALS AND METHODS

This study took place at the KBS LTAR's ACSE located in Southwest Michigan (42.4024, −85.3790). Prior to experimentation, corn (*Zea mays*) was planted for one season. The experiment was established in Fall 2021, whereby perennial forages and native prairie (NP) were planted. The experiment is a randomized complete block design with four replicates (Figure 1). Treatment is the main effect: BAU, ASP, and NP. The BAU treatment consists of a corn–soybean *(Glycine max)* rotation and is managed with standard agricultural practices, including tillage and regular scheduled fertilizer applications, mirroring the prevailing regional practices. The ASP treatment has a high diversity crop rotation that includes corn–soybean–wheat (*Triticum aestivum*)–canola (*Brassica napus*), and perennial forage (five species), along with multiple cover crops. Each cash crop phase of the rotation is present every year and represents the sub-factor. The NP treatment is permanent with plots in each block. There are a total of 32 plots.

More information on the various treatments and experimental design can be found in Figure 1 and Robertson et al. (2024).

2.1 | Soil sampling during each phase of the crop rotation

Soils (Kalamazoo Loam) from each of the 32 plots were collected in December 2022 and mark the end of the first official growing season in ASCE. From each plot a 1.91-cm diameter soil probe was used to collect 10 cores at a depth of 10 cm. Cores were composited and stored at 4˚C until processing. During sampling, the crop phases (hereafter referred to as crop) were BAU corn and soybeans; ASP corn, soybeans, wheat, canola, and perennial forage; and NP. In 2022, the BAU systems were tilled, had standard fertilization, and had pests that were conventionally managed. The ASP crops were no-till, organic fertilizer was applied to corn, and integrated pest management was deployed (Figure 1).

2.2 | Soil health parameters

Biotic soil health indicators included nematode communities, POXC, soil protein, and minC. Nematodes were extracted from 50 g of soil using the Baermann funnel extraction method (Baermann, 1917). Extracted nematodes were fixed in 4% formalin. Nematode abundance was quantified using an inverted microscope. For each sample the first 100 nematodes were identified to trophic group and family. Before analyses of other soil health indicators, soils were sieved at 2 mm and air-dried. POXC was quantified by methods adapted from Weil et al. (2003) and Culman et al. (2012). Soil protein was quantified by methods adapted from Hurisso et al. (2018) and Moebius-Clune et al. (2016). Lastly, minC was quantified via a 24-h assay measuring CO_2 respired from rewetted soil (Franzluebbers et al., 2022; Hurisso et al., 2016).

2.3 | Statistical analyses

All analyses were performed using R software (R Core Team, 2021). General linear mixed models (*glmmTMB*) were used to determine if soil health parameters differed by treatment (main factor) and crop (sub factor), both of which were independent variables. The NP treatment was aggregated with ASP for analyses. The *DHARMa* and Shapiro tests were used for model verification. Analysis of variance and *emmeans* using Tukey's honestly significant difference were used for post hoc analyses. For all models, block was treated as a random effect, and significance was set at $p < 0.05$.

Non-metric multidimensional scaling using Bray–Curtis dissimilarity matrices and permutational multivariate analysis of variance (PERMANOVA) were used to determine compositional differences for nematode communities at the trophic level. In the PERMANOVA, treatment and crop were independent variables. Significant results were presented at trophic level and visualized as ordination and relative abundance plots. All graphs were made with *ggplot2*.

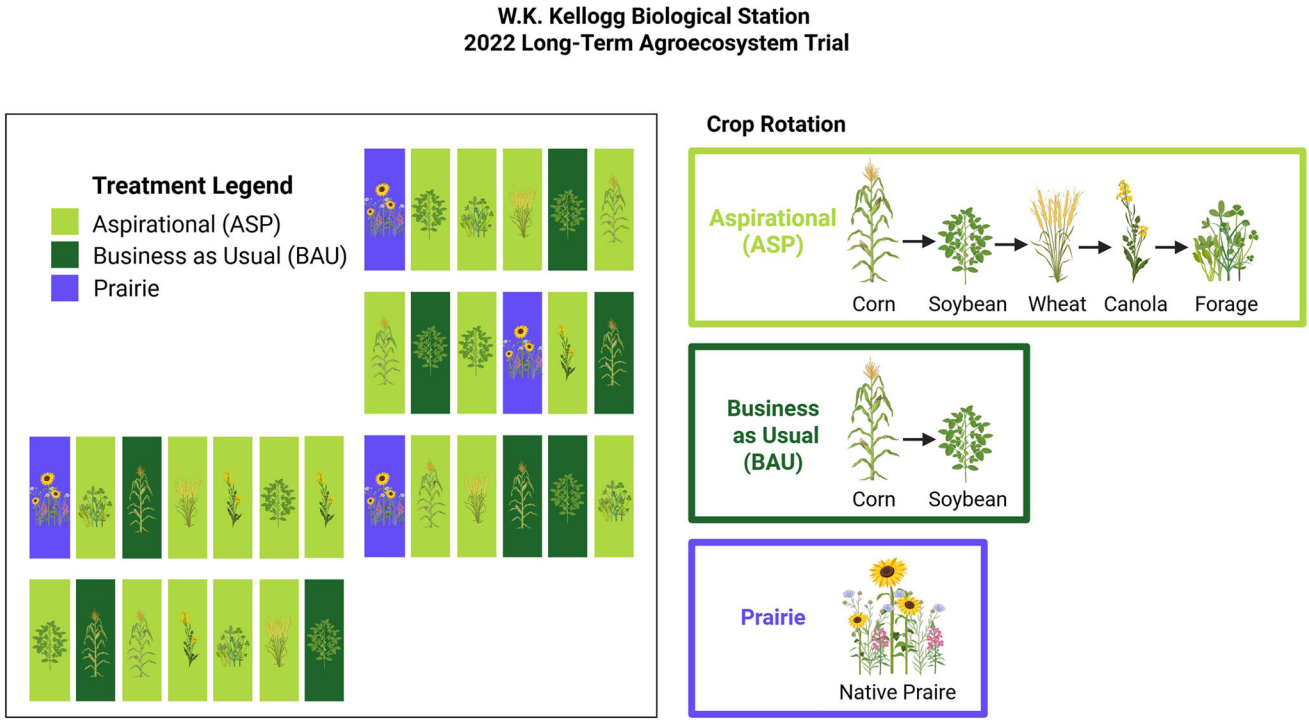
Core Ideas

- Nematode abundance is highest in treatments with high plant diversity.
- Perennial forage nematode communities are compositionally distinct from soybean and corn nematode communities.
- Mineralizable carbon (minC) is marginally higher in high plant diversity treatments and the aspirational corn treatment than conventionally managed crops.

3 | RESULTS AND DISCUSSION

One year post establishment, nematodes were the only indicator that significantly responded to treatment (Figure 2D; $p < 2.2e\text{-}16$). For instance, perennial forage and NP supported more nematodes than ASP soybeans, all treatments of corn, ASP wheat, and ASP canola. BAU soybeans also had high nematode abundance. Additionally, ASP corn supported more nematodes than BAU corn ($p < 0.05$). In contrast, treatment did not have a significant effect on other soil health indicators (Figure 2B,C). However, minC was marginally influenced by crop (Figure 2A; $p < 0.1$). Perennial forage and NP had the highest rate of minC compared to other crops, although perennial forage versus canola was similar, and ASP corn had a trend of higher minC than BAU corn.

The increase in nematode abundance combined with the slight increase in minC in high plant diversity and ASP corn treatments are likely an indication of higher soil health one growing season after a management shift. Conversely, higher nematode abundance despite low minC in the BAU soybeans (Figure 2A,D) may be an artifact of this specific crop rather than a sign of a substantive shift in soil health. Previous work found that legumes have a higher abundance of nematodes than non-legumes, but this did not translate to significant increases in soil C (Ikoyi et al., 2023). This indicates nematodes may be competing for a limited, homogenous pool of resources in soybeans, whereas crop and plant diversity is often tied to increased soil fertility and nutrient availability as well as long-term nematode community success and soil health (Dybzinski et al., 2008).



2021-2022 Management Practices

	ASP	BAU
Tillage Prior to Planting	No	Yes
Cover crop installed Fall 2022	Yes	No
Fertilization	Organic N (minimal rate in corn only)	Inorganic N (standard rates)
Pest Management	Integrated Pest Management	Conventional

FIGURE 1 Depiction of the aspirational cropping system experiment (ACSE) at the W. K. Kellogg Biological Station. The aspiration treatment includes a five-crop rotation and the business-as-usual treatment includes a two-crop rotation. The native prairies are permanent. The map depicts the crop status for each plot in 2022. In addition to a high-diversity crop rotation, the aspirational treatment also had integrated pest management, no-till, cover crops, and variable fertilizer inputs. The business-as-usual treatment had chisel plow tillage, scheduled pesticide applications, and flat rates of fertilizer. Management practices implemented in 2021–2022 (before, during, and after growing season) are depicted in the table.

Our results are in line with previous research showing a long-term increase in nematode abundance after a change in management intensity (i.e., ASP vs. BAU). For instance, long-term studies of agroecosystems that incorporate sustainable agricultural practices saw an increase in nematode abundance and soil health along with improved crop and soil productivity (DuPont et al., 2009; Liu et al., 2016). Additionally, increasing plant diversity appears to have the strongest effect on nematode abundance, which aligns with previous work where there was a strong positive relationship between the two (Cortois et al., 2017; Dietrich et al., 2021; Ikoyi et al., 2023). Evidence from the two corn treatments also indicates a positive effect of other management changes (i.e., no-till; reduced inputs). Moreover, these factors, together with perenniality, likely compounded the positive effect of plant diversity on nematode abundance in the NP and perennial forage treatments relative to the other ASP treatment (Martin & Sprunger, 2021). Accordingly, tillage and organic amendments affected nematode abundances in bananas, rice, and row crops, and these abundances increased further as crop rotational diversity increased (Freckman & Ettema, 1993; Nguyen et al., 2020; Zhong et al., 2016). Additional studies also show a strong connection between soil nematode density and trophic diversity and a combination of crop rotational diversity and reduced tillage (Carter et al., 2009; Neher et al., 2019).

The nematode communities within perennial forage were compositionally distinct from soybean and corn communities, regardless of treatment (Figure 3A; $p < 0.05$). Those differences were driven by higher numbers of bacterivore species and lower numbers of herbivores in perennial

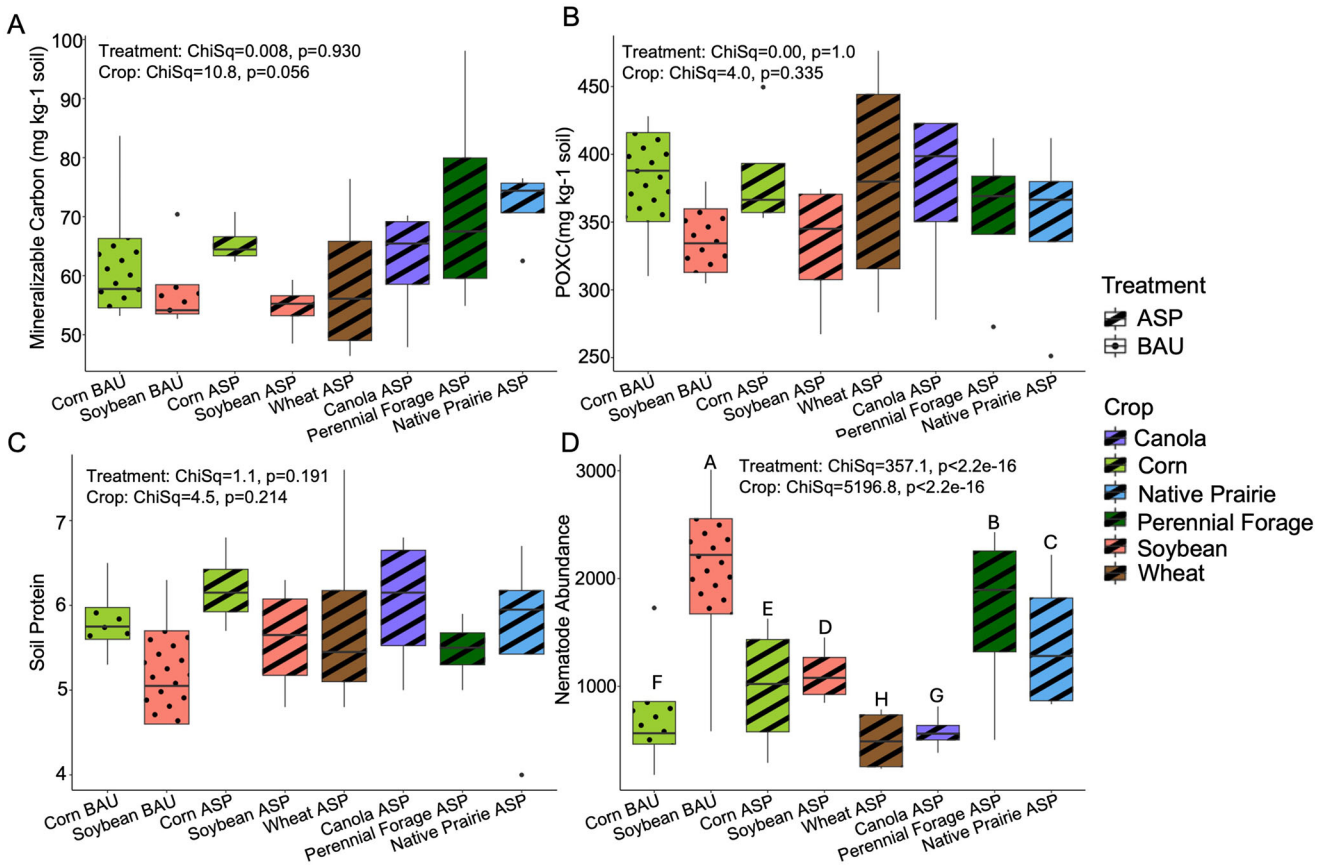
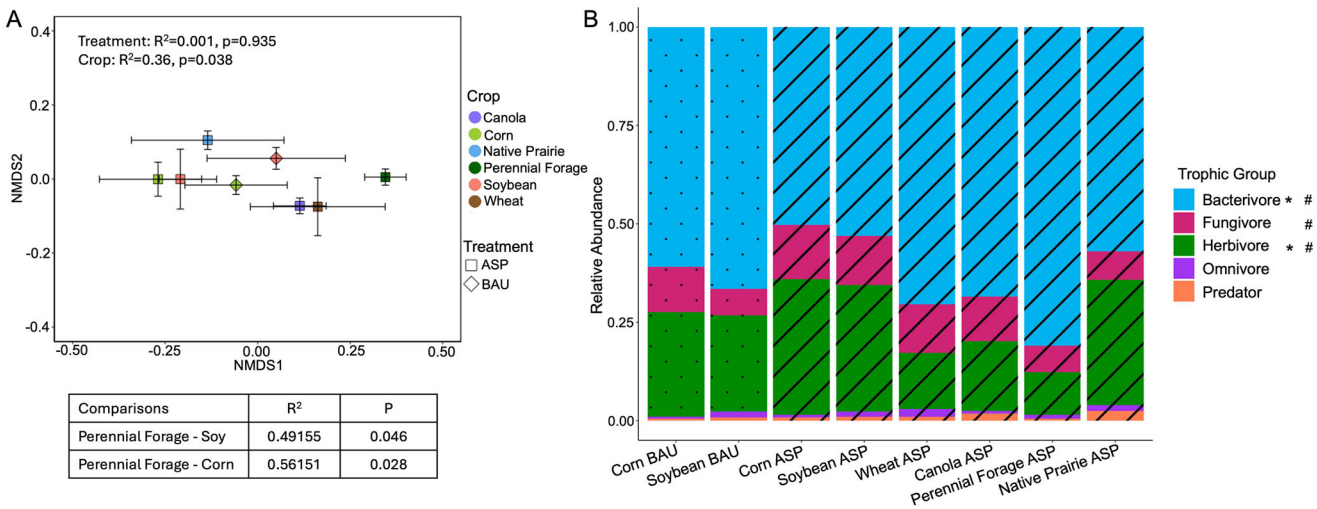


FIGURE 2 Mineralizable C (A), permanganate oxidizable carbon (POXC) (B), soil protein (C), and nematode abundance (D) from the aspirational (ASP) and business-as-usual (BAU) treatments across all crop types. Color represents crop types and pattern indicates treatment. General linear mixed models were used to determine significant differences caused by treatment ($df = 1$) and crop types ($df = 5$). Letters indicate significant differences ($p < 0.05$).



Comparisons	R^2	P
Perennial Forage - Soy	0.49155	0.046
Perennial Forage - Corn	0.56151	0.028

FIGURE 3 Nematode community composition (A) and relative abundance (B) at the trophic level. Statistical analyses for the ordination plot are reported according to permutational multivariate analysis of variance (PERMANOVA) where crop ($df = 5$) type (i.e., combined aspirational [ASP] and business-as-usual [BAU] for corn and soy reported in table) significantly affected composition, but treatment ($df = 1$) did not. Centroids were calculated and plotted as larger data points with individual repetitions being smaller and more transparent. Statistical analyses of the relative abundance by general linear models were reported with symbols on the figure legend. The "*" symbol indicates a marginal effect ($p < 0.1$) of treatment, and the "#" symbol indicates a significant effect ($p < 0.05$) of crop type.

forage (Figure 3B; $p < 0.05$). The major herbivore families found in all treatments were Pratylenchidae and Tylenchidae. The composition of the NP was not different despite the similar increase in nematode abundance as perennial forage. Evidently, changes in nematode composition are more highly variable in response to management shifts than nematode abundance (Gonzalez et al., 2025). While nematode composition may not respond consistently to rapid change in management, shifts in composition, regardless of time, should be considered a sign of soil health improvement given the strong relationship between trophic complexity and soil health (Ferris et al., 2001; Martin & Sprunger, 2022; Pires et al., 2023).

Here we have demonstrated that researchers may be able to use nematode communities, specifically nematode abundance, in combination with marginal shifts in other soil health parameters to indicate early changes in soil health. These results will improve our ability to guide farmers in sustainable soil health management. However, while nematode communities appear to be a reliable and expedient marker of soil health, skill, and labor required to identify nematodes is still a concern. Future work should continue to focus on technologies (i.e., artificial intelligence) that could more rapidly identify nematodes to provide an inexpensive, reliable soil health indicator for farmers.

AUTHOR CONTRIBUTIONS

Kaitlin Gattoni: Formal analysis; visualization; writing—original draft; writing—review and editing. **Meredith Mann**: Data curation; visualization; writing—review and editing. **Christine D. Sprunger**: Conceptualization; funding acquisition; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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