

# SOIL HEALTH RESPONSES TO INTERSEEDED COVER CROPS AND NITROGEN STRATEGIES IN THE NORTHERN CORN BELT

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## ABSTRACT

Cover cropping and nitrogen (N) management are often promoted for improving soil health, yet their combined influence under interseeded systems in temperate regions remains less understood. To address this gap, a field study was conducted in South Dakota at two no-till corn–soybean rotation sites (Brookings and Beresford) established on clay loam soils. Cover crop treatments included a no cover, a single grass species, and a multi-species mixture of grass and broadleaf species interseeded at the V6 stage of corn, combined with three nitrogen (N) rates (low, medium, and high) applied 10 days after planting. Soil health was assessed at three growth stages (V6, R1, and R6) using indicators such as active carbon (Active C), aggregate stability, soil organic carbon (SOC), soil organic matter (SOM), potentially mineralizable nitrogen (PMN), nitrate-N, and ammonium-N. Cover crops produced relatively little biomass (maximum 0.9 Mg ha<sup>-1</sup>, average 0.5 Mg ha<sup>-1</sup>), which likely explains why no significant effects on soil health indicators were observed. In contrast, higher N fertilization rates increased PMN, nitrate-N, and ammonium-N, reflecting greater nutrient availability. Sampling time also shaped responses: Active C and SOM peaked at V6, indicating strong early-season microbial activity and fresh residue inputs, whereas SOC and aggregate stability were highest at R6, suggesting improved soil structure later in the season. Overall, short-term cover cropping had a minimal influence on soil health, while the nitrogen rate and sampling time exerted significantly stronger effects on nutrient dynamics and soil properties.

## INTRODUCTION

Soil health underpins sustainable crop production by supporting nutrient cycling and environmental resilience (Davis et al., 2023; Tahat et al., 2020). However, intensive agriculture often depletes nutrients and accelerates erosion, prompting interest in conservation practices such as cover cropping and improved N management (Teng et al., 2024). While both practices independently benefit soil quality, their combined effects under temperate rainfed systems remain less understood.

The benefits of cover crops are well established in tropical regions (Farmaha et al., 2022). However, their adoption in temperate production systems is constrained by short growing seasons, cool soils, and early frosts (Blanco-Canqui et al., 2015). These factors limit biomass production and reduce potential gains in SOM, microbial activity,

aggregation, and nutrient cycling (Ruis et al., 2019). Interseeding cover crops into standing crops helps overcome these challenges by extending the growing window. Research suggests that V6–V7 corn stages strike a balance between canopy openness and minimal crop competition (Brooker et al., 2020; Peterson et al., 2019). The choice of species is also critical as grasses promote SOM and aggregation through high C: N residues, legumes contribute N through fixation, and brassicas enhance rooting and reduce leaching (Blanco-Canqui & Ruis, 2020).

Additionally, nitrogen management interacts with cover crops to influence soil processes. At high fertilizer rates, grasses can reduce excess residual N, while brassicas help limit leaching losses; in contrast, at moderate rates, legumes become important contributors of biologically fixed N that complements fertilizer inputs. These dynamics mean that the soil health effects of fertilization depend not only on the rate applied but also on the functional traits of the cover crop species present (Geisseler & Scow, 2014; Finney et al., 2016).

Another layer of complexity arises from the timing of soil sampling. Soil indicators such as Active C, PMN, and inorganic N pools fluctuate across the season, with early stages reflecting microbial activity and nutrient release, and later stages capturing soil structural improvement (Hurisso et al., 2018; Kong & Six, 2010). Despite recognition of these dynamics, few studies have assessed how cover crop mixtures, N rates, and sampling times interact across a full season in temperate systems. Accordingly, the objective of this study is to assess the combined effects of cover crop composition, N fertilization rate, and sampling timing on soil health indicators in a no-till corn–soybean rotation in South Dakota.

## **MATERIALS AND METHODS**

The study was conducted from 2021 to 2022 at two no-till corn–soybean rotation sites established in 2012 near Brookings and Beresford, South Dakota. Both sites have clay loam soils, although Brookings is primarily composed of Egan–Clarno–Tetonka, and Beresford also includes Egan–Trent silty clay loams. Average long-term precipitation is ~500 mm annually.

Both sites followed a split-plot design with four replications. The whole plot received one of the three cover crop treatments: no cover, annual ryegrass, or a four-species mixture (perennial ryegrass, crimson clover, turnip, and radish). Subplots received one of the three N rates: low (0 kg N ha<sup>-1</sup>), medium (75 kg N ha<sup>-1</sup> at Brookings, 100 kg N ha<sup>-1</sup> at Beresford), or high (150 and 200 kg N ha<sup>-1</sup>, respectively). Cover crops were interseeded at the V6–V7 stage of corn using a high-clearance planter. Fertilizer was surface-applied 7–10 days after planting as SUPERU® stabilized urea.

Pre-plant samples (0–15 cm and 15–60 cm) were collected to establish baseline fertility. In-season samples (0–15 cm) were taken at V6, R1, and R6. The indicators

measured included Active C, SOM, SOC, PMN, aggregate stability, nitrate-N, and ammonium-N. Standard laboratory methods were used: POXC for Active C, loss-on-ignition for SOM, dry combustion for SOC, anaerobic incubation for PMN, wet sieving for aggregate stability, and flow injection analysis for inorganic N.

Data were analyzed in R (version 4.4.1). Linear mixed-effects models (lme4, lmerTest) were used to test the effects of cover crops, N rate, and sampling time, with site-year and block included as random factors. When significant effects ( $p < 0.05$ ) were detected, mean separations were performed using Sidak-adjusted comparisons with the emmeans package.

## RESULTS AND DISCUSSION

Seasonal temperature and rainfall patterns strongly influenced soil biological responses. At both sites, precipitation in 2021 was close to or slightly below the 30-year average; however, the distribution varied. Brookings recorded moderate early deficits followed by excess rainfall in late summer, while Beresford experienced severe June drought and wetter conditions later. These fluctuations likely disrupted synchronization between N supply and crop demand, reducing microbial activity early but increasing late-season N losses.

Cover crops had no significant effect on soil health indicators measured (Table 1). Similar short-term studies have shown that measurable improvements in SOM, SOC, or aggregation often require longer than four years of consistent cover cropping (Blanco-Canqui & Ruis, 2020; Decker et al., 2022). Low biomass production in this study (maximum  $0.9 \text{ Mg ha}^{-1}$ , average  $0.5 \text{ Mg ha}^{-1}$ ) also contributed to the lack of response, as previous research suggests at least  $3\text{--}5 \text{ Mg ha}^{-1}$  is necessary for detectable improvements (Kaspar & Bakker, 2015; Nichols et al., 2020). The corn–soybean rotation may have further limited effects compared to more diverse systems that return greater organic inputs (Reisner et al., 2021). In addition, bulk sampling to 15 cm may have diluted near-surface changes, as differences in SOM and SOC are often most pronounced in the top 3–5 cm (Franzluebbers, 2002).

Table 1 F-test significance of site-year, cover crops, nitrogen rate, and sampling time, and their interactions on soil parameters, 2021–22.

Cover crop	Active C	PMN	SOM	WSA	SOC
	mg kg <sup>-1</sup>		LOI %	%	
No Cover	436a	47a	4.2a	16.8a	2.02a
Single Species	439a	47a	4.2a	17.3a	2.01a
Mixed Species	443a	47a	4.2a	17.2a	1.99a

*Note:* Within each column, means followed by the same letter are not significantly different at  $p > 0.05$

Despite minimal treatment effects, strong interactions between site-year and sampling time were evident. Active C peaked early (V6) (Figure 1), reflecting microbial stimulation, then generally declined by R6, though patterns varied by year. PMN

followed the expected seasonal declines from preplant to mid-season, with late increases in the wetter 2021 season, but continued reductions under the 2022 drought. Aggregate stability generally improved through R1 and declined by R6, again reflecting shifts in biological activity and soil moisture. SOM rose modestly in 2021 but decreased in 2022, indicating sensitivity to seasonal climate fluctuations. SOC showed both increases and declines across site-years, highlighting the importance of within-season dynamics and environmental context. Together, these results highlight the significance of sampling time as a crucial factor influencing observed soil health trends.

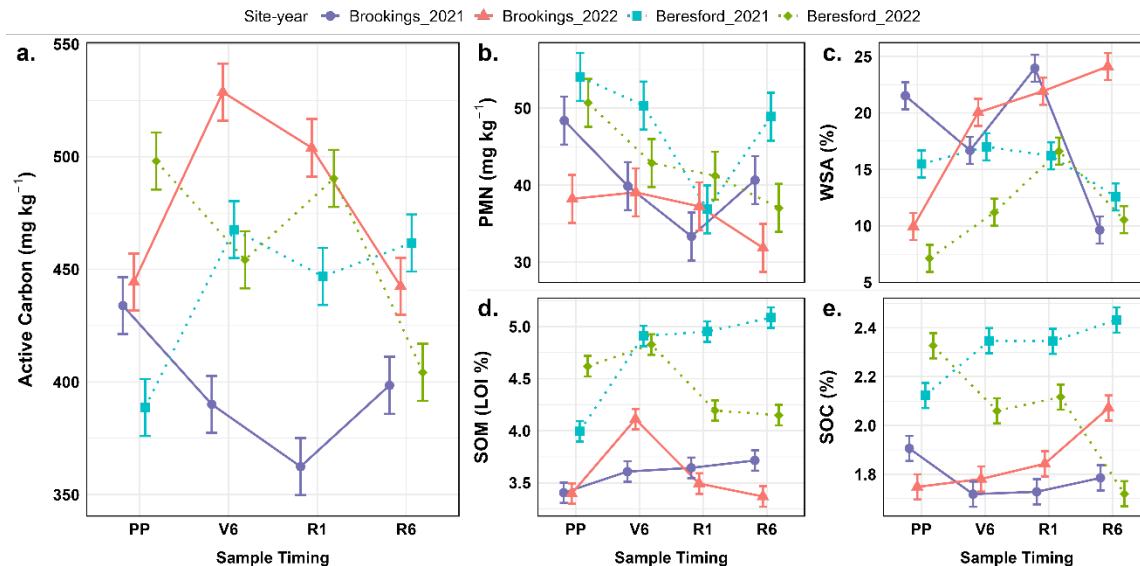


Figure 1 Seasonal dynamics of (a) active C, (b) PMN, (c) WSA, (d) SOM, (e) SOC across site-years and sample timings.

Note: Corn growth stages include V6, R1, R6 (Corn growth stages) (Abendroth et al., 2011)

Abbreviations: Active C, active carbon; PMN, potentially mineralizable nitrogen; WSA, water stable aggregates; SOM, soil organic matter; LOI, loss on ignition; SOC, Soil organic Carbon; PP, pre-plant.

Soil inorganic N responded strongly to N rate and sampling time, with nitrate-N showing more consistent changes than ammonium-N (Figure 2). In low-N plots, nitrate-N concentrations remained stable ( $\sim 2\text{--}13 \text{ mg kg}^{-1}$ ), while medium and high N plots peaked at V6 after fertilization ( $40$  and  $64 \text{ mg kg}^{-1}$ , respectively) (Figure 2a). Concentrations then generally declined with crop uptake through R1 and R6, though occasional late-season increases reflected mineralization or rewetting effects. Across all timings, nitrate-N increased predictably with the application of N.

Ammonium-N was more variable and transient (Figure 2). Levels also peaked at V6 in fertilized plots ( $10 \text{ mg kg}^{-1}$  at medium N and  $20 \text{ mg kg}^{-1}$  at high N), but declined more inconsistently thereafter, often due to rapid nitrification and environmental sensitivity (Figure 2b). Overall differences across N rates were minor ( $4\text{--}19 \text{ mg kg}^{-1}$ ) compared to nitrate-N ( $20\text{--}70 \text{ mg kg}^{-1}$ ). This confirms that nitrate is the more persistent,

management-responsive pool, whereas ammonium is short-lived and closely tied to microbial transformation and soil conditions.

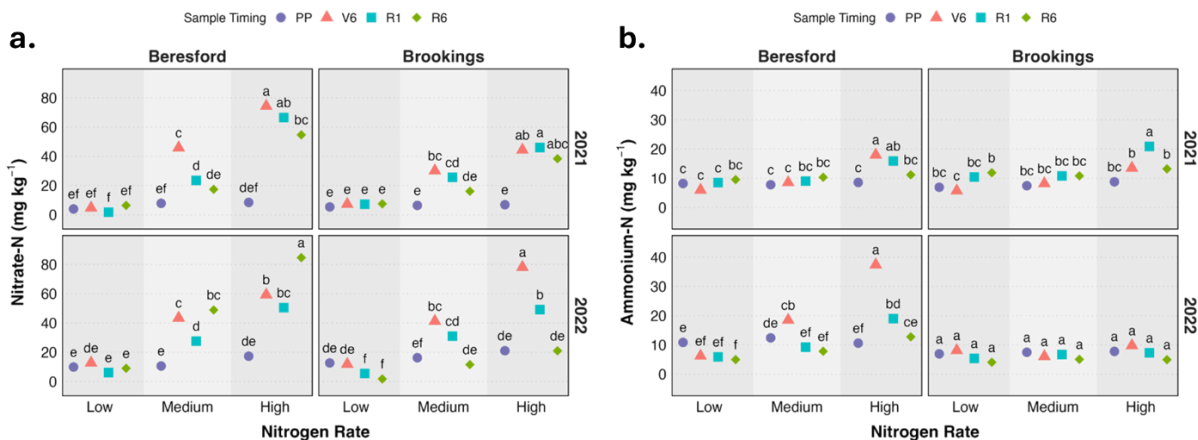


Figure 2 Inorganic soil-N concentrations across four site-years at Brookings and Beresford in 2021 and 2022.

Note: Corn growth stages include V6, R1, R6 (Corn growth stages) (Abendroth et al., 2011)

Abbreviations: PP, pre-plant.

## CONCLUSIONS

Four years of interseeding cover crops had no measurable effect on soil health indicators such as active C, aggregate stability, SOM, or PMN. These results are consistent with previous findings, which report that soil health improvements often require longer-term adoption, higher biomass inputs ( $>3 \text{ Mg ha}^{-1}$ ), and more diverse rotations. The limited biomass ( $<1 \text{ Mg ha}^{-1}$ ) and use of composite 0–15 cm samples likely contributed to the absence of detectable changes in this study.

In contrast, the nitrogen rate and sampling time had a strong influence on soil health responses. Inorganic-N exhibited clear seasonal and site-specific dynamics, with nitrate-N responding more consistently and to a greater magnitude than ammonium-N. Other soil parameters (Active C, PMN, SOM, SOC, WSA) also varied across the season, with V6 capturing peak biological activity and R6 reflecting improved soil structural properties. These results highlight that sampling timing is crucial for accurately interpreting soil health outcomes.

Overall, the study demonstrates that while short-term interseeding has a limited impact on soil health, N management and sampling strategies play a central role in shaping soil nutrient pools and biological processes. Long-term trials, finer-depth sampling, and inclusion of more diverse rotations are needed to fully evaluate the soil health potential of interseeded cover crops in temperate corn–soybean systems.

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