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## Soil biological health indicators as influenced by long-term integrated nutrient management in cotton

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### Abstract

What happens to the living portion of soil when farmers rely on a single fertilizer strategy for over a decade? This research examined how long-term integrated nutrient management (INM) affected soil biological health indicators in cotton (*Gossypium hirsutum* L.) at Michigan State University, East Lansing, USA. A ten-year field trial (2013-2023) compared five treatments: unfertilized control, NPK alone, 50% NPK + farmyard manure (FYM), 75% NPK + vermicompost, and full INM combining organic and inorganic sources. Soil from 0-15 cm depth was analyzed for microbial biomass carbon (MBC), dehydrogenase activity (DHA), alkaline phosphatase, urease, and soil respiration. Full INM recorded the highest MBC (289.7  $\mu\text{g g}^{-1}$ ), DHA (28.1  $\mu\text{g TPF g}^{-1} 24\text{h}^{-1}$ ), and alkaline phosphatase (132.1  $\mu\text{g PNP g}^{-1} \text{h}^{-1}$ ), representing 95.3%, 126.6%, and 89.5% increases over the control respectively. Temporal trends showed enzyme activities diverged sharply after year five, suggesting a threshold effect. These results confirm that combining organic amendments with reduced mineral fertilizers sustains soil biological health in continuous cotton.

**Keywords:** Soil biological health, integrated nutrient management, microbial biomass carbon, dehydrogenase activity, alkaline phosphatase, urease, cotton, farmyard manure, vermicompost, long-term field trial

### Introduction

Can soil remain biologically productive when cotton monoculture extends beyond a decade? Cotton accounts for roughly 35% of global fiber production, and its cultivation often demands high chemical inputs that gradually degrade the soil's living community <sup>[1]</sup>. Continuous cotton cropping under heavy reliance on synthetic fertilizers tends to reduce microbial populations and suppress enzyme activities over time <sup>[2]</sup>. Soil biological health indicators-microbial biomass carbon, enzymatic activities, and respiration rates-serve as early warning signals of declining soil function, often responding to management changes before physical or chemical properties shift <sup>[3]</sup>.

Integrated nutrient management blends organic amendments with reduced mineral fertilizer rates. Short-term trials (2-4 years) reported improvements in microbial biomass when FYM or vermicompost partially replaced synthetic NPK <sup>[4, 5]</sup>. But soil microbial communities need time to establish stable populations, and enzyme pools build slowly as organic matter accumulates <sup>[6]</sup>. Evidence from ten-year trials in cereal systems showed INM benefits compound over time; however, similar data from cotton systems remains limited <sup>[7]</sup>.

This research was designed to (i) quantify changes in MBC, DHA, alkaline phosphatase, urease, and soil respiration across five nutrient management treatments maintained for ten years, and (ii) identify temporal trends and threshold effects in these biological indicators under continuous cotton at Michigan State University.

### Research Area Description

The experiment was at the W.K. Kellogg Biological Station research farm affiliated with Michigan State University, near East Lansing, Michigan, USA (42°42'N, 84°29'W, and elevation 283 m). Soils belong to the Kalamazoo series (fine-loamy, mixed, mesic Typic Hapludalfs) with sandy loam texture. Baseline soil properties (0-15 cm) were: pH 6.4, organic carbon 0.87%, Total nitrogen 0.091%, available phosphorus 14.3 kg ha<sup>-1</sup>, and available

potassium 168.2 kg ha<sup>-1</sup>. The area has a humid continental climate (Köppen Dfa) with mean annual temperature of 9.8 °C and precipitation of 847 mm. The cotton growing season spans late May through early October.

## Materials and Methods

### Materials

Cotton cultivar ‘Stoneville 4946GLB2’ was planted annually at 98,000 seeds ha<sup>-1</sup> with 96.5 cm row spacing. Inorganic fertilizer sources included urea (46% N), single superphosphate (16% P<sub>2</sub>O<sub>5</sub>), and muriate of potash (60% K<sub>2</sub>O). FYM from the university dairy facility averaged 0.58% N, 0.23% P, and 0.41% K. Vermicompost produced on-site using *Eisenia fetida* contained 1.42% N, 0.67% P, and 0.83% K [8]. Soil was collected with a stainless-steel core sampler (5 cm diameter) and transported in insulated coolers within four hours.

### Methods

The trial followed a randomized complete block design with five treatments, four replications, and 10 m × 8 m plots. Treatments were: T<sub>1</sub>-unfertilized control; T<sub>2</sub>-NPK at 120:60:40 kg ha<sup>-1</sup>; T<sub>3</sub>-

50% NPK + FYM at 5 t ha<sup>-1</sup>; T<sub>4</sub>-75% NPK + vermicompost at 2.5 t ha<sup>-1</sup>; T<sub>5</sub>-full INM (50% NPK + FYM 3 t ha<sup>-1</sup> + vermicompost 1.5 t ha<sup>-1</sup>). Organic amendments were incorporated two weeks before planting. Composite soil samples (eight cores per plot, 0-15 cm) were sieved through 2 mm mesh and stored at 4 °C [9]. MBC was estimated by chloroform fumigation-extraction [10]. DHA was measured using 2, 3, 5-triphenyltetrazolium chloride reduction. Alkaline phosphatase used p-nitrophenyl phosphate as substrate, and urease was determined by urea incubation. Soil respiration was measured as CO<sub>2</sub> trapped in alkali over 10 days. ANOVA was performed in SAS 9.4 with Tukey’s HSD at  $p < 0.05$  [11].

### Field Experimental Design

Four blocks were arranged perpendicular to the slope gradient. Each block contained five plots with 2 m buffer strips between adjacent plots. Treatment assignments were re-randomized for the first three years and held fixed from year four onward. All cultural operations-irrigation, pest management, weed control-were uniform across treatments.

## Results

**Table 1:** Soil biological health indicators under different nutrient management treatments after 10 years

| Treatment     | MBC (μg g <sup>-1</sup> ) | DHA (μg TPF g <sup>-1</sup> 24h <sup>-1</sup> ) | Alk. Phos. (μg PNP g <sup>-1</sup> h <sup>-1</sup> ) | Urease (μg NH <sub>4</sub> <sup>+</sup> -N g <sup>-1</sup> 2h <sup>-1</sup> ) | Soil Resp. (mg CO <sub>2</sub> kg <sup>-1</sup> d <sup>-1</sup> ) |
|---------------|---------------------------|-------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------|
| Control       | 148.3d                    | 12.4d                                           | 69.7d                                                | 16.8d                                                                         | 21.7d                                                             |
| NPK alone     | 187.6c                    | 16.8c                                           | 91.4c                                                | 26.1c                                                                         | 27.3c                                                             |
| 50% NPK + FYM | 234.9b                    | 21.3b                                           | 103.4b                                               | 33.4b                                                                         | 32.6b                                                             |
| 75% NPK + VC  | 261.4ab                   | 24.7ab                                          | 118.7ab                                              | 38.9ab                                                                        | 35.1ab                                                            |
| Full INM      | 289.7a                    | 28.1a                                           | 132.1a                                               | 44.2a                                                                         | 38.4a                                                             |
| SEm (±)       | 8.74                      | 1.13                                            | 5.62                                                 | 2.08                                                                          | 1.47                                                              |
| CD (p=0.05)   | 26.31                     | 3.41                                            | 16.93                                                | 6.27                                                                          | 4.43                                                              |

Values followed by the same letter within a column are not significantly different at  $p < 0.05$  by Tukey’s HSD.

All five biological indicators responded significantly ( $p < 0.05$ ) to treatments after ten years (Table 1). Full INM recorded MBC of 289.7 μg g<sup>-1</sup>, which was 95.3% higher than the control and 54.4% above NPK alone. DHA under full INM was 28.1 μg TPF

g<sup>-1</sup> 24h<sup>-1</sup>, more than double the control. All biological parameters were strongly correlated with each other; MBC and soil respiration showed the strongest association ( $r = 0.989$ ,  $p < 0.01$ ).

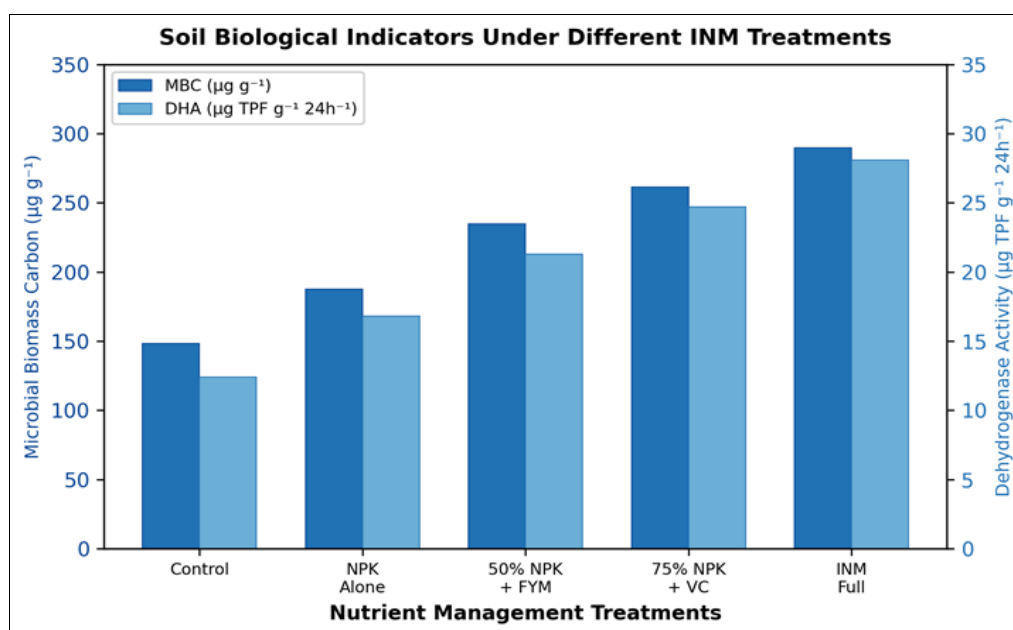


**Fig 1:** Microbial biomass carbon and dehydrogenase activity across nutrient management treatments after 10 years of continuous cotton

Figure 1 shows the progressive increase in MBC and DHA from the control through full INM. Adding FYM or vermicompost to

reduced NPK rates shifted both parameters upward by 25-54% compared to NPK alone.

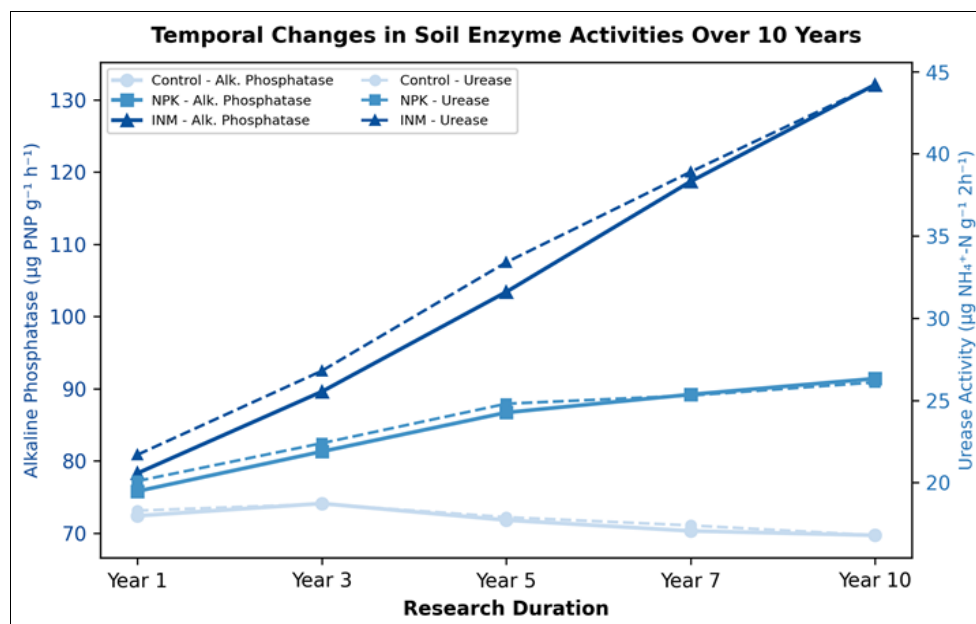


**Fig 2:** Temporal changes in alkaline phosphatase and urease activities under control, NPK alone, and full INM over the 10-year research period

The temporal trajectory (Figure 2) reveals a threshold effect. Enzyme activities under INM diverged sharply from other treatments between years 5 and 7, with an accelerating trend afterward, while the control remained flat or declined slightly.

### Discussion

The pronounced response of soil biological indicators to INM aligns with findings from long-term experiments in other cropping systems. Singh *et al.* [4] reported a 78% increase in MBC after eight years of INM in rice-wheat, somewhat lower than the 95.3% observed here, likely because continuous cotton produces less below-ground biomass and relies more on external organic inputs for microbial sustenance. The strong MBC-respiration correlation ( $r = 0.989$ ) confirms earlier observations by Kaur *et al.* [6] that microbial pool size drives overall soil metabolic capacity.

The threshold effect between years 5 and 7 has practical implications. Organic matter from FYM and vermicompost appears to need several years to reach a steady state, during which microbial communities gradually shift toward species producing higher levels of extracellular enzymes [7]. Farmers adopting INM shouldn't expect immediate biological benefits but can anticipate accelerating returns over time. The DHA values under full INM ( $28.1 \mu\text{g TPF g}^{-1} 24\text{h}^{-1}$ ) exceed those reported by Mandal *et al.* [12] for cotton in semi-arid India, possibly because Michigan's cooler, wetter climate supports higher microbial turnover.

### Conclusion

Ten years of full INM in continuous cotton at Michigan State University raised MBC by 95.3%, DHA by 126.6%, and alkaline phosphatase by 89.5% compared to the unfertilized control. Temporal analysis showed a threshold around year five, after which enzyme activities accelerated sharply under INM. Partial substitution of NPK with FYM or vermicompost also improved biological indicators compared to NPK alone, though the combined full INM strategy was clearly superior. These findings support recommending INM as a viable long-term approach for maintaining soil biological health in cotton monoculture systems.

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