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# Perennial grain Kernza<sup>®</sup> fields have higher particulate organic carbon at depth than annual grain fields

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## Abstract

Conversion from annual to perennial grains such as intermediate wheatgrass Kernza<sup>®</sup> could sequester soil organic carbon (SOC). To date, no studies have quantified SOC under Kernza on working farms. We sampled three sites with paired fields under annual grains and converted to Kernza 5–17 years ago to 100 cm and compared their SOC stocks as distributed between mineral-associated organic matter (MAOM) and particulate organic matter (POM). POM-C was higher under Kernza cultivation but total and MAOM-C were similar. Our findings suggest that Kernza increases SOC at depth as POM. Further study is needed to assess whether this will result in long-term SOC sequestration.

**Key words:** Kernza<sup>®</sup> (*Thynopyrum intermedium*), soil organic matter, soil carbon sequestration, cropping system

## Résumé

Passer des céréales annuelles à des céréales vivaces comme l'agropyre intermédiaire Kernza<sup>®</sup> permettrait de séquestrer le carbone organique du sol (COS). Jusqu'à présent, aucune étude n'a quantifié le COS dans les exploitations agricoles qui cultivent Kernza. Les auteurs ont prélevé des échantillons jusqu'à une profondeur de 100 cm à trois endroits où des champs servant à la culture de céréales annuelles avaient été convertis à la culture du Kernza 5–17 années plus tôt. Ensuite, ils ont comparé les réserves de COS sous forme de matière organique liée aux minéraux (MOAM) et de particules de matière organique (PMO). La concentration de C-PMO était plus importante dans les parcelles de Kernza, mais la teneur totale et la concentration de C-MOAM étaient similaires. Ces résultats laissent croire que Kernza augmente la quantité de COS en profondeur sous forme de PMO. Des études plus poussées seraient nécessaires pour déterminer s'il y a subséquentment séquestration à long terme du COS. [Traduit par la Rédaction]

**Mots-clés :** Kernza<sup>®</sup> (*Thynopyrum intermedium*), matière organique du sol, séquestration du carbone du sol, système cultural

## Introduction

Annual grain agriculture accrues a soil organic carbon (SOC) debt due to the paucity of inputs and reliance on frequent disturbance compared to the displaced natural ecosystems whose perennial vegetation built stores of SOC. Converting from annual to perennial grain crops offers the potential to repay that SOC debt as such systems have minimal disturbance, reduced erosion, deeper root systems, and longer periods of plant growth (Crews et al. 2018). Studies measuring the rate of SOC accumulation when converting from annual cropping to grassland or perennial biofuel vegetation range from 0.3 to 1.88 Mg C ha<sup>-1</sup> year<sup>-1</sup> (Crews and Rumsey 2017), leading perennials to be promoted as an SOC sequestration strategy. Perennial vegetation may increase SOC in two main ways: (i) by providing continuous, living cover to the soil, it can reduce erosion and enhance SOC inputs, and (ii) with

deeper and more robust rooting systems, perennial vegetation adds SOC to depths where it is more likely to become stabilized root debris, may undergo slower decomposition due to reduced microbial activity in subsoils, and root exudates may bond to the more available mineral surfaces. Fresh root inputs at depth, however, may also prime the mineralization of existing SOC and result in little or no net SOC increase.

Kernza<sup>®</sup>, a grain produced by domesticated forms of intermediate wheatgrass (*Thynopyrum intermedium*), is grown on 1600 ha in the United States and is the only perennial grain available to American farmers currently. While the sequestration potential of Kernza production has been estimated using net ecosystem exchange flux measurements (de Oliveira et al. 2018), no studies have yet quantified the effect of conversion of annual grains to Kernza on SOC stocks to depth at a field scale.

We conducted an observational study to assess whether Kernza fields increased SOC compared to annual row crops by sampling three fields with the longest known history of Kernza cultivation in Kansas, United States and adjacent fields remaining under annual grains. Detecting changes to SOC is difficult due to the inherent heterogeneity of soils and the long time-horizons for change. Examining SOC stocks as distributed between chemically and functionally distinct fractions of mineral-associated organic matter (MAOM (<53  $\mu\text{m}$ ) and particulate organic matter (POM (>53  $\mu\text{m}$ )) enhances our ability to detect differences, especially for the relatively small POM fraction (Cotrufo and Lavelle 2022).

POM originates from fragmented plant and microbial structural debris (de Oliveira et al. 2018). Unless it is occluded in stable aggregates, chemical recalcitrance is its only form of protection from further microbial decomposition in agricultural soils, making it vulnerable to disturbance such as tillage. As POM generated from grass residues has a C:N higher than that of the microorganisms feeding on it, POM decomposition likely has a low microbial use efficiency and results in little long-term SOC accumulation. However, POM formed from root input in subsoils can accumulate due to the limited microbial activity and stabilize by aggregate occlusion.

In contrast, MAOM forms from root exudates and residues' leachates either through direct sorption to minerals or after microbial utilization and subsequent adsorption of microbial necromass to minerals. Due to its origins, MAOM tends to increase with microbial turnover and plant inputs with high proportions of metabolic components and N such as from legumes (de Oliveira et al. 2018). The mineral association renders much of the MAOM-C inaccessible to further microbial processing and resistant to disturbance. Therefore, MAOM-C is on average older than POM-C, and it accounts for the majority of SOC in annually cropped agricultural ecosystems (Cotrufo and Lavelle 2022 and references therein).

To better appreciate the effect of conversion to Kernza on SOC and N dynamics, we quantified the amount of SOC and N as MAOM and POM in four depth increments from 0 to 100 cm. We hypothesized that perennial vegetation would have greater overall soil organic matter (SOM) at depth as well as a higher proportion of C as POM due to the deeper rooting systems of perennials and the tendency for root tissues to contribute chiefly to POM. Given our perennial fields were not intercropped with legumes, we did not expect significant MAOM increases.

## Methods

### Experimental site and design

The three study sites were chosen for having adjacent plantings of Kernza and annual crops. Two sites were farms, while the third was an experimental research trial including a restored native prairie treatment (McKenna et al. 2020). Details on study sites are provided in Table 1. All sites are located in Kansas, United States on silt loam soils and a typical continental climate.

### Soil sampling

Annual and Kernza fields were sampled by NRCS soil survey crews in December 2019 using stratified random sampling based on soil type from the USDA Web Soil Survey using a truck-mounted, 3.81 cm diameter hydraulic probe (Giddings Machine Company, Inc., Windsor, CO). Three samples from each soil type were collected at the vertex of an equilateral triangle with side lengths of 6 m, and each sample consisted of three composited cores sampled at the vertex of an equilateral triangle with lengths of 1 m (Spencer et al. 2011). Each core was divided into four depth increments (0–15, 15–30, 30–60, and 60–100 cm) and composited in the field.

### Laboratory analyses

Soils were ground by hand using a weighted wheel to a maximum size of 2 mm and dried at 35–37 °C within one week of collection. Moisture content was determined on a subsample of ~20 g soil dried at 120 °C. We calculated bulk density based on the total sample mass minus soil water content in the core volume calculated from the sample depth increment and soil core diameter. Coarse fragments >2 mm were negligible.

Further, we separated POM and MAOM by wet sieving at 53  $\mu\text{m}$  ~8 g of oven-dried soil after mechanical dispersion by shaking with glass beads and 30 mL of 0.5% sodium hexametaphosphate for 18 hours (Cotrufo et al. 2019). Mass recovery after fractionation ranged between 99% and 102%.

As many samples contained inorganic C (IC), samples that produced bubbles ( $\text{CO}_2$ ) with two drops of 1 mol L<sup>-1</sup> hydrochloric acid were acidified prior to analyses on the elemental analyzer to remove all IC. We quantified the %C and %N of bulk soil, POM, and MAOM fractions on an elemental analyzer (CN analyzer Costech 4100, Italy). Fraction C and N recovery compared to bulk soil were as follows: C mean 101% and N mean 98%. Mid-infrared (MIR) spectroscopy was used to estimate soil pH and percent clay (Seybold et al. 2019), which were used in the regression analysis.

### Statistical analyses

We assessed the effect of crop on SOC and N using mixed-linear effects model of analysis of variance (ANOVA) with Kenward–Roger correction. We log-transformed the SOC and N data to account for the non-normal distribution of residual values and analyzed each fraction separately. Fixed terms in the model included the categorical effect of crop, depth, interaction of crop and depth, and the continuous variable of MIR-estimated percent clay. We created a random variable that combined the site with the soil-stratification pairing to account for variability introduced by site and soil history.

As the regression model cannot account for nonlinearity in soil profiles at depth and the nonindependence of vertically nested measurements, we additionally tested for differences in SOC using bootstrapped resampling with local least-squares-based polynomial smoothing (LOESS) regres-

**Table 1.** Mean soil organic matter C and N stocks by depth.

| Field characteristics                                      |                |   |                                       |                      |           |                     |                     |
|--|----------------|---|---------------------------------------|----------------------|-----------|---------------------|---------------------|
|  |                | Mentor                                    | Ellsworth                             | Salina               |           |                     |                     |
| Soil classification  |                | Cumulic Haplustoll; Fluventic Haplustolls | Udic Arguistolls; Cumulic Haplustolls | Fluventic Haplustoll |           |                     |                     |
| Field size (ha)  |                |   |                                       |                      |           |                     |                     |
| Annual   |                | 24  | 10                                    | 0.14                 |           |                     |                     |
| Perennial  |                | 1   | 11                                    | 0.14                 |           |                     |                     |
| Mean bulk density (g cm <sup>-3</sup> )                    |                |   |                                       |                      |           |                     |                     |
| Annual   |                | 1.27 (0.09)                               | 1.42 (0.1)                            | 1.23 (0.1)           |           |                     |                     |
| Perennial  |                | 1.29 (0.07)                               | 1.37 (0.1)                            | 1.23 (0.05)          |           |                     |                     |
| Mean est. % clay   |                |   |                                       |                      |           |                     |                     |
| Annual   |                | 42 (19)                                   | 24 (9)                                | 31 (10)              |           |                     |                     |
| Perennial  |                | 37 (14)                                   | 25 (7)                                | 36 (10)              |           |                     |                     |
| Mean est. pH   |                |   |                                       |                      |           |                     |                     |
| Annual   |                | 7.77 (0.4)                                | 6.51 (0.8)                            | 7.89 (0.3)           |           |                     |                     |
| Perennial  |                | 7.58 (0.8)                                | 6.77 (0.5)                            | 7.67 (0.4)           |           |                     |                     |
| A. rotation  |                | Sorghum–Soy–Wheat                         | Fallow–Sorghum–Oat–Wheat              | Wheat–Sorghum–Soy    |           |                     |                     |
| A. management  |                | Annual tillage; winter grazed             | No-till; winter grazed                | Annual tillage       |           |                     |                     |
| A. fertilization (kg ha <sup>-1</sup> year <sup>-1</sup> ) |                | 80 N <sup>†</sup>                         | 90 N                                  | 84–123 N; 56 P       |           |                     |                     |
| P. fertilization (kg ha <sup>-1</sup> year <sup>-1</sup> ) |                | None                                      | Manure <i>rate unknown</i>            | None                 |           |                     |                     |
| Year P. planted  |                | 2011                                      | 2014                                  | 2002                 |           |                     |                     |
| P. field prior use   |                | Annual cropping                           | Annual cropping                       | Alfalfa              |           |                     |                     |
| Soil organic matter (Mg ha <sup>-1</sup> )                 |                |   |                                       |                      |           |                     |                     |
| Depth (cm)   | <i>n</i> cores | Bulk OC*                                  | Bulk N                                | MAOM-C               | MAOM-N    | POM-C***            | POM-N               |
| Annual   |                |   |                                       |                      |           |                     |                     |
| 0–15   | 18             | 28.7 (11.5)                               | 3.0 (0.9)                             | 23.5 (10.5)          | 2.5 (0.9) | 4.6 (2.1)           | 0.3 (0.2)           |
| 15–30  | 18             | 24.3 (12.6)                               | 2.5 (1.1)                             | 21.6 (11.3)          | 2.3 (1.0) | 1.0 (0.3)           | 0.07 (0.03)         |
| 30–60  | 18             | 33.1 (16.8)                               | 3.7 (2.0)                             | 26.7 (15.7)          | 3.5 (1.8) | <b>1.2***</b> (0.6) | 0.09 (0.05)         |
| 60–100   | 18             | 32.9 (12.8)                               | 3.9 (1.7)                             | 32.7 (13.8)          | 3.7 (1.4) | 2.2 (3.7)           | <b>0.09*</b> (0.07) |
| Perennial Kernza <sup>®</sup>                              |                |   |                                       |                      |           |                     |                     |
| 0–15   | 18             | 31.1 (8.4)                                | 3.1 (0.6)                             | 23.8 (6.9)           | 2.6 (0.6) | 6.4 (1.9)           | 0.4 (0.1)           |
| 15–30  | 18             | 22.6 (10)                                 | 2.2 (0.8)                             | 24.5 (9.1)           | 2.0 (0.7) | 1.9 (1.0)           | 0.1 (0.08)          |
| 30–60  | 18             | 35.8 (17.8)                               | 3.7 (1.6)                             | 35.0 (19.1)          | 3.5 (1.5) | <b>2.8***</b> (2.3) | 0.1 (0.05)          |
| 60–100   | 18             | 40.9 (20.7)                               | 4.0 (1.8)                             | 39.2 (21.2)          | 4.0 (1.7) | 2.0 (1.0)           | <b>0.1*</b> (0.1)   |
| Restored prairie   |                |   |                                       |                      |           |                     |                     |
| 0–15   | 3              | 33.6 (0.5)                                | 3.40 (0.1)                            | 25.7 (0.9)           | 2.7 (0.1) | 5.1 (0.8)           | 0.4 (0.04)          |
| 15–30  | 3              | 24.2 (1.3)                                | 2.55 (0.1)                            | 20.8 (1.8)           | 2.3 (0.2) | 2.0 (0.5)           | 0.1 (0.03)          |
| 30–60  | 3              | 24.0 (2.6)                                | 2.63 (0.2)                            | 23.7 (3.8)           | 2.6 (0.2) | 2.4 (0.8)           | 0.2 (0.02)          |
| 60–100   | 3              | 52.7 (2.8) <sup>‡</sup>                   | 5.31 (0.3)                            | 51.1 (1.9)           | 5.3 (0.2) | 1.4 (0.5)           | 0.07 (0.02)         |

**Note:** The three sites in this study were managed as annual (A.) crops prior to the establishment of perennial (P.) Kernza in the year indicated for each site. Samples were paired by soil series for the annual and Kernza fields. For C and N stocks, all values are given in Mg ha<sup>-1</sup>; values in parentheses indicate the standard deviation averaged across site for field characteristics and depth for stocks. Est. means “estimated” from mid-infrared spectroscopy. POM is particulate organic matter and MAOM is mineral-associated organic matter. Asterisks indicate significant differences based on analysis of variance (ANOVA) between annual and perennial management, where \*\*\* indicates  $p < 0.001$  and \* indicates  $p < 0.05$ .

<sup>†</sup>This amount is an estimate based on typical application rates in the region.

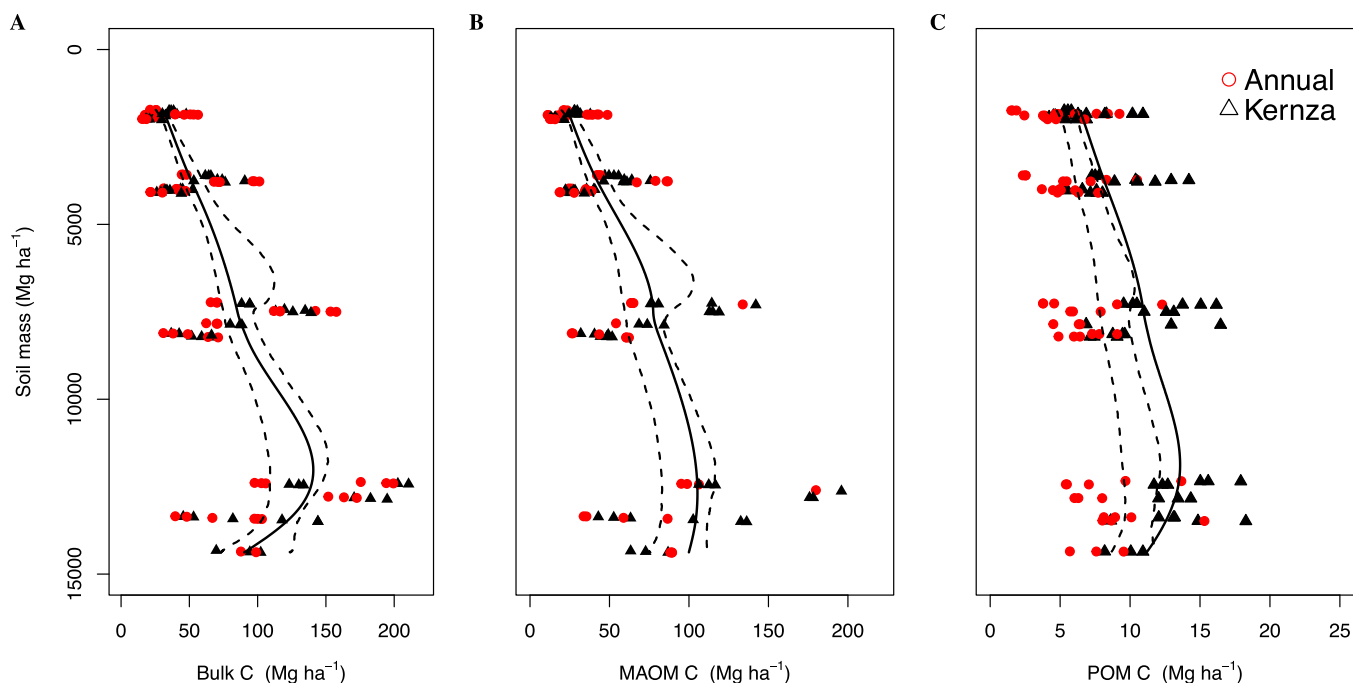
<sup>‡</sup>The Salina site had a buried A horizon ~80 cm depth under the restored prairie.

sion (Keith et al. 2016). We calculated the cumulative amount of SOC for each sample using an equivalent soil mass (ESM) approach (von Haden et al. 2020). Using these values, we created a bootstrapped population based on combined samples from under Kernza and annual crops on SOC with replacement ( $n = 1000$ ). We used the bootstrapped dataset to generate a 95% confidence interval (CI) for the combined management and used a LOESS regression to model the effect of the Kernza cropping system on SOC.

## Results and discussion

We set out to assess the effect of Kernza on SOC stocks, hypothesizing that the perennial vegetation would lead to enhanced SOC primarily as POM due to its deep root system and year-round presence. We found bulk SOC was higher under Kernza than in annual cropping systems ( $t = 2.59$ ,  $p = 0.03$ ) with fields planted to Kernza having  $\sim 4 \pm 2$  Mg ha<sup>-1</sup> more SOC than annually cropped fields across the 0–100 cm soil

**Fig. 1.** Bootstrapped local least-squares-based polynomial smoothing (LOESS) regression of measured soil organic carbon (SOC) in (A) bulk soil, (B) mineral-associated organic matter (MAOM), and (C) particulate organic matter (POM) along the 0–100 cm soil profile. The equivalent soil mass (ESM) portrays to sum of mass over the depth sampled ( $y$ -axis) based on the bulk density and estimated mineral mass of soil for each depth increment sampled. The cumulative soil carbon ( $x$ -axis) is calculated based on the ESM and percent carbon for each depth and soil fraction. Bulk soil is the whole soil sample. MAOM,  $<53 \mu\text{m}$ ; POM,  $>53 \mu\text{m}$ . Triangles represent Kernza vegetation; circles are annual vegetation. Dashed lines represent the 95% confidence interval (CI) based on the bootstrapped sampling ( $n = 1000$ ) with replacement of pooled soil C values from both annual and Kernza vegetation. The solid black line represents the model of soil C from the Kernza vegetation only. The model falling outside of the CI suggests significant differences in the two vegetation types. [Color online]



profile. There were no differences in bulk soil N. Assuming fields planted to Kernza had similar soil C to the annual fields at planting, this would suggest an average SOC gain with Kernza of  $0.4 \pm 0.2 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ , which is aligned with other estimates of converting to perennial vegetation (Crews and Rumsey 2017). The SOC accrual rate under Kernza may have been faster as farmers described selecting Kernza fields in part due to low yield expectations for annual crops in those areas, suggesting that the assumption of similar starting SOC values may not be valid.

Examining the MAOM and POM fractions provided insight into how the annual and perennial vegetation impacted SOC and N in soil (Fig. 1). Both POM-C and POM-N were higher in Kernza fields ( $t = 19.26$ ,  $p < 0.001 \text{ C}$ ;  $t = 4.51$ ,  $p < 0.001 \text{ N}$ ) compared to those annually cropped (Table 1). The same was true for POM-C in the restored native prairie ( $t = 2.75$ ,  $p = 0.02$ ), though not POM-N ( $t = 1.57$ ,  $p = 0.3$ ). MAOM-C tended to be higher under Kernza ( $F = 2.6$ ,  $p = 0.07$ ), though MAOM-N did not vary by vegetation. Given the potential for nonlinear changes in soil characteristics and the interdependence of the soil depths, we used the bootstrapped LOESS regression (BLR) to assess the overall difference in bulk SOC and fractions (Fig. 1). We found that POM-C was consistently higher for Kernza, while there were no differences in bulk or

MAOM-C. These findings support our hypothesis that perennial vegetation promotes greater SOC primarily as POM. Soil under Kernza is not tilled after planting, promoting aggregation and reducing microbial access to surface residues. Reduced mixing and aggregate occlusion could promote greater POM in the surface soils while the deeper root structural inputs could enhance POM formation at depth. There was a greater difference in POM-C in the two sites with regular tillage, supporting the hypothesis that reduced tillage promotes the preservation of POM-C (Cotrufo and Lavalley 2022).

As root exudates and their associated microbial communities are known to support MAOM formation, we may have expected Kernza also to enhance MAOM-C with its deeper, denser, and longer lived root systems (Cotrufo and Lavalley 2022). We saw little change in MAOM-C, however. While detecting differences in this larger pool of soil C is more difficult, there may not be significant increases to MAOM despite increased root activity since the additional C without additional N inputs may lead to priming.

## Conclusion

Kernza demonstrated the potential to increase soil C and N at depth primarily as POM on working farms as hypoth-

esized. As POM decomposes readily when conditions are favorable and there were scant differences in MAOM, it is unclear from this study whether planting Kernza monoculture will result in long-term C sequestration. Promoting SOM accrual as MAOM could be enhanced by supplying the perennial system with greater N and labile C inputs such as by intercropping legumes with similarly deep root systems as Kernza. As this was a small, observational study initiated after Kernza was established, future studies would benefit from sampling prior to conversion as well as utilizing an approach that combines mechanistic, manipulative experiments and robust field sampling with cost-effective techniques such as MIR spectroscopy. Such work could elucidate the potential for the deep soil POM-C to become occluded in aggregates and be considered long-term SOC storage as well as assess the rate of SOC accrual with this emerging perennial grain. Finally, incorporating the representation of perennial crops in new generation models that represent POM and MAOM dynamics is auspicious to forecast the effects of SOC stocks and soil health and inform their adoption at scale.

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### Data availability

Data are available in the GitHub repository (doi: 10.5281/zenodo.6588654, <https://github.com/slylyvp/KernzaC>).

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## Author contributions

L.v.P. wrote the manuscript and performed the analysis and visualization; L.v.P., B.N., and B.S. performed the investigation; L.v.P. and B.N. curated data; M.F.C., T.E.C., B.N., and B.S. conceived of the research project and contributed funding to this project. All authors reviewed the manuscript.

## Competing interests

The authors declare no competing interests and note that M.F.C. is a co-founder of Cquester Analytics LLC, which offers consultant and analytical services for accurate soil C measurements and monitoring.

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