Effect of cover crops on the yield and nutrient concentration of organic kale (*Brassica oleracea* L. var. *acephala*)

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Kale is a leafy green vegetable regularly grown using non-organic agricultural systems. In recent years, organic kale demand has increased at near-doubling rates in the USA due to its perceived nutritional value. The objective of this study was to determine the effect of organic cover cropping systems on subsequent kale biomass production and nutrient composition (protein, mineral, and prebiotic carbohydrate concentrations) and to assess organic kale as a potential whole food source of daily essential mineral micronutrients and prebiotic carbohydrates. A single 100-g serving of fresh organic kale can provide mineral micronutrients (43–438 mg Ca; 11–60 mg Mg; 28–102 mg P; 0.5–3.3 mg Fe; 0.3–1.3 mg Mn; 1–136µg Cu; and 0–35µg Se) as well as 5.7–8.7 g of total prebiotic carbohydrates, including sugar alcohols (0.4–6.6 mg), simple sugars (6–1507 mg), raffinose and fructooligosaccharides (0.8–169 mg), hemicellulose (77–763 mg), lignin (0–90 mg), and unknown dietary fiber (5–6 g). Fresh organic kale has low to moderate concentrations of protein (1.3–6.0 g/100 g). Study results indicate that Starbor and Red Russian are the most suitable kale cultivars for organic production without considerable biomass and nutrient composition losses. Among the cover crops, faba bean results in the highest mineral, protein, and prebiotic carbohydrate concentrations in subsequent kale crops but ryegrass increases kale biomass production. Results also demonstrated a significant interaction between kale variety and organic cover crop with respect to biomass and nutrient concentration. Future organic nutritional breeding of kale is possible by selecting cultivars that perform well following different cover crops.

Originating from the eastern Mediterranean, kale is one of the oldest leafy brassica vegetables. Kale was not introduced to the USA until the early 1980s, but since then has been marketed in grocery stores as different varieties and at various stages of maturity, packed as fresh food ready to eat or cook. Kale is ranked 15th on the list of “powerhouse” fruits and vegetables (a 100-g serving provides ≥10% of the daily value of 17 essential nutrients). These “powerhouse” fruits and vegetables are strongly associated with reduced risk of heart disease and other non-communicable diseases. Consequently, kale is gaining popularity in communities around the world but remains an understudied vegetable, especially in terms of organic production.

Since the early 1990s, American consumer demand for organic vegetables has significantly increased. Today, the US organic vegetable industry is worth approximately $29 billion annually and is growing as a result of human health concerns, such as breast cancer, liver disease, and heavy metal toxicity. Organic produce also plays a predominant role in detoxification and obesity prevention therapies. However, a primary criticism of organic agriculture is lower production and nutrient composition compared to non-organic systems. US-certified organic agriculture production has increased since the introduction of the Organic Foods Production Act of 1990. The United States Department of Agriculture (USDA) National Organic Standards Board defines organic agriculture as “an ecological production management system that promotes and enhances biodiversity, biological cycles, and

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higher than other varieties, followed by Lacinato (3 of 9) and Scarlet (2 of 9) systems (4 of 9) (followed faba bean in terms of some mineral concentrations that were significantly higher than other cropping systems. Kale grown following faba bean had the most mineral concentrations (7 of 9 minerals) that were significantly higher than all other systems. Red Russian had the most prebiotic carbohydrate concentrations (9 of 12 carbohydrates) that were significantly higher than other cropping systems, followed by faba bean (6 of 12), hairy vetch (5 of 12), and Lynx winter pea, and ryegrass covers. Starbor and Lacinato showed higher protein concentration in most cases than the other varieties.

A significant (P < 0.05) interaction was evident between different cover cropping systems and kale cultivars in terms of mean prebiotic carbohydrate concentrations (Table 4). Kale grown following the crimson clover cover cropping system had the most prebiotic carbohydrate concentrations (9 of 12 carbohydrates) that were significantly higher than other cropping systems, followed by faba bean (6 of 12), hairy vetch (5 of 12), and Lynx winter pea (5 of 12) (Table 4). The crimson clover cover cropping system yielded kale cultivars with RFO + FOS concentrations significantly higher than all other systems. Red Russian had the most prebiotic carbohydrate concentrations (3 of 12) that were significantly higher than other cultivars, followed by Darkibor (4 of 12) and Scarlet (4 of 12). Darkibor had significantly higher RFO concentrations than all other cultivars (Table 5).

Different cover cropping systems and kale cultivars had significant (P < 0.05) effects on mean mineral concentrations. Kale grown following faba bean had the most mineral concentrations (7 of 9 minerals) that were significantly higher than for other cropping systems. Phosphorous and Mn concentrations were significantly higher in all kale cultivars following faba bean cover than all other systems. Hairy vetch, crimson clover, and ryegrass followed faba bean in terms of some mineral concentrations that were significantly higher than for other cropping systems (4 of 9) (Table 6). Overall, Starbor had the most mineral concentrations (5 of 9) that were significantly higher than other varieties, followed by Lacinato (3 of 9) and Scarlet (2 of 9) (Table 7).

Discussion
Organic cover cropping systems have a significant effect on subsequent kale biomass production and nutrient composition. Our study results clearly show that ryegrass is the most suitable cover crop for subsequent kale biomass production (42,846 kg/ha) followed by faba bean (33,624 kg/ha); the Lynx winter pea cover crop is the least suitable for subsequent organic kale production (16,224 kg/ha; Fig. 1). Similar results reported for annual
ryegrass as a cover crop for subsequent vegetable production\textsuperscript{20,21} indicate 80% of the ryegrass biomass establishes within the first month of the growing period and provides greater carbon and N biomass quantities to subsequent vegetable crops than legume cover crops, which require temperatures below 15–18 °C and longer times\textsuperscript{17}.

Although grass cover crops establish faster than legumes, organic kale produced after faba bean cover had higher levels of protein (4.7 g/100 g), minerals (Ca, Mg, P, and Mn), and prebiotic carbohydrates (mannose, nystose, arabinose, xylose, and lignin) than kale produced after ryegrass cover (Tables 4 and 6; Fig. 1). Therefore, organic kale production is possible by optimizing the legume cover cropping system. However, further studies are required to confirm planting dates and cultivar effects on nutritional quality considering different organic farm locations and climatic regions.

The highest concentrations of K, P, Mg, Fe, and Mn were observed in the curly kale cultivar Starbor, followed by Lacinato for P, Mn, and Zn; Scarlet for Ca and Mg; and Red Russian for Zn (Table 7). Further, these kale cultivars were also high in Cu; however, Se concentrations were below the detection limit for most cultivars except Red Russian and Starbor (Table 7). Therefore, organic kale production is possible by optimizing the legume cover cropping system. However, further studies are required to confirm planting dates and cultivator effects on nutritional quality considering different organic farm locations and climatic regions.

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Data from this study provide evidence that organic kale, grown using different cover cropping systems, is a whole food source of the essential minerals Ca, Mg, P, Fe, Mn, Cu, and Se as well as significant levels of prebiotic carbohydrates.
Table 2. Analysis of variance of prebiotic carbohydrate and mineral concentrations for kale cultivars grown in organic cover crops. Sorbitol (Sor), Mannitol (Man), Glucose (Glu), Fructose (Fru), Sucrose (Suc), Mannose (Mann), Stachyose and Raffinose (Sta + Raf), Verbascone and Kestose (Ver + Kes), Nystose (Nys), Arabinose (Ara), Xylose (Xyl), Total prebiotic carbohydrates (TP). **Significant at P < 0.05, *significant at P < 0.1.

Table 3. %RDA of different minerals in a 100-g serving of fresh organic kale grown in SC, USA. *Percent recommended daily allowance (%RDA) was calculated based on National Academy of Sciences (2004)33. Values are based on fresh weight (85% moisture), n = 108 per nutrient.

Figure 1. Variation of leaf biomass and protein content of six kale cultivars grown in six different organic cover cropping systems. *leaf biomass and protein within varieties for different cover crops are significantly different at p < 0.05.
Overall mineral concentrations in six different kale cultivars. Means within a column followed by different letters are significant at $P < 0.05$. Faba bean (FB); Windham winter pea (WWP); hairy vetch (HV); Lynx winter pea (LWP); crimson clover (CC); ryegrass (RG).

Table 4. Prebiotic carbohydrate levels in organic kale cultivars in response to different cover crops. Means within a column followed by different letters are significant at $P < 0.05$. Faba bean (FB); Windham winter pea (WWP); hairy vetch (HV); Lynx winter pea (LWP); crimson clover (CC); ryegrass (RG).

Table 5. Overall prebiotic carbohydrate concentrations of six different kale cultivars. Means in columns followed by different letters are significant at $P < 0.05$.

Table 6. Mineral concentrations in organic kale cultivars in response to different cover crops. *Means within a column followed by different letters are significantly different at $p < 0.05$. Faba bean (FB); Windham winter pea (WWP); hairy vetch (HV); Lynx winter pea (LWP); crimson clover (CC); ryegrass (RG); not detectable levels (ND).

Table 7. Overall mineral concentrations in six different kale cultivars. *Means within a column followed by different letters are significantly different at $p < 0.05$. ND, not detectable levels.
The health and nutritional sectors are paying increasing attention to organic kale. However, little research has been done to assess the nutritional value of organic kale. Data from the present study support the notion that organic kale can provide significant essential minerals and adequate prebiotic carbohydrates quantities with moderate to low levels of protein. Other organic vegetable studies show that protein content increases with increased biomass production and nutritional quality of subsequent spring organic kale production.

In conclusion, organic cover cropping systems have a significant effect on subsequent kale biomass production as well as protein, essential minerals, and prebiotic carbohydrate concentrations. For kale biomass production, ryegrass is the most suitable cover crop followed by faba bean. Kale cultivars Lacinato and Starbor have high protein levels and Darkibor, Red Russian, and Scarlet are abundant in prebiotic carbohydrates and minerals. Further opportunities should be pursued to improve the nutritional and production values of organic kale as well as to provide more significant quantities of nutrients to kale consumers.

### Materials and Methods

#### Materials.
- Standards, reagents, acids, and high-purity solvents used for kale sample and reagent preparation were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and VWR International (Radnor, PA, USA) and used without further purification. Water, distilled and deionized (ddH₂O) to resistance of ≥18.2 MΩ (Milli-Q Water System, Millipore, Milford, MA), was used for kale sample and reagent preparation.

#### Field design.
- The experimental field design was a complete randomized block design with three replicates of six commercial kale cultivars grown in six cover cropping systems (n = 108; Table 8).

#### Land preparation.
- Organic field experiment was conducted at Clemson University, SC, USA. Certified organic seeds for cover crops faba bean (Windsor), crimson clover, hairy vetch, and ryegrass were obtained from Johnny’s Select Seeds, ME, USA. Seeds for the two winter field pea cultivars, Windham and Lynx, were obtained from the USDA-ARS field pea breeding program at Washington State University, WA (Table 8). Cover crop plants were established in the Clemson University USDA Certified Student Organic Farm in October 2016 using protocols and recommendations developed by the USDA Sustainable Agriculture Research and Education Program (Table 8)26. Each cover crop was planted using a small seed drill in 7 × 8 m plots at a seeding rate of 8–100 lb/ac with a seeding depth of 1–2 cm depending on the cover crop species (Table 7).26 No fertilizer or any organically certified chemicals were added to the cover crop plots. Weeds were manually removed every week, and cover crops were irrigated using a drip irrigation system (0.4 gal/h for 30 min at 8–9 am). Cover crops were crimped 15 days before kale transplanting, and the planting rows were prepared using a small tractor.

#### Kale experiment.
- For kale transplants, organic kale seeds were grown in an organically certified poly house for three weeks, and then six kale cultivars with three replicates per row were planted in each cover crop plot on March 24, 2017 (Table 8). Six commercially grown kale cultivars were selected based on market class, consumer demand, disease resistance, drought tolerance, biological yield, and our current breeding and selection research (Table 8).22,27. Weeds were removed manually every week, and plants were irrigated twice a day using a drip irrigation system (1.5 L/h for 30 min). No organic fertilizer was added. Field-planted kale seedlings became infested with fire ants during the first week of planting, which was controlled using two applications of organically certified *Bacillus thuringiensis* (B.t.; 1 mL/L). Kale plants were harvested at physiological maturity (ready to eat) in mid-May 2017. A single kale plant per replicate was harvested, the stem removed, and then the remainder weighed for leaf biomass production. Fresh kale leaf samples (250 g) were then taken randomly from the entire harvested plant of each of three independent replicated field plots and subjected to protein, mineral, and prebiotic analysis.

### Table 8.

| Cover crop; seeding rate; seeding depth | Total number (n) |
|----------------------------------------|-----------------|
| 1. Faba bean (FB); 100 lb/ac; 2.5 cm   | 54               |
| 2. Windham winter pea (WWP); 100 lb/ac; 2.5 cm | 54               |
| 3. Hairy vetch (HV); 25 lb/ac; 1 cm    | 54               |
| 4. Lynx winter pea (LWP); 100 lb/ac; 2.5 cm | 54               |
| 5. Crimson clover (CC); 20 lb/ac; 1 cm  | 54               |
| 6. Ryegrass (RG); 110 lb/ac; 2.5 cm    | 54               |

| Kale cultivars | Replications | Total number (n) |
|----------------|--------------|-----------------|
| Curly kale: Darkibor, Red Russian, Scarlet, Starbor | 3            | 108             |
| Dinosaur kale: Black Magic, Lacinato               |              |                 |
carbohydrate analyses. A total of 108 replicate kale leaf samples were collected. The moisture content of fresh sub-samples (105 °C for 16 h) was determined, and the remaining samples immediately stored at −40 °C until analysis. Before each analysis, the oven-dried samples were finely ground using a mortar and pestle. Nutrient composition data are reported on a fresh weight basis (85% moisture).

**Protein analysis.** Total nitrogen level was determined on oven-dried finely ground kale leaf samples using a LECO FP3000 CNS analyzer; protein content was calculated as N × 6.25.

**Mineral concentrations.** Mineral concentrations were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Thermo 6500 Duo instrument (Thermo Fisher Scientific, PA, USA) after modified HNO₃-H₂O₂ digestion. Aliquots (500 mg) of oven-dried samples were digested with 6 mL of 70% HNO₃ overnight at room temperature. They were then heated to 90 °C for one h, after which 3 mL of 30% H₂O₂ were added and the sample held at 90 °C for 15–20 min. Finally, 3 mL of 6 M hydrochloric acid was added, and the samples held at 90 °C for another 5 min. Samples were then filtered and made up to 10 mL in Milli-Q water. Detection limits were 30 μg/L for K, Ca, and Mg and 5 μg/L for Fe, Zn, Mn, Cu, and Se. Analytical quality assurance was accomplished using authentic calibration standards and the NIST standard reference material peach leaves 1547. The percentages of recommended daily allowance values (%RDA) for each mineral were calculated for a 100-g portion of fresh kale.

**Prebiotic carbohydrate concentrations.** Oven-dried finely ground kale leaf samples (~500 mg) were extracted as per the method reported by Muir et al. This method involved extraction with distilled water at 80 °C for one h. The resulting extract was passed through a 13 mm × 0.45 μm nylon syringe filter (Chromatographic Specialties, Brockville, ON, Canada). Analyses for sugar alcohols, monosaccharides, disaccharides, and oligosaccharides were performed using high-performance liquid-liquid partition chromatography (ICS-5000 Dionex, Sunnyvale, CA, USA) as described by Feinberg with pulsed amperometric detection (PAD, Dionex) in series with electrochemical detection (Thermo Fisher Scientific, Inc.). Carbohydrates were separated by a CarboPac PA-100 4 × 250 mm column in series with a CarboPac PA-100 4 × 50 mm guard column (Dionex, Sunnyvale, CA, USA). The mobile phase flow rate was maintained at 1 mL/min. Solvents used for elution were 100 mM sodium hydroxide/600 mM sodium acetate (solvent A), 200 mM sodium hydroxide (solvent B), and ddH₂O (solvent C). The initial 16 min used 50% B and 50% C, followed by a gradient change to 3% A, 48.5% B, and 48.5% C. At 18 min, the gradient changed linearly to 16% A, 42% B, and 42% C. Finally, at 19 min, the gradient returned to 50% B and 50% C. The silver-silver chloride electrode was set at 2.0 μA. The minimum detectable level (signal to noise ratio ≥ 3) of each analyte was 0.1 ppm. Peak areas for a reference lentil sample were routinely analyzed with an error of less than 5%, and linear calibration models for standards also had errors <5%. Concentrations of carbohydrates in the filtrate (C) were determined from the calibration model used to calculate concentrations for sample dry matter in the expression $X = (C \times V) / m$, where $X$ is the concentration of the carbohydrate in the sample (corrected for moisture), $V$ is the final diluted volume, and $m$ is the mass of the dry sample aliquot. Hemicellulose was measured as described above after digesting a 500-mg sample with 5 mL of 7% (w/w) hydrochloric acid (HCl) at 55 °C for 120 min. Lignin was determined using an aliquot (0.9 mL) of extract added into a cuvette with 0.1 mL of neutral ferric chloride (5 g in 100 mL) solution, the absorbance of which was then measured at 690 nm in a spectrophotometer (GENESYS 20, Thermo Scientific, Ashville, NC, USA). The concentration of lignin (equivalent to gallic acid) was measured using a standard curve. All data are presented as mg/100 g fresh weight.

**Statistical analysis.** Data from the complete randomized block design represented three replicates of six kale cultivars grown in six cover cropping systems (n = 108). Replicates and cultivars were considered as random factors. Class variables include cultivar, replication, and cover cropping treatment. A mixed model analysis of variance was performed using the PROC GLM procedure of SAS version 9.4. Means were separated by Fisher’s protected least significant difference at $P < 0.05$.

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Author Contributions

D.T.: project P.I., with overall responsibility for experimental design, data analysis, data interpretation, manuscript writing, revisions, and final editing; N.S.: technical assistance to nutritional composition analysis and data table preparation; N.J.: contributed to data interpretation and wrote a section of this manuscript; R.M.: contributed to experimental design, manuscript revisions, and final editing; P.T.: oversaw analytical chemistry assays, data interpretation, and final editing.

Additional Information

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