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Evaluating Carbon Sequestration and Soil Organic Carbon Enhancement with Innovative Slow-Release Micronutrient Products

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Abstract

This study investigates soil organic carbon enhancement and greenhouse gas mitigation using innovative slow-release micronutrient fertilizers in both greenhouse and field trials for wheat (*Triticum aestivum*) cultivation. In the greenhouse trial cultivating spring wheat, CO_2 and N_2O emissions, soil carbon levels, yield, and above-ground biomass were measured to determine the relative carbon balance and to assess the viability of Soileos and Nutreos products, two innovative slow-release fertilizers designed for carbon sequestration. Additionally, four field trials were conducted using different wheat varieties, comparing total soil carbon in fields treated with the Soileos Zinc product to the Grower Standard Practice (GSP). In greenhouse trials, Soileos and Nutreos fertilizers promoted soil health by enhancing microbial activity, as evidenced by increased soil respiration rates and final soil carbon content. The relative carbon balance of treatments using slow-release Soileos micronutrient fertilizer and Nutreos micronutrient seed coatings improved by 15% - 25% over the GSP, compared to a 2% - 13% improvement in treatments using sulfate-based micronutrient fertilizers. In field trials, the average total soil organic carbon in soils treated with the slow-release Soileos fertilizer improved by about 11% compared to the GSP, aligning with greenhouse results. Additionally, wheat yield increased in three out of four field trials using Soileos Zinc micronutrient. Consequently, these findings suggest that Soileos and Nutreos slow-release fertilizers can enhance soil carbon sequestration. By enhancing soil health and promoting soil organic carbon in greenhouse and field trials within a single growing season, these fertilizers contribute to an improved carbon balance in agricultural production.

Keywords

Field Trial, Soil flux, Sustainable, Agriculture, Wheat

1. Introduction

Fertilizers play a crucial role in addressing global food security challenges and meeting the increasing demand for agricultural products. However, their inefficient use contributes to significant environmental issues, such as soil acidification, eutrophication, nutrient runoff, and greenhouse gas emissions from agricultural practices. Agricultural activities account for a substantial share of global greenhouse gas emissions, estimated between 10.7 and 12.0 $GtCO_2e$ annually [1]. Nutrient deficiencies, like copper in wheat (*Triticum aestivum*), can severely impact crop productivity [2], and optimized zinc levels enhance soil enzyme activities critical for a sustainable agroecosystem [3] [4]. Despite their importance, current nutrient delivery systems in industrial agriculture are inefficient [5]. Moreover, conventional, water-soluble micronutrient fertilizers often contribute to groundwater pollution, while non-soluble fertilizers limit crop nutrient uptake due to less bioavailability [6]. Improving nutrient use efficiency, reducing runoff and environmental pollution, and boosting biomass and crop yields are crucial for food security and mitigating greenhouse gas emissions [7].

To address these challenges, innovative approaches, such as slow and controlled-release fertilizers (SRFs and CRFs), are being developed worldwide. Many of the commercially available SRFs and CRFs use synthetic, thermoplastic coatings that do not readily degrade in the soil and contribute to microplastic pollution [8]. Efforts are underway to establish regulations for biodegradable polymer materials in these fertilizers, emphasizing the need for environmentally friendly alternatives [9]. Consequently, developing and commercializing environmentally friendly, biodegradable SRFs and CRFs fertilizers are paramount. Similarly, bio-based slow-release micronutrient seed coatings are emerging as substitutes for water soluble minerals and improving nutrient longevity around the seeds [10].

This study presents the efficacy and carbon sequestration impact of novel biopolymer based slow-release micronutrient fertilizers, Soileos Zinc and Soileos Copper as granular applications and Nutreos Zinc as seed coatings (Soileos and Nutreos are trade names with reserved copyright) in greenhouse and field trials for cultivating wheat. Soileos and Nutreos are developed using proprietary technology [11]. Through this patented technology, micronutrients are bound to biopolymers from food processing co-products, such as pea, lentil, or oat hulls, to minimize the minerals' water solubility and enhance nutrient longevity in soil. The product's mode of action is available online [12]. This study reports greenhouse and field trials cultivating wheat and measuring soil organic carbon compared to grower standard practice (GSP). In greenhouse trials, spring wheat (*Trit-*

icum aestivum L.) was treated with Soileos and Nutreos products, and the results were compared with those when conventional micronutrient sulfate sources and GSP control were applied. During the cultivation, soil respiration, yield, above-ground biomass, soil carbon levels, net greenhouse gas (CO_2 and N_2O) emissions, and estimated post-harvest soil carbon balance were evaluated. In the field trials, Soileos Zinc was applied as a source of slow release (SRF) zinc micronutrient to four wheat varieties grown in four different fields in Manitoba, Canada. Yield and soil organic carbon content were monitored and compared to the crops treated with GSP, in which no Soileos Zinc product was added. The results of greenhouse and field trials both highlight the value of this innovative technology in enhancing nutrient longevity in the soil.

2. Materials and Methods

2.1. Greenhouse Study

Greenhouse trial techniques and methodologies are explained here.

2.1.1. Experimental Location and Setup

The study was conducted at the research greenhouse of Simon Fraser University (SFU), Burnaby, BC, Canada, at 370 m above sea level on spring wheat (*Triticum aestivum L.*, AAC Brandon) from November 2023 until March 2024. The studies were conducted peat moss and perlite in a 70:30 percent ratio and $\rho_{\mathrm{bulk}} = 150\ \mathrm{g\ cm^{-3}}$ under a 16/8 h day/night photoperiod, $450 \pm 50\ \mathrm{\mu mol \cdot m^{-2} \cdot s^{-1}}$ PAR, average temperature of 22/18˚C ± 2˚C, and relative humidity of 45 ± 5%. The physicochemical characteristics of the media and irrigation water are presented in **Table 1**.

Table 1. The physicochemical characteristics of (a) irrigation water, (b) potting media, and (c) field trial soil. CEC is cation exchange capacity in (meq/100g), OM is Organic Matter, and all the elements are in ppm.

(a)	Conductivity ($\mathrm{mS.cm^{-1}}$)	pH	Na	Cl	Ca	K	N	P	Cu
	0.05	6.5	2.1	2.2	5.8	~0	~0	~0	~0
(b)	CEC	pH	OM (%)	N	P	K	Zn	Cu	
	16	6.8	48.4	1	3	17	1.6	0.2	
(c)	Trial	pH	CEC	OM (%)	P	K	Zn	Cu	
	(a) Winnipeg	7.8	50.3	6.1	5.5	513.0	0.8	1.7	
	(b) Hamiota	8	41.6	4.1	21.3	222.8	1.5	0.9	
	(c) Altamont	6.3	25.5	6	18.8	205.5	1.4	0.5	
	(d) Deloraine	6.2	23.4	4.7	15.5	235.3	0.9	1.1	

2.1.2. Flux Chambers and Temp/Moist Sensors Installation

The automated long-term soil gas (CO_2 and N_2O) flux measurement systems from

LICOR Biosciences, consisting of fifteen LI-8100 Long-Term Opaque Flux Chambers, two LI-8250 Multiplexers, two LI-7810 CH_4/CO_2/H2O Trace Gas Analyzers and two LI-7820 N_2O/H_2O Trace Gas Analyzers were carefully installed prior to sowing the seeds. 21,315 data points were collected for each CO_2 and N_2O fluxes during the greenhouse crop growth cycle. The average daily soil temperature during the wheat cropping cycle was 21.7˚C, and the average daily minimum and maximum air temperatures were 17.3˚C and 24.6˚C, respectively.

2.1.3. Experimental Treatments and Applications

The experiment employed a factorial design based on fertilizer type (Soileos, sulfate) and seed coating (Nutreos, uncoated), utilizing a completely randomized design scheme with five treatments, including: T1: grower standard practice (GSP), which contains conventional macro and micro nutrients, with no additional zinc and copper; T2: Soileos Zinc and Soileos Copper blend with Nutreos Zinc coated seeds; T3: Soileos Zinc and Soileos Copper blend with untreated seeds; T4: zinc and copper sulfate blend with untreated seeds; and T5: zinc and copper sulfate blend with Nutreos Zinc coated seeds, with three replications.

The application rate of elemental zinc and copper fertilizer was 4.48 $kg \cdot ha^{-1}$ and 2.80 $kg \cdot ha^{-1}$, respectively. While the application rate of Nutreos coating was 0.5 g Zn per kg of seeds. For all treatments except for the GSP (T1), the peat moss/perlite media was transferred to a Ryobi electric cement mixer, where the soil was treated and thoroughly mixed with the fertilizer for 90 seconds. Then, the potting media was transferred to the reservoirs. Subsequently, the potting media was packed, and the soil depth was between 20 ± 1 cm.

2.1.4. Seed Sowing and Growing Cycle

Nutreos Zinc coated (T2 and T5) and uncoated (T1, T3, and T4) seeds were sown manually by hand into the potting media in their respective trays on November 7, 2023, at a seeding rate of 101 $seeds \cdot m^{-2}$ (188/tray). Six rows of seeds were planted in each tray, spaced 5 cm apart with 11.5 cm between rows, considering room for the flux chambers and plants were grown until March 4, 2024.

2.1.5. Plant and Soil Carbon Analysis

Soil samples were collected, ground, and sieved (2 mm mesh size) at the end of the growing cycle after harvest. An independent, accredited third-party lab analyzed the carbon contents of soil samples using the TMECC combustion method [13]. Relative soil relative carbon balance ($C_{balance(rel.)}$) was calculated following protocols outlined by [14], with Equation (1) below:

$$C_{balance(rel.)} = \frac{C_{s(final)}}{C_{s(initial)}} \times 100\% \tag{1}$$

where C_s is the soil carbon stock, meaning the total amount of carbon stored in the soil, $C_{s\,(\text{initial})}$ is the soil carbon stock before sowing the seeds, and $C_{s\,(\text{final})}$ is the soil carbon stock after harvest ($mol \cdot m^{-2}$). Above ground carbon stock (kg/plot) was calculated by applying a carbon conversion factor of 0.45 to the above ground biomass [15].

2.2. Field Study

2.2.1. Experimental Location and Setup

The field study was conducted at four different locations in Manitoba, Canada as presented in Figure 1.

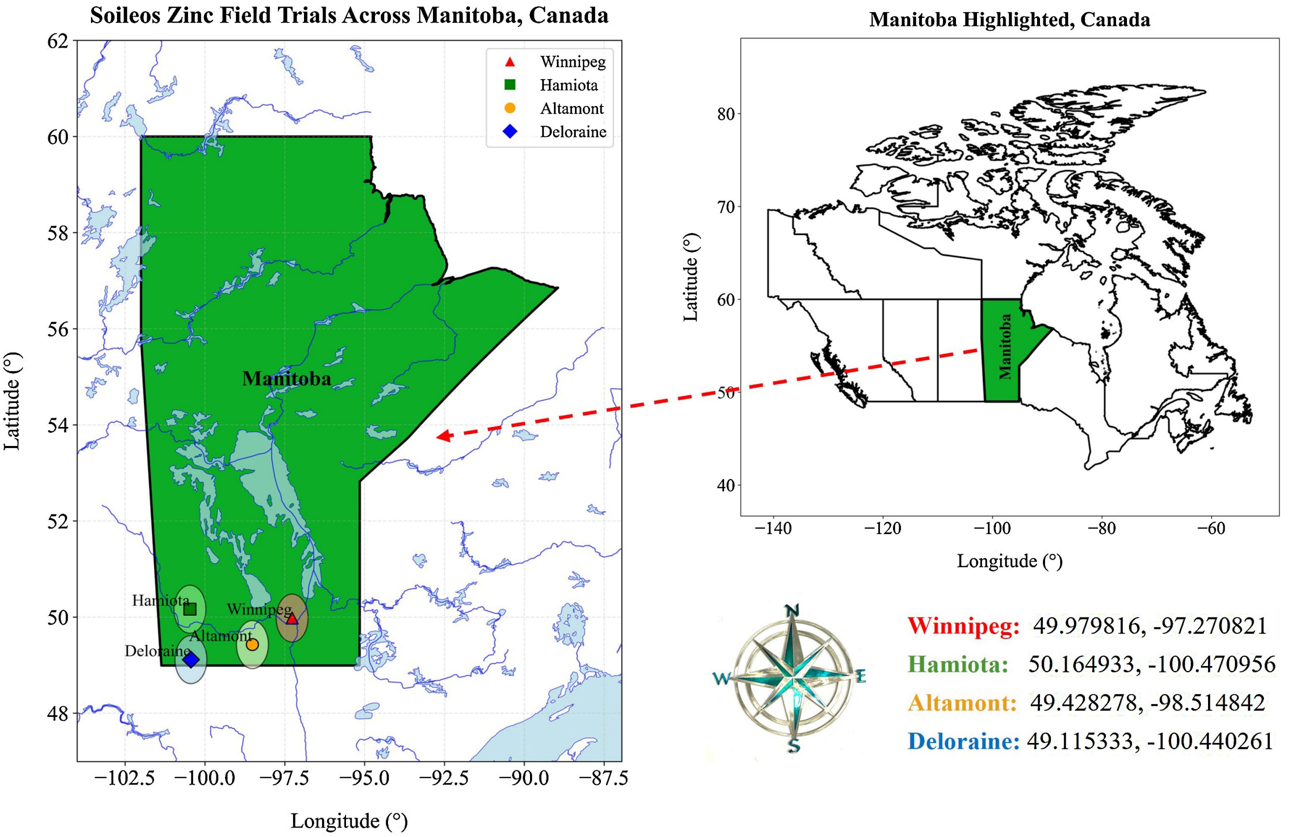

Figure 1. Trial locations in Winnipeg, Hamiota, Altamont and Deloraine in Manitoba, Canada.

Different varieties of spring wheat (*Triticum aestivum L.*) seeds were grown in each location. The mean total precipitation during the growing cycle was 291 ± 24 mm. Soil sampling and analysis were conducted after harvest at each site (a composite sample from a depth of 0 - 30 cm) for both Soileos Zinc treated and untreated fields. The physicochemical characteristics of the soil are represented in Table 1.

2.2.2. Treatments and Application

The experiment was conducted using an 8.09 ha (20 ac) replicated strip trial including FT1: grower standard practice (GSP), which contains conventional macro and micronutrients, excluding zinc, and FT2: GSP with Soileos Zinc. Agronomic practices are represented in Table 2.

2.2.3. Total Organic Carbon (TOC)

At the end of the growing cycle and after harvest, a composite sample from each treatment was collected using a random sampling method from eight different

Table 2. Agronomic practices of field trials.

Trial	Variety	Area (m^2)	Sowing Date, 2024	Harvest Date, 2024	N ($kg \cdot ha^{-1}$)	P ($kg \cdot ha^{-1}$)	Zn ($kg \cdot ha^{-1}$)
(a) Winnipeg	AAC-Hockley	410,432	May 14	Sep 01	157.92	56.04	1.12
(b) Hamiota	Starbuck	260,577	May 10	Sep 06	146.71	44.83	1.12
(c) Altamont	Manness	69,161	May 20	Sep 12	148.95	39.23	1.12
(d) Deloraine	Elite	401,367	May 14	Sep 04	123.29	33.63	1.12

locations. The composite samples were then sent to a third-party lab for carbon measurement, where the total percentage of organic carbon (TOC) was compared to Soileos and GSP.

2.2.4. Yield

At harvest, wheat yield (total grain weight/strip) was recorded for each treatment at all four locations. Yield data were collected using a calibrated grain yield monitor mounted on a combine harvester. The final yield (bushels per acre) was calculated, converted to $kg \cdot ha^{-1}$ and compared between the Soileos Zinc-treated (FT2) and the GSP (FT1) plots.

2.3. Data Analysis and Statistical Methods

Statistical analysis was performed using two-way ANOVA followed by an effect size (η_p^2) test with a 95% confidence interval. Analyses were conducted using the R Statistical language (version 4.3.0; R Core Team, 2023) [16], with the following packages: agricolae (version 1.3.5) [17] and effect size (version 1.0.0) [18] for statistical analysis, dlookr (version 0.6.1) [19] for data inspection and exploration, dplyr (version 1.1.4) [20] for data manipulation and ggplot2 (version 3.5.1) [21] for data visualization.

3. Results

3.1. Greenhouse Study

3.1.1. CO_2 and N_2O Flux

As explained in 2.1.2 section, emitted CO_2 and N_2O were automatically measured during the crop growth cycle. The emission patterns of CO_2 and N_2O were different between treatments, and there were different observed peaks of CO_2 and N_2O fluxes throughout the entire growing cycle (**Figure 2**). Moreover, the interaction of treatments with soil moisture dynamics and irrigation appeared to play a critical role in regulating emission peaks, consistent with findings by Shah *et al.* [22].

3.1.2. Yield and Biomass

Above-ground dry biomasses for the uncoated wheat seed treatments of the T1, T3, and T4 fertilized plots were 0.91, 1.1, and 0.96 kg/plot, respectively. For the Nutreos Zinc-coated treatments, the above-ground biomasses of the T2 and T5 fertilized plots were 1.16 and 1.01 kg/plot, respectively. The application of Soileos

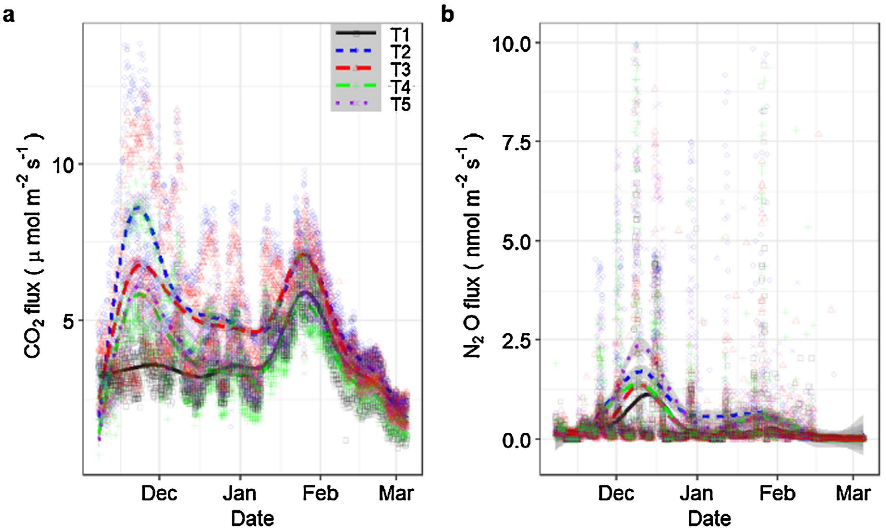

Figure 2. Scatter plot of 21,315 data points measured for each a. CO_2 (μmol·m^{-2}·s^{-1}) and b. N_2O fluxes (nmol·m^{-2}·s^{-1}) from *T. aestivum* (AAC Brandon) plots in potting media for T1-T5 over the entire growth cycle.

Zinc and Soileos Copper, combined with Nutreos Zinc-coated seeds (T2), increased the above-ground biomass of the harvested wheat crops compared to treatments that did not receive seed coating (Figure 3a). A similar trend was observed for spike weight, as illustrated in Figure 3b. The dried spike weights (DSW) for T1, T3, and T4 were 0.40, 0.51, and 0.43 kg/plot, respectively. While the Nutreos Zinc-coated wheat seed treatments, the DSW of the T2 and T5 were 0.50 and 0.47 kg/plot, respectively. In general, zinc and copper fertilization numerically increased wheat yield in this study compared to GSP (T1). More specifically, the fertilizer source had a large effect ($\eta_P^2 = 0.59$) on DSW, T2 and T3 (Soileos Zinc and Soileos Copper), demonstrated larger DSW than T1 (GSP) and T4 and T5 (zinc and copper sulfate). Similarly, the fertilizer source had a large effect ($\eta_P^2 = 0.32$) on the above-ground biomass, T2 and T3 (Soileos Zinc and Soileos Copper), had higher above-ground biomass than T4 and T5 (zinc and copper sulfate), while T1 (GSP) had the lowest above-ground biomass. It also appeared that Nutreos Zinc had a small effect on the above-ground biomass ($\eta_P^2 = 0.04$), where further increases in biomass were observed in treatments where seeds were coated with Nutreos Zinc compared to uncoated seeds.

After harvesting the wheat, soil samples were collected, and the soil carbon content was analyzed as detailed in section 2.1.7. The results are presented in Figure 3c and Figure 3d, as well as in Table 3.

3.2. Field Study

3.2.1. Yield

Generally, fields treated with Soileos (FT2) demonstrated increases in wheat yields compared to the GSP (FT1) from +3.3% to −0.2%. More specifically, on average, FT2 increased wheat yield by 0.9% compared to FT1. The largest increase in yield for FT2 compared to FT1 occurred at Deloraine (+3.3%), Winnipeg (+0.6%),

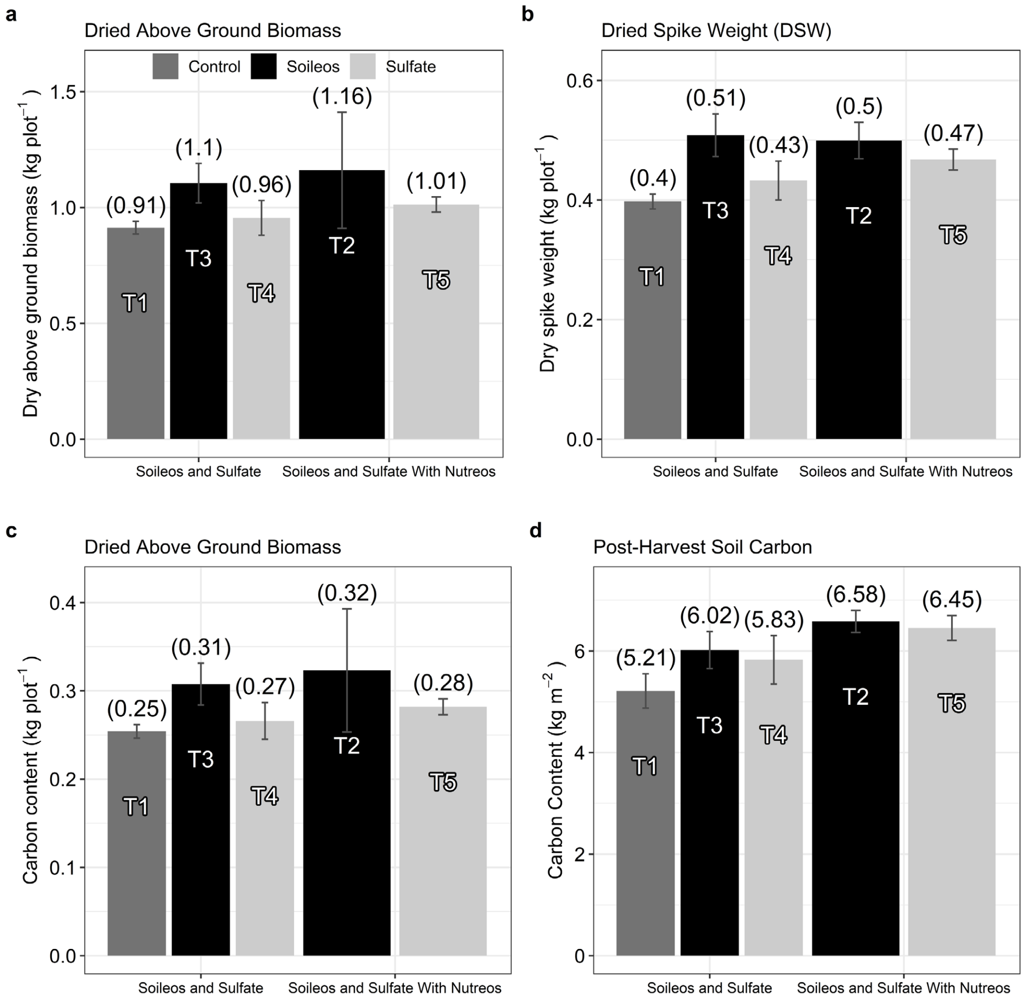

Figure 3. a. Above-ground dry biomass; b. spike weight; c. above-ground biomass carbon content; d. soil carbon content of *T. aestivum* (AAC Brandon) plots. Error bars are the standard error of the mean.

Table 3. Post-harvest soil carbon contents ($C_{s\ final}$), and calculated cumulative CO_2, N_2O emitted during the growing cycle.

Treatment	Cs final* (kg·m^{-2})	Cs final (mol·m^{-2})	CO_2 em. (mol·m^{-2})	N_2O em. (mmol·m^{-2})
T1	5.2 ± 0.3	434.0 ± 28.2	40.7 ± 2.6	3.8 ± 0.2
T2	6.6 ± 0.2	547.9 ± 18.2	58.4 ± 8.0	6.3 ± 2.8
T3	6.0 ± 0.4	501.0 ± 30.4	54.6 ± 7.2	4.5 ± 1.2
T4	5.8 ± 0.5	485.1 ± 39.7	42.0 ± 4.8	4.6 ± 1.3
T5	6.5 ± 0.2	537.2 ± 20.5	44.7 ± 5.1	6.6 ± 1.9

*Initial soil carbon was 5.3 kg·m^{-2} (444.0 mol·m^{-2}).

Altamont (+0.02%), and then Hamiota (-0.2%). At Winnipeg, the wheat yield was measured at 5683 and 5716 kg·ha^{-1} for FT1A (GSP) and FT2A (Soileos), respectively. Altamont had wheat yields measured at 4542 and 4543 kg·ha^{-1} for FT1C (GSP) and FT2C (Soileos), respectively. At Deloraine, the wheat yields were measured to be 5568 and 5749 kg·ha^{-1} for FT1D (GSP) and FT2D (Soileos), respectively. The only trial where FT1 had higher wheat yields than FT2 was Hamiota, where FT1B had a slightly higher yield (5739 kg·ha^{-1}) compared to FT2B (5730 kg·ha^{-1}) as seen in **Figure 4**.

3.2.2. Total Organic Carbon

The post-harvest soil organic carbon (SOC) of wheat fields treated with FT2 was also higher than FT1 (**Figure 5**), The mean soil organic carbon contents for FT1

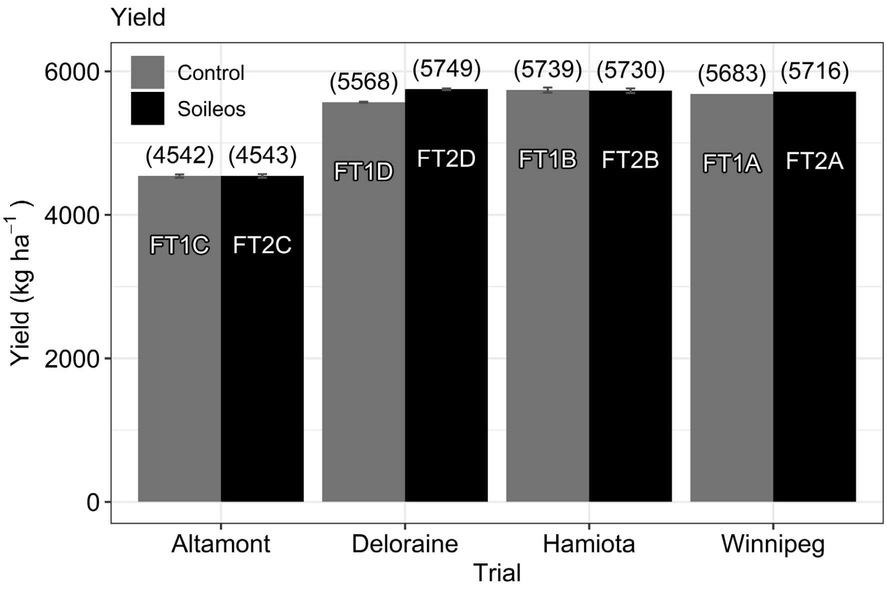

Figure 4. Comparison of mean yield of *T. aestivum* fields fertilized with (FT2) and without (FT1) Soileos in Manitoba, MB, in the year 2024. Error bars are standard errors of the mean.

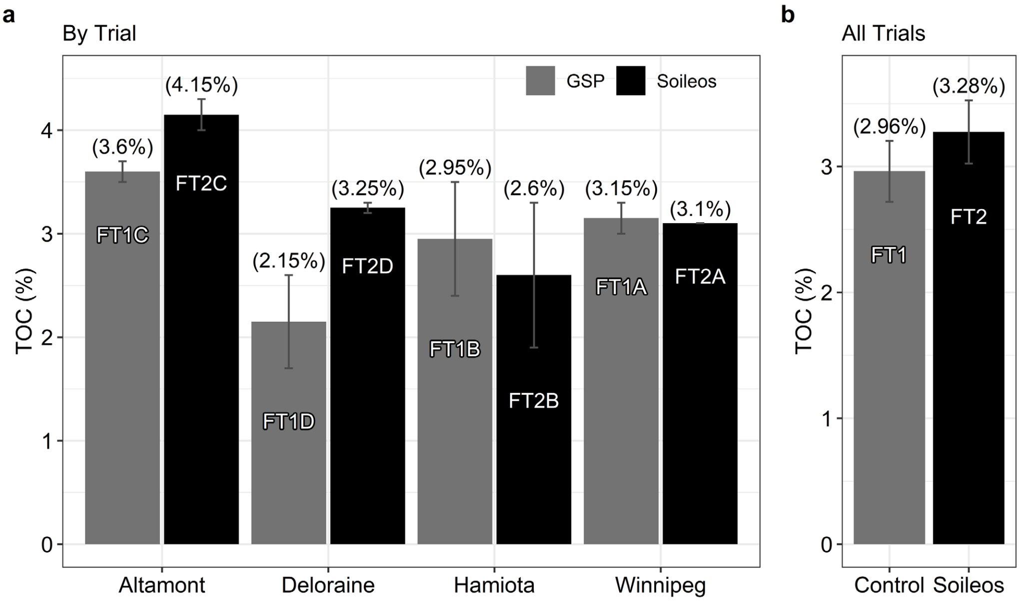

Figure 5. Comparison of total organic carbon contents in the fields fertilized with (FT2) and without (FT1) Soileos in Manitoba, MB, in the year 2024 a. by trial and b. all trials together. Error bars are standard errors of the mean.

and FT2 were 2.96 ± 0.31 and 3.28% ± 0.13%, respectively. Interestingly, the Deloraine field, which had the greatest difference in yield between FT1D and FT2D, also had the greatest difference in SOC between treatments; FT2D had ~51% more SOC than FT1D. SOC contents for FT1D and FT2D were 2.15% ± 0.45% and 3.25% ± 0.05%, respectively. Furthermore, at Hamiota, FT2 exhibited lower wheat yields than FT1 and demonstrated a similar trend in SOC content. FT2B had 11.9% less SOC than FT1B, the measured SOC for each treatment was 2.60% ± 0.70% and 2.95% ± 0.55%, respectively.

4. Discussion

4.1. Effect of Soileos and Nutreos Slow-Release Fertilizer on Yield

As shown in **Figure 3**, in the greenhouse trial, T2 and T3 increased dried spike weight (DSW) yield by 25% - 27% per plot compared to T1, and 16% - 19% per plot compared to plots treated with zinc and copper sulfate, T4. Wheat plots treated with zinc and copper sulfate (T4) showed a 7.5% increase in DSW per plot compared to the GSP (T1). When applying a Nutreos Zinc seed coating, T5, the DSW increased by 17% per plot compared to T1. Nutreos Zinc-coated seeds did not demonstrate yield improvements in addition to Soileos products in this study.

The findings in the field trials are also consistent with the greenhouse trial, where three of four trials had yield improvements when treated with Soileos Zinc compared to GSP. In the field, the average yield improvement was 0.9%, which was lower than the greenhouse results. This may be due to the relatively low amount of precipitation (291 ± 24 mm) during the growing season across the trials. Soileos releases micronutrients through a microbially mediated process, and the relatively dry conditions may have inhibited some soil microbial activity needed to release zinc gradually during the growing season, reducing the impact of Soileos on yield in this experiment. Nonetheless, the impact of additional zinc and copper on wheat yield in deficient soils agrees with the previous reports [23].

4.2. Effect of Soileos and Nutreos Slow-Release Fertilizer on Soil Biological Activity in the Greenhouse Trial

Zinc and copper play critical roles in soil biological activity and ecosystem functioning. These elements influence a wide range of biological and chemical processes, including enzyme activity, redox reactions, and nutrient cycling, all of which have implications for carbon dioxide (CO_2) emissions from soil [24] [25]. Zinc is a structural and catalytic cofactor for over 300 enzymes [26]. Similarly, copper is an essential cofactor in enzymatic activities that mediate redox reactions and are vital for the degradation of complex organic compounds [27]. Both zinc and copper contribute to microbial metabolic processes. Microorganisms in the soil depend on these micronutrients to drive energy production and nutrient assimilation. Zinc's role in stabilizing ribosomes and transcriptional processes supports microbial growth and activity, while copper's redox properties enable electron transfer reactions essential for microbial respiration [28]. Enhanced microbial activity, in turn, accelerates the breakdown of organic matter, releasing nutrients such as nitrogen and phosphorus for plant uptake [29]. These processes collectively improve soil fertility and support sustainable agricultural productivity. Among these proven micronutrient impacts, the micronutrient delivery system is highly impactful on nutrient efficiency. Slow-release micronutrient fertilizers offer significant advantages over fast-release counterparts in terms of enhancing soil microbiome populations. Unlike fast-release fertilizers that quickly release nutrients into the soil, which can lead to imbalances and excesses that are detrimental to microbial diversity, slow-release formulations provide nutrients gradually over an extended period. This gradual release supports a more stable and balanced environment for microbial communities to thrive. Recent research has highlighted that slow-release fertilizers can promote a healthier soil microbiome by sustaining nutrient availability without causing abrupt changes in soil chemistry. This stability encourages the proliferation of beneficial microbes that contribute to nutrient cycling, organic matter decomposition, and overall soil health [30]-[32].

However, the enhanced biological activity increases the rate of organic matter decomposition, leading to higher CO_2 and N_2O emissions. Microbial respiration, fueled by the breakdown of carbon-rich substrates, is a significant source of CO_2 and N_2O in soils [33] [34]. Additionally, zinc's role in dehydrogenase activity accelerates carbon cycling, further contributing to gaseous carbon loss [35] [36]. These influences are demonstrated in this study, as the fertilizer source had a large effect ($\eta_P^2 = 0.41$) on the cumulative CO_2 emissions and a medium effect ($\eta_P^2 = 0.07$) on cumulative N_2O emissions. The cumulative CO_2 and N_2O emission data indicated that wheat plots fertilized with Soileos Zinc and Soileos Copper (T3) promote higher soil biological activities compared to the GSP (T1) and wheat plots fertilized with conventional zinc and copper sulfate (T4). The cumulative CO_2 emissions of wheat plots fertilized with Soileos Zinc and Soileos Copper were calculated to be 54.6 ± 7.2 mol·m^{-2}, while wheat plots fertilized with zinc and copper sulfate were calculated to be 42.0 ± 4.8 mol·m^{-2}. Applying Nutreos Zinc to wheat seeds had a small effect ($\eta_P^2 = 0.03$), further increasing cumulative CO_2 emissions in both Soileos Zinc and Soileos Copper (T2) and zinc and copper sulfate (T5) fertilized wheat plots to 58.4 ± 8.0 and 44.7 ± 5.1 mol·m^{-2} (**Figure 6a**), respectively.

The effect of zinc and copper fertilizer sources and seed coating on the cumulative N_2O emissions is mentioned in **Figure 6b**. All treatments were amended identically and received the same amount of nitrogenous fertilizer (in the form of urea). However, the GSP treatment (T1), which received no zinc or copper fertilization, emitted less N_2O than treatments fertilized with zinc and copper (3.8 ± 0.2 mmol m^{-2}). The cumulative N_2O emissions of wheat plots fertilized with Soileos Zinc and Soileos Copper (T3) and zinc and copper sulfate (T4) were calculated to be 4.5 ± 1.2 and 4.6 ± 1.3 mmol m^{-2}, respectively. A similar trend in cumulative CO_2 emissions (**Figure 6a**) was also observed in the N_2O emissions ($\eta_P^2 = 0.08$), applying Nutreos Zinc to wheat seeds further increased N_2O emissions in both T2 and T5 fertilized wheat plots to 6.3 ± 2.8 and 6.6 ± 1.9 mmol m^{-2}, respectively. The observed differences in cumulative CO_2 and N_2O emissions between treatments were not statistically significant, which is reasonable because although soil respiration and microbial growth are closely related, the relationship is not perfectly linear. Soil respiration measures the carbon dioxide released from the soil, which is influenced not only by microbial growth but also by other processes such as root respiration and the decomposition of organic matter [37]. Generally, the increased CO_2 emissions in T3 and T2 demonstrated increased biological activities, which are indicators of soil health.

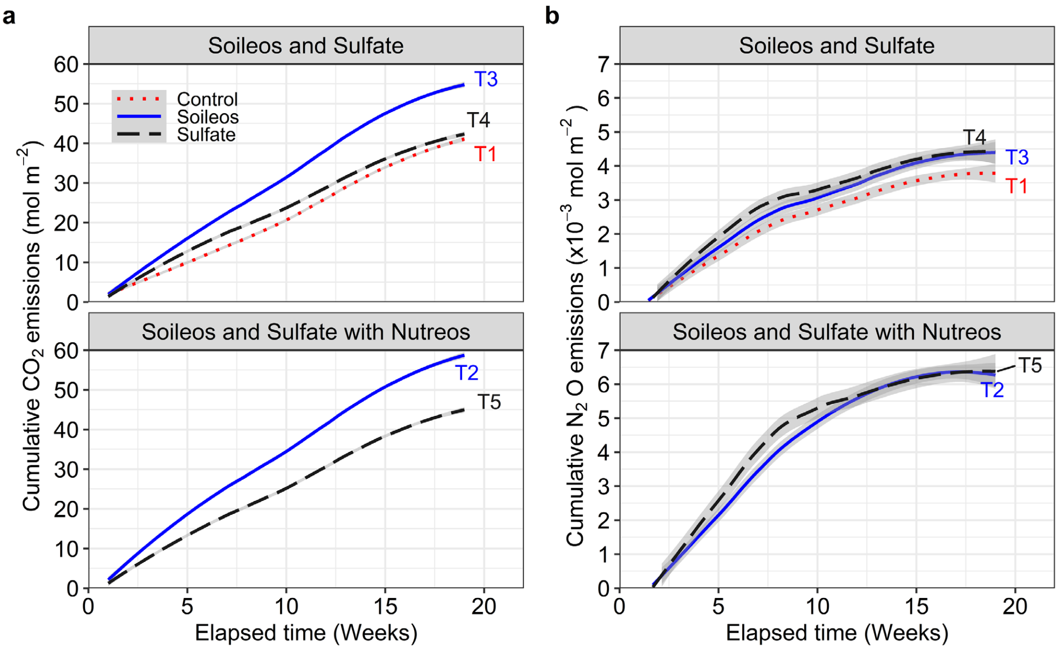

Figure 6. Calculated cumulative a. CO_2 and b. N_2O emissions (mol·m^{-2}) from *T. aestivum* (AAC Brandon) plots in potting media fertilized T1-T5 over the entire growth cycle. The gray area around the lines represents the standard errors.

4.3. Effect of Soileos and Nutreos Slow-Release Fertilizer on Soil Relative Carbon Balance in Greenhouse and Field Trials

4.3.1. Soil Carbon Balance in the Greenhouse Trial

The carbon content of the above-ground biomass presented in Figure 3c was calculated as outlined in section S3. The soil was sampled before seeds were sown and post-harvest for each treatment, with three replicates. Soil samples were sent to a third-party lab for accurate carbon content measurements. Numerically, the differences in post-harvest soil carbon were observed, although not statistically significant (Figure 3d). GSP (T1) had the lowest post-harvest soil carbon content (5.2 ± 0.3 kg·m^{-2}), while plots that received zinc and copper fertilization demonstrated higher post-harvest soil carbon of 6.0 ± 0.4 and 5.8 ± 0.5 kg·m^{-2} for T3 and T4, respectively. Further increases in final soil carbon content were observed when fertilizing with zinc and copper and coating seeds with Nutreos Zinc, where the post-harvest soil carbon content was 6.6 ± 0.2 and 6.5 ± 0.2 kg·m^{-2} for T2 and T5, respectively. It is important to note again that each plot contained 125 kg of soil, and the total Soileos per related plot was only 15 g, and coated seeds received a total of 0.04 g of Nutreos Zinc per related plot. Furthermore, this study's growth media/soil was a 70/30 peat moss/perlite, containing 59.7% organic matter as discussed in sec. 2.1. Since the total application of Soileos and Nutreos was very low and the growth media already contained a high level of organic matter, the impact of the trace amount of additional cellulosic carbon on the final soil carbon content is negligible. Therefore, differences in soil carbon content between treatments are due to additional micronutrients, the fertilizer source, and/or seed coating, which demonstrated a large effect, $\eta_P^2 = 0.18$, related to fertilizer source and medium effect, $\eta_P^2 = 0.12$ of seed coating on the soil carbon content. Plots treated with Soileos Zinc and Soileos Copper (T2 and T3) demonstrated higher final soil carbon contents than all other treatments. This effect is likely due to the higher efficacy and longevity of micronutrients in the soil when using slow-release micronutrient fertilizers compared to conventional water-soluble sulfate sources.

The relative soil carbon balance (final carbon content relative to the initial soil carbon content) of the Negative GSP (T1) was 97.8% (**Figure 7**). Plots fertilized with zinc and copper increased the relative soil carbon balance compared to the GSP (T1). Soileos Zinc and Soileos Copper (T3) and zinc and copper sulfate (T4) had a relative carbon balance of 112.8% and 109.3%, respectively. Coating wheat seeds with Nutreos Zinc enhanced the relative soil carbon balance in both T2 and T5, estimated at 123.4% and 121.0%, respectively.

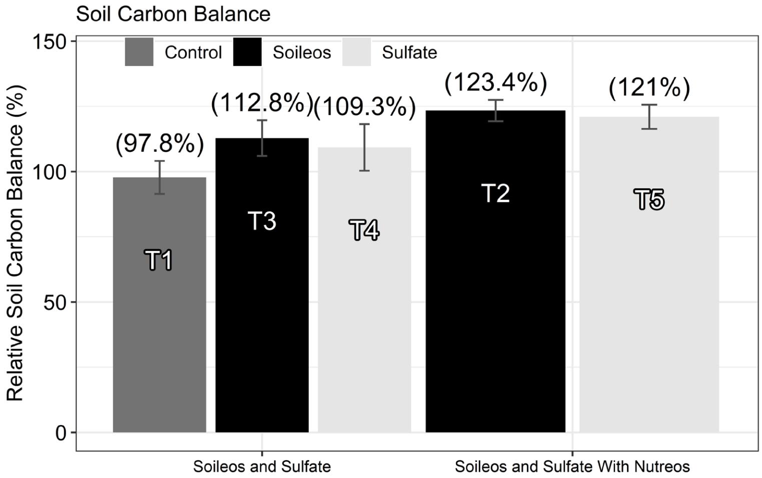

Figure 7. Calculated relative post-harvest soil carbon balance of T. aestivum (AAC Brandon) plots for treatments T1-T5. Error bars are standard errors of the mean.

As shown in **Figure 7**, GSP (T1), with no additional Zinc and Copper micronutrients, has shown carbon depletion. Micronutrients, in general, have increased soil relative carbon balance compared to T1, and the maximum measured soil carbon balance was related to T2, treated with both Soileos and Nutreos. Nutreos has improved the relative carbon balance in both Soileos and Sulfate, T2 and T5, treatments. This indicates that adding micronutrients prevents soil depletion and improves soil carbon balance. Moreover, using slow-release Soileos and Nutreos fertilizers has an improved impact on soil carbon balance compared to the conventional water-soluble sulfate source of micronutrients.

4.3.2. Soil Carbon in Field Trial

The final soil organic carbon contents of plots treated with Soileos were generally higher than GSP, indicating a positive impact of Soileos on the final soil total organic carbon content. The findings in the field trials were consistent with the greenhouse trial. The estimated CO_2 sequestered in Soileos plots relative to GSP was calculated assuming the mass of the field topsoil was 907 t·ac^{-1}. As shown in Figure 8, the average estimated CO_2 sequestered in Soileos plots (FT2) was 10.4 t·ac^{-1} compared to GSP (FT1).

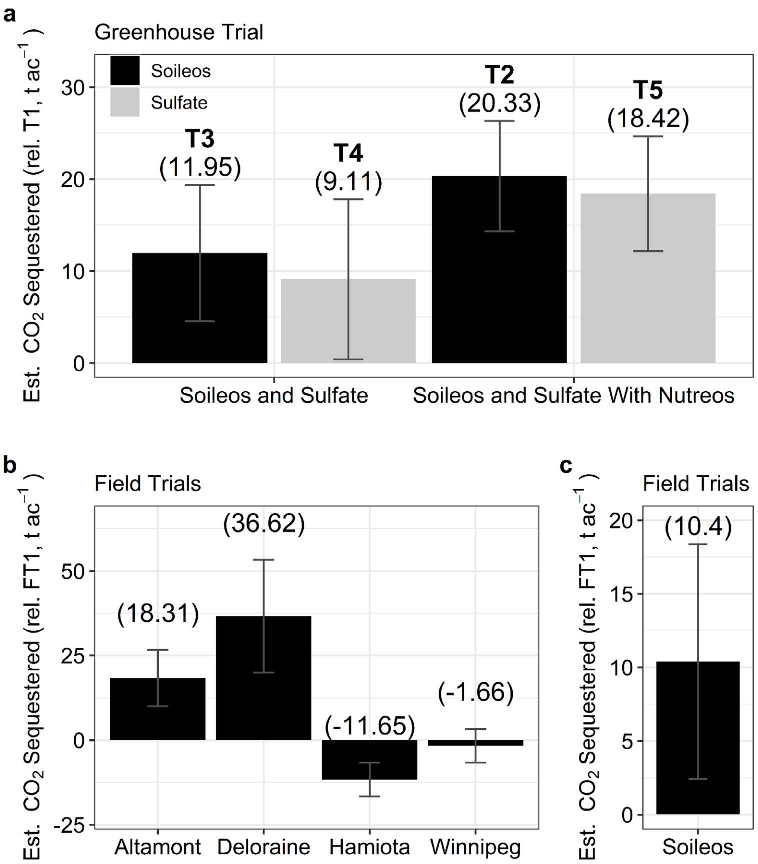

Figure 8. Estimated equivalent of CO_2 sequestered in the greenhouse trial for T1-T5, b. estimated equivalent of sequestrated CO_2 in the soil for FT2 per different field trials compared to the GSP (FT1) and c. the average estimated sequestrated CO_2 for all field trials treated with Soileos (FT2) compared to GSP (FT1). Error bars are standard errors of the mean.

Unsustainable practices from agricultural intensification deplete the soil's carbon pool. The annual average rate of depletion of soil organic carbon (SOC) is often much greater than the rate of SOC sequestration. But adopting sustainable agricultural management practices may unlock tremendous potential for agricultural soils to store atmospheric carbon [38]. Utilizing Soileos and Nutreos as micronutrient fertilizers and seed coatings promotes sustainable agricultural practices. Management strategies such as increasing fertilizer use efficiency via slow-release fertilization, which increases plant biomass, lead to increases in soil carbon stocks [39]. Therefore, delivering micronutrients using Nutreos and Soileos products with higher longevity in the soil for plants had positive effects on wheat crop growth and, thus, the final soil carbon balance. This environmentally positive impact is important for soil carbon sequestration.

4. Conclusions

Our findings underscore the effectiveness of Soileos and a combination of Soileos and Nutreos seed coating, as sustainable slow-release crop micronutrient sources, demonstrating numerical increases in crop yields, above and below ground biomass, and relative carbon balance compared to both GSP and sulfate-based fertilizers. The higher rates of CO_2 respiration observed in these Soileos and Nutreos trials, indicative of enhanced soil microbial activity, were positively correlated with increased soil carbon biomass production. In the greenhouse trials, Soileos improved soil microbial activity by 34% compared to the GSP and by 29% compared to sulfate-based sources. Combining Nutreos with Soileos further enhanced microbial activity, showing increases of 44% over the GSP and 29% over sulfate. The relative carbon balance increased by 15% and 3% when using Soileos compared to the GSP and sulfate treatments, respectively. Additionally, combining Nutreos with Soileos improved the carbon balance by 26% relative to the GSP and by 2% relative to sulfate. Field trial results support the greenhouse results on the Soileos carbon sequestration impact. The estimated CO_2 sequestered in the field when using Soileos was 10.4 ± 8.0 $t \cdot ac^{-1}$ relative to GSP, while the estimated CO_2 sequestered in plots treated with Soileos in the greenhouse was 12.0 ± 7.4 $t \cdot ac^{-1}$ relative to GSP. This makes Soileos and Nutreos valuable tools for producers seeking to improve soil health and participate in carbon farming initiatives and regenerative agriculture systems.

While Soileos shows benefits in terms of yield enhancement and carbon sequestration over one growing season, we are continuing long-term field trials and data collection to further validate its impact over multiple growing seasons. Additionally, future trials will be run by focusing on the integration of Nutreos seed coatings, which have shown promising results in previous greenhouse studies, to evaluate their combined effect when used alongside Soileos fertilizers. The results will be published afterward.

Data Availability Statement

The data supporting this study's findings is available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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