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Spent Coffee Grounds (SCGs) as a Soil Amendment: The Effects of Composting Time on Early Sunflower Development

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Abstract: The unsustainability of current and future agricultural use has led to increased interest in using organic waste products, such as spent coffee grounds (SCGs) and compost, as alternative nutrient supplements. While SCGs are initially phytotoxic, upon composting, they can be utilised as a soil amendment; however, the minimum length of composting time required is not well known. Two glasshouse experiments were conducted to identify the concentration where raw SCGs are toxic to sunflower seedling growth and to assess the age and concentration at which composted SCGs are most effective for crop soil addition. Both raw and composted SCG substrates demonstrated higher water-holding capacities and electrical conductivity levels than commercial soil mix, though differences were observed in pH, with raw SCGs being acidic and composted SCGs being neutral. Concentrations of raw SCGs $\geq$ 35% caused large reductions in germination, plant height, cotyledon and true leaf emergence. SCGs composted for 6 months were non-toxic for sunflower seedlings and most effective at $\geq$35%, which could potentially reduce composting times by 50%. The addition of raw or composted SCGs also reduced the root/shoot ratio, though the cause and effects are currently unknown.

Keywords: spent coffee grounds; circular economy; phytotoxicity; composting; nature-based solutions

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1. Introduction

Fertilisers are essential for maintaining crop yield in contemporary agriculture [1]. Of the nutrients provided by fertilisers, nitrogen is generally the main limiting soil nutrient for plants provided by these amendments [2,3].

Chemical fertilisers are unable to replace all nutrients lost from agricultural soils from repeated harvests [4], making the indefinite use of chemical fertilisers unsustainable. Long-term reductions in the quality of agricultural products have been recorded [5], and waterway eutrophication from nutrient runoff has been well described [6,7], which may have consequences for human health [6,7]. Furthermore, fertiliser prices can serve as an economic sink [8], having increased drastically due to recent geopolitical changes [6,8] and fossil fuel costs. Fertilisers also account for 2.1% of global greenhouse gas (GHG) emissions [9].

As a result of the growing unsustainability of synthetic fertiliser use [10], there has been increasing interest in the use of organic waste products. These wastes, such as garden waste, food waste and biosolids [11], can reduce environmental costs and waste management if properly utilised [12]. Composting facilitates the breakdown of these wastes into plant-usable forms and the stabilisation of organic materials [11], while also reducing potentially harmful bacteria, parasites and viruses [13]. Organic wastes provide a range of organic compounds and minerals suitable for agriculture [14], making them of growing interest as a soil fertiliser amendment [13]. Despite these benefits, disconnect between the agricultural and waste management sectors, as well as the absence of a general business

model, have restricted large-scale prevalence [13]. As such, providing evidence to support the value of compost for agricultural use is hoped to help reduce these barriers.

An organic waste of current interest in research is spent coffee grounds (SCGs), produced as waste from the coffee brewing process [15,16]. Coffee is one of the most consumed beverages globally [17] and one of the most traded commodities [15], with its production serving as a major source of revenue and employment for developing countries such as Brazil, Vietnam and Columbia [18]. This large-scale production and consumption results in large amounts of SCGs, equating to 650 kg from each tonne of coffee produced [15,16], with the majority of globally produced SCGs (54.2%) currently discarded into landfill [19]. With at least six million tonnes of SCGs produced worldwide from just instant coffee [20], this raises landfill waste and costs and produces GHG [20]. Utilising spent coffee grounds as a soil amendment not only benefits plant growth through germination stimulation and increased leaf number [17,21] but also improves soil properties through improvements in nutrient availability, soil water-holding capacities and soil pH [22]. Furthermore, the use of SCGs keeps them out of the waste system and also supports the circular economy concept [13], which aims to minimise waste and repurpose resources for current and future sustainable development. The disposal of raw SCGs directly after use can cause potential soil and water contamination [17] due to the release of components such as phenols, caffeine and tannins [22]. Composting SCGs is known to break down these toxic components [23], producing a material suitable for plant growth [24]. Currently, 34.1% of global SCGs are utilised as composted soil amendments [17]. Composted SCGs increase plant biomass and production, as well as enhancing plant health [25], especially in combination with other composted waste products [18]. However, composting raises challenges: CO_2 is released during the composting process [26], and it is time-consuming, meaning that SCGs are typically unusable for 12 months after collection. Means by which the composting process could be shortened whilst still reducing phytotoxicity, or otherwise simplified, would be of significant benefit.

The effects of raw SCGs on plant growth are mostly attributed to their phytotoxic components: caffeine, tannins and chlorogenic acid [27]. Most studies have cited negative effects on plant growth [23], particularly at higher concentrations, through reductions in plant growth and biomass [22]. However, some studies have citied positive effects on plant growth, although only at concentrations under 10% raw SCG [21,28]. Despite negative effects of plant growth being observed most commonly at high raw SCG concentrations, a definitive concentration at which toxicity is present has yet to be identified.

SCGs have several properties beneficial for plant growth. Both raw and composted SCGs have high water-holding capacities and high levels of organic matter and macronutrients [28]. There are, however, notable differences in properties between raw and composted SCGs, with raw SCGs having lower pH values [22] and electrical conductivity (ranging from 2600 to 4500 µS [21]). Composted SCGs have a pH ranging from slightly acidic to neutral [25], making them suitable for many crop species; however, they also have much higher electrical conductivity than raw SCGs, which has potential to negatively influence plant growth [17]. The biggest difference in properties between raw and composted SCGs, however, is the presence of toxic components in the former. Composting has been shown to significantly reduce these components [23] to levels where they show no effects on plant growth [27], which offers potential for the growth of agricultural plants such as sunflowers (Helianthus annuus). Sunflowers are fast growing, agriculturally relevant and adaptable to a variety of different climate and soil conditions [29]. Furthermore, sunflowers are an important oilseed crop [30], and sunflower stalks are finding growing use as a bio-based building material, showing similar properties to other bio-based composites including industrial hemp [31].

Despite their wide availability, the agricultural use of SCGs has been restricted to date due to the general perception of their phytotoxicity, along with an aversion to time and costs associated with composting, which can range between 12 months and up to 2 years [32] before use. As such, this project aims to facilitate the broader use of SCGs

in agriculture by quantifying the effects of raw and composted SCGs on plant growth at different concentrations over an extended period of time, identifying the features of any phytotoxicity that arises, and determining whether composting time could be reduced without introducing phytotoxic effects.

2. Materials and Methods

2.1. Spent Coffee Grounds' (SCGs') Composting Process

The composted spent coffee grounds (SCGs) used in these trials were collected in situ, from a farm that currently incorporates this material in commercial crops of garlic and industrial hemp. The composted SCGs used for this experiment were commercially collected from a range of Sydney cafes, including the Campos and Kua Coffee chains, which were transported to Hartley Vale Farm (−33.528, 150.244; Hartley Vale, NSW, Australia). These SCGs were composted alongside but separated from dehydrated food waste (DFW) in open windrows (Figure 1), where organics are placed in long, narrow piles and are mechanically recirculated on a bi-weekly basis. Samples were collected from fresh (SCGCF), 6-month composted (SCGCM) and 12-month composted (SCGCY) windrows. The 12-month sample was tested as this is the age at which composted SGM materials are used in practice. We selected the 6-month treatment to test whether residual toxicity was present at this stage to determine whether composting time could be reduced. Samples of SCGCF, SCGCM and SCGCY were collected from depths of 0–10 cm, 40–60 cm and >100 cm deep, respectively. Samples were transported and stored before analysis in sealed containers to minimise moisture loss. Sub-samples were sieved (710 μm) and refrigerated (4 °C) for soil profile analysis.

Figure 1. Process of open windrows.

2.2. Glasshouse Experiments

Experiments were conducted in a climate-controlled glasshouse located on the rooftop of Building 4 at the University of Technology Sydney, Australia (S 33°52′57.638″ E 151°12′2.256″), with the focus of comparing raw and composted SCG regarding their effects on plant growth.

For each glasshouse experiment, a substrate mix was created to fill the required pots for each treatment (1720 g for the raw treatments and 450 g for composted treatments). This

comprised of a commercial soil mix that met the Australian Standard AS4454 for Composts, Soil conditioners and Mulches (Garden Basics Soil Mix, Pinegro Products, Goulburn NSW, Australia) and the SCG substrate (raw or composted). This commercial soil mix was selected in lieu of agricultural field substrates collected from the farm or commercial potting mix to represent a general-purpose soil with an acceptable level of plant nutrients, but without the high levels found in potting mix, or the site-specific characteristics that would typify substrates collected from a specific region. Each glasshouse trial tested the SCG concentration mixes of 0%, 2.5%, 5%, 10%, 20% and 35% SCGs by mass; however, an extra SCG concentration of 50% was added to the composted SCG treatments. This extra concentration was included due to the literature citing that the effectiveness of composted SCG was only observed at higher concentrations above 10% [33], as well as to determine whether there was an upper concentration limit of SCG compost's effectiveness. The proportion of each substrate used for each soil mix reflected the treatment (e.g., 35% raw used 65% commercial soil) and was weighed out using a scale into a prepared tray. Mixes were homogenised using a cement mixer for two minutes before being measured out evenly and compacted into the prepared 150 mm diameter pots for each treatment. The raw SCG glasshouse experiment used 10 pots for each treatment, while the composted SCG trials used 5 pots for each treatment, resulting in 15 pots total for each tested concentration across the three compost ages. These differing pot numbers were due to the initial raw SCG glasshouse trial testing two different pot sizes, tube pots (length: $5 \times 5 \times 12.5$ cm) and circular pots (length: $9.5 \times 9.5 \times 14$ cm), in case pot size resulted in any differences in results. As no significant differences were observed, the larger pot size was not used. After the first experiment, it was clear that the smaller sample size of 5 pots provided sufficient statistical power, and consequent experiments thus used the smaller number of independent replicates. For each glasshouse experiment, pot placement was randomised amongst treatments to avoid spatial confounding during plant growth.

Each glasshouse trial was conducted separately, with the raw treatments planted on the 5th of December 2023 and the composted treatments planted on the 25th of January 2024. Pot soils were moistened to field capacity, and two sunflower seeds were planted in each pot, 3 cm deep and 1 cm apart. For each glasshouse trial, plants were observed for four weeks after the initial planting date and were watered to field capacity thrice weekly. Observations of live plant measurements (detailed within the Results section) were conducted every weekday upon first observing germination (12 December 2023 for the raw treatments and 30 January 2024 for composted treatments). The observation period for each glasshouse experiment was 2nd of January 2024 for the raw treatments and 22nd of February for the composted treatments.

After completion of the observation period, sunflower plants were taken to the laboratory to record the final destructive plant measurements. This was conducted on the 3rd of January 2024 for raw treatments and the 23rd of February for the composted treatments. Here, soil from each pot was loosened and individual plants were removed. Each pot's soil was separated from the plants and roots and retained for analysis. Plants including roots were dried with damp paper towels and left to air dry. Upon drying, the root and shoot lengths of plants were measured and fresh weights recorded. Plants were dried in an air oven at 40 °C for 48 h, after which, dry weight was recorded. Root and shoot masses were obtained by weighing each plant after drying. Before drying, each plant was divided at the soil surface into roots and shoots, and each were individually weighed using a scale. After drying, this process was repeated for each plant, with the recorded weights being used to determine the root/shoot ratio of each plant.

2.3. Soil Analysis

Soil analysis consisted of moisture content percentage (MC%), pH, electrical conductivity (EC), total carbon (TC) and total nitrogen (TN). Each soil analysis tested the five different substrates used in the glasshouse experiments, commercial soil mix, raw SCGs, SCGCF, SCGCM and SCGCY. TC and TN tested all treatments used in the glasshouse

experiments; however, this was not feasible for other soil analysis methods. For MC%, pH and EC, five samples of each substrate were tested. MC% was calculated as the difference between fresh weight (determined on samples taken directly from the glasshouse trials) and the dry weight of the same samples dried in an air oven at 40 °C to constant weight, multiplied by 100 for a percentage value. Substrate samples for the moisture content assay ranged from 10 to 20 g per sample, with five samples used per substrate.

pH was determined using the $CaCl_2$ suspension method, as it has known accuracy, following the Department of Sustainable Natural Resources Soil Survey Standard Test Method [34]. This method involved using a calibrated Milwaukee Sharp pH probe (Milwaukee Instruments, Rowville Victoria, Australia) to measure the pH of a 1:5 soil/0.01M $CaCl_2$ suspension, consisting of 10 g of air-dried soil and 50 mL of 0.01M $CaCl_2$, which had settled for 30 min after being mechanically shaken for 1 h at 15 rpm.

EC was also measured using the Department of Sustainable Natural Resources Soil Survey Standard Test Method [34]. This method used a calibrated Milwaukee Sharp EC meter (Milwaukee Instruments, Rowville Victoria, Australia) to measure the electrical conductivity of a 1:5 soil/water suspension, consisting of 10 g of air-dried soil and 50 mL of deionised water, which had been mechanically shaken for 1 h at 15 rpm.

For quantifying TC and TN, sample preparation involved air-drying samples of each substrate at 40 °C for 7 d, followed by sieving (710 µm) to improve sample homogeneity. Total carbon and nitrogen analyses were conducted using the LECO TruMac LECO TC/TN Macro Determinator (Model 630-300-900, LECO Corporation, Castle Hille NSW, Australia), which oxidises samples using an induction furnace, and quantification using an infrared detector. Before sample analysis, three blank samples were loaded into the machine to ensure the machine's calibration curve remained consistent. Due to the absence of an a priori expectation of C and N ratios, the correct standard needed to be determined before sample analysis. One sample from three of the tested substrates, these being control soil, SCGCF and SCGCM, were tested to determine the standard. From these data, it was determined that the 'wheat flour standard method' was suited for all future samples. For testing, three 200 mg samples of each substrate were measured into sample boats, weighed and loaded into the machine, which recorded TC and TN values.

2.4. Statistical Analysis

All data were collated in Microsoft Excel for statistical analysis. All data were tested for normality and homogeneity of variance before analysis and transformation (log(1 + data)) was conducted if necessary, though this was only required for first true leaf emergence for raw SCGs and the total carbon and total nitrogen data.

Data were analysed using PAST 4.14 [35]. Mean comparisons were made using ANOVA or PERMANOVA, as noted in the Results section. For data that did not consist of a mean, which include germination rates, cotyledon emergence and true leaf set emergence, data were statistically analysed using Chi Squared tests. For example, germination rate data sets had the frequency of germination compared to a null data set using Chi Squared tests. Statistical significance was accepted at $\alpha = 0.05$.

3. Results

3.1. Raw Spent Coffee Ground (SCG) Toxicity

Germination rates after 16 days were significantly different amongst the different concentrations of raw SCGs (ANOVA $p = 0.000$; Figure 2). The 0% concentration (i.e., control soil) and 5% concentration had the highest germination rates overall of 90% and 85%, respectively, after 16 days, with significant comparisons with all other treatments (Tukey's post hoc $p < 0.000$). The lowest germination was for 35% raw SCG, which was only 30% at the final measurement, confirming an inhibitory effect of raw SCG. Raw SCG concentrations from 2.5 to 20% performed worse than the 0% control, but still maintained a final germination percentage of ≥70%. Note that minor plant losses were recorded in the 0

and 10% treatments, whilst almost half of the plants in the 35% treatment did not survive 16 d.

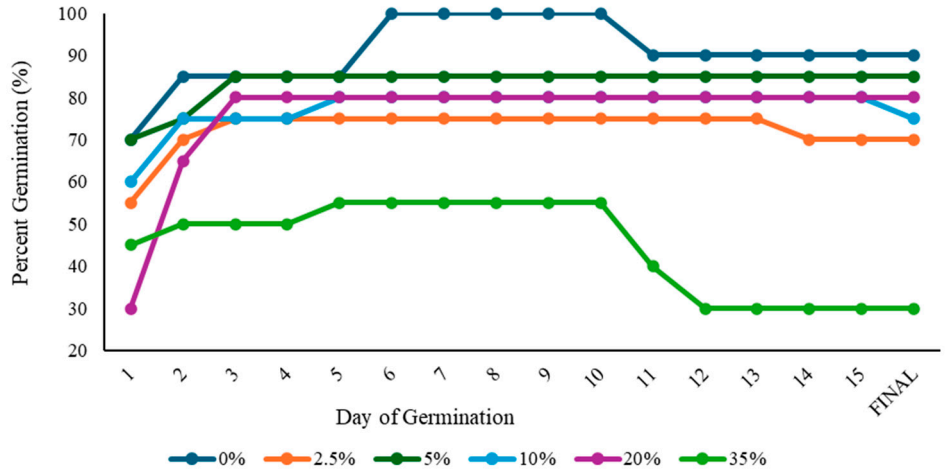

Figure 2. Differences in germination percentage amongst different raw spent coffee ground concentrations. Germination reflects the process where a plant develops from a seed into a seedling. For each day of observation, a total count of all plants that exhibited this condition was taken for each concentration and, as such, error bars were not used for this graph. Reductions in germination reflect plants that died over the course of the experiment and were thus not included in future observations.

A significant reduction in plant height was recorded in the 35% raw SCG group (Figure 3), which was almost 20% shorter than the next lowest value of 16.3 cm for the 20% treatment, with values in all other treatments ranging from 16 to 18 cm.

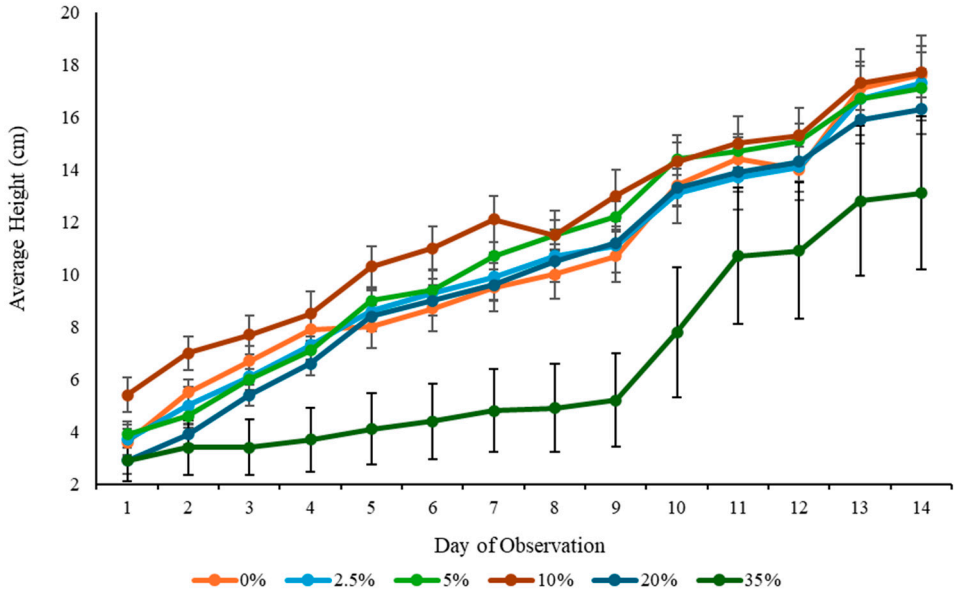

Figure 3. Differences in height amongst various raw spent coffee ground concentrations (error bars are the SEM). Height improves a plant's access to sunlight, making it able to photosynthesise easier, but can also reduce stem maintenance, making it more vulnerable to weather conditions (e.g., wind and rain).

The 35% raw SCG treatment showed the lowest cotyledon emergence of 6/20 plants (Figure 4), which was significantly different to all other concentrations ($p = 0.000$), which performed similarly.

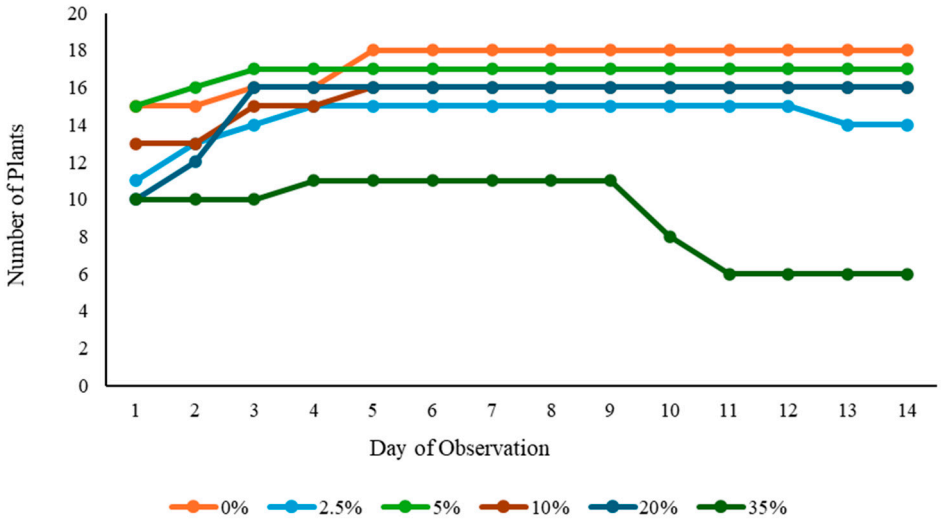

Figure 4. Cotyledon emergence of sunflowers amongst raw spent coffee ground concentrations. Cotyledon represents when plant leaves initially emerge from the seed, providing the plant with nutrients until it is able to photosynthesise. For each day of observation, a total count of all plants that exhibited this condition was taken for each concentration and, as such, error bars were not used for this graph. Reductions observed in the graph reflect plants that died over the course of the experiment and were not included in future observations.

First true leaf emergence was also significantly (p = 0.000) retarded in the 35% raw SCG treatment (Figure 5), with an anomalous low level also recorded in the 2.5% SCG concentration.

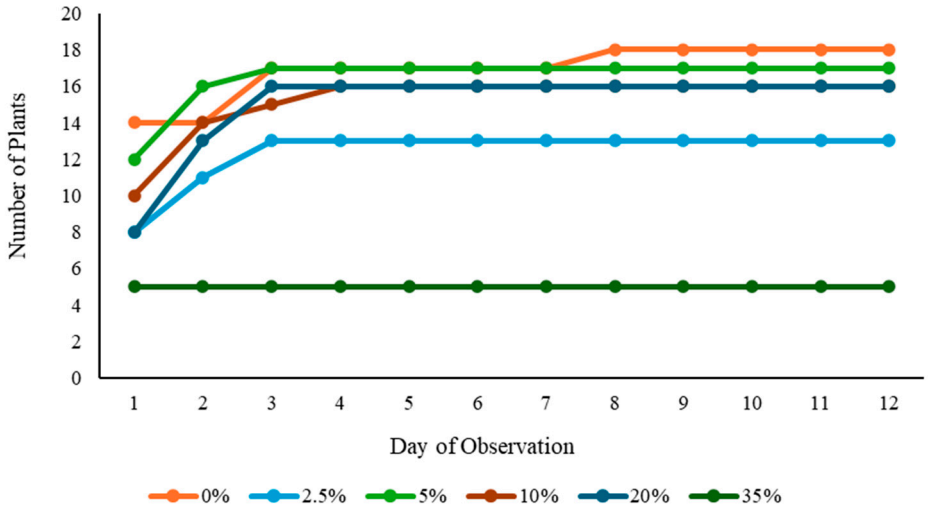

Figure 5. Number of sunflowers that experienced first true leaf emergence in soils that contained differing concentrations of raw spent coffee grounds. First true leaves are counted as the second observed leaf set on plants (as the first leaves are the cotyledon leaf set) and are the first adult leaves on a plant, allowing the plant to become a photosynthetic organism. For each day of observation, a total count of all plants that exhibited this condition was taken for each concentration and, as such, error bars were not used for this graph.

Patterns were more muted in the second true leaf emergence data set (Figure 6), with significant differences only detected between the 10% and 20% groups (p = 0.023) and the 10% and 35% treatments (p = 0.009).

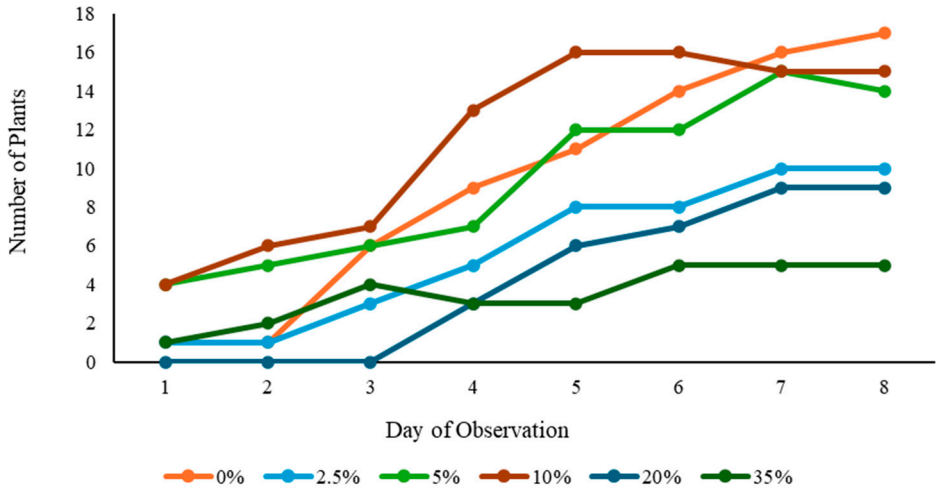

Figure 6. Second true leaf set emergence of sunflowers in soils that contained differing concentrations of raw spent coffee grounds. Second true leaves are counted as the third observed leaf set on plants (as the first leaves are the cotyledon leaf set and second are the first true leaves) and further improve a plant's ability to photosynthesise. For each day of observation, a total count of all plants that exhibited this condition was taken for each concentration and, as such, error bars were not used for this graph. Reductions observed in the graph reflect plants that died over the course of the experiment and were not included in future observations.

Plant damage was assessed subjectively and included any physical symptoms such as incompletely opened leaves, necrotic tissue, soft rots, etc. Our a priori expectation that higher concentrations of raw SCG would lead to higher numbers of damaged plants due to their higher toxicity did not occur, with no clear pattern between raw SCG treatments (ANOVA $p = 0.795$).

The total root and shoot lengths of the sunflowers were anticipated to be lower at higher concentrations due to toxicity limiting plant growth. However, no statistically significant trends were recorded for average root nor shoot length (ANOVA $p = 0.460$ and 0.259, respectively).

At the end of the experimental period, differences in the average fresh weight of sunflower seedlings amongst the different raw SCG concentrations were detected (ANOVA $p = 0.042$; Figure 7). However, Tukey's test revealed that there was only one significant comparison, between the 0% and 10% groups. Notably, the fresh weight of the 0% concentration plants was considerably lower than the 10% SCG treatment plants, potentially indicating some level of growth enhancement from raw SCG.

The patterns detected in wet weight amongst treatments were somewhat replicated in the dry weight analysis, with the highest average dry weight recorded for the 10% concentration, which was significantly higher than the 5%, 20% and 35% treatments (Tukey's $p = 0.043$, 0.011, 0.026). The 0% treatment, however, demonstrated dry weights that were not significantly lower than the 10% group ($p > 0.05$).

Root mass amongst the raw SCG treatments was highest in the 0% control, at 0.054 g, with the second highest value being 0.039 g in the 10% raw SCG plants (Figure 8). The lowest value was found in the 35% group, with an average root mass of 0.014 g. ANOVA confirmed that these findings were significant (ANOVA $p = 0.000$), with Tukey's post hoc analysis showing that all concentrations, excluding 10%, led to the development of significantly lighter root systems to the control soil treatment. There was a general tendency for higher concentrations of raw SCG to lead to smaller root masses in the seedlings.

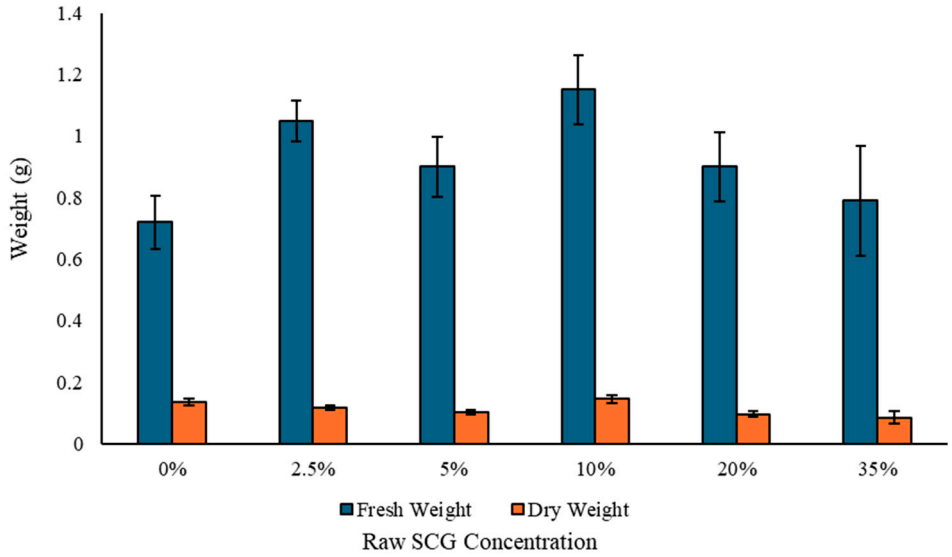

Figure 7. Comparisons of sunflower fresh weight and dry weight grown in soils containing different raw spent coffee ground concentrations (error bars are the SEM). Fresh weight is a useful measurement for evaluating the yield of plants, while dry weight is a measurement of biomass that removes any fluctuations caused by plant water content.

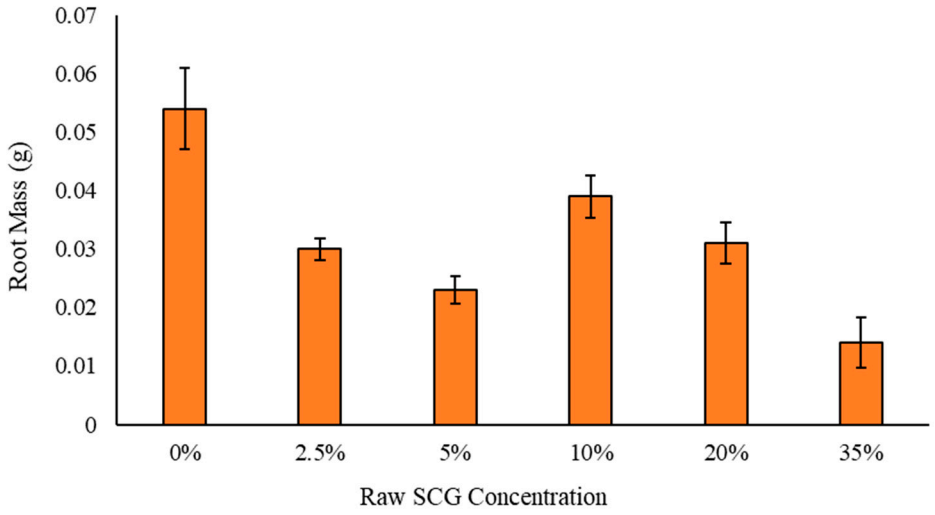

Figure 8. Root mass of sunflowers grown in different concentrations of raw spent coffee grounds (error bars are the SEM). Root mass is an important parameter to measure a plant's response to belowground environmental changes.

Shoot mass displayed a different pattern to root mass. Plants grown in 10% raw SCG had greater shoot mass than the 5%, 20% and 35% conditions (Tukey $p = 0.018$, 0.002 and 0.016), but there was no consistent pattern between SCG concentrations and the shoot mass produced (Figure 9).

Due to the unexpected patterns detected for the previous metrics, it was decided that calculating the root/shoot mass ratio may provide revealing findings (Figure 10). Any addition of raw SCGs was found to reduce the root/shoot ratio of the sunflowers (i.e., greater shoot mass at the expense of root mass), with significant differences observed between the 0% reference and all other raw SCG treatments (ANOVA, $p = 0.000$). The effect of altering root/shoot ratios in plants grown in an SCG-amended substrate has not been previously recorded and contributes to our understanding of the nature of the 'toxic' effects of raw SCG.

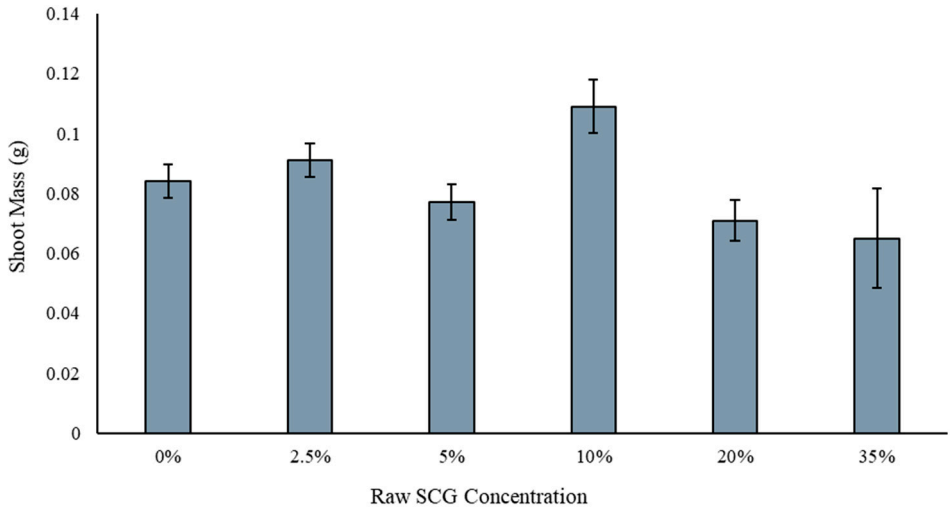

Figure 9. Shoot mass of sunflowers grown in different concentrations of raw spent coffee grounds (error bars are the SEM). Shoot mass can be used to assess a plant's response to aboveground environmental changes.

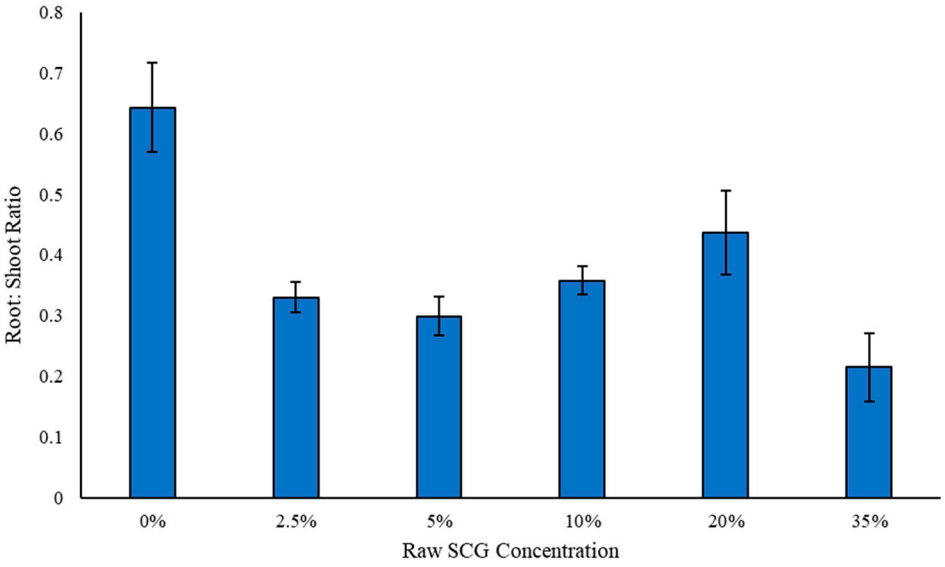

Figure 10. Comparisons of root/shoot ratio of sunflowers grown in soils that contained varying concentrations of raw spent coffee grounds (error bars are the SEM). Root/shoot ratio provides a ratio of below-ground and aboveground biomass, providing a holistic assessment of how plants respond to the surrounding environmental conditions.

Overall, live plant measurements revealed that higher concentrations of raw SCGs detrimentally affected early sunflower growth. Final values for raw SCG 0% were the highest for every live plant variable, excluding height, which was the second highest. The highest tested concentration, raw SCG 35%, displayed toxicity to sunflower growth, notably decreasing sunflower germination, plant height, cotyledon and true leaf set emergence. All other raw SCG concentrations performed worse overall than raw SCG 0% but performed significantly higher than raw 35% SCG, excluding the 2.5% and 20% concentration for second true leaf emergence. Destructive plant measurements revealed that higher concentrations, 20% and 35%, of raw SCGs resulted in reductions in dry weight, excluding 10%, where it was highest. Higher concentrations of raw SCG exhibited a growth retardation effect, resulting in smaller root masses of sunflower seedlings, also reflected in shoot mass. Interestingly, the root/shoot ratios of plants significantly decreased with any addition of raw SCG; however, the implications of this finding are currently unexplored.

3.2. Effectiveness of Spent Coffee Ground (SCG) Compost

For the first 2 days after planting, there were significant differences in the rate of germination amongst groups (χ^2 $p < 0.05$, Figure 11), with higher percentages of SCG compost associated with faster initial rates of germination (Pearson correlation between % composted SCG and germination, $p < 0.05$). However, these effects were not significant from day three onwards. These findings thus show no pathological effects of composted SCGs, but rather are evidence that higher proportions of composted SCG lead to enhanced germination rates.

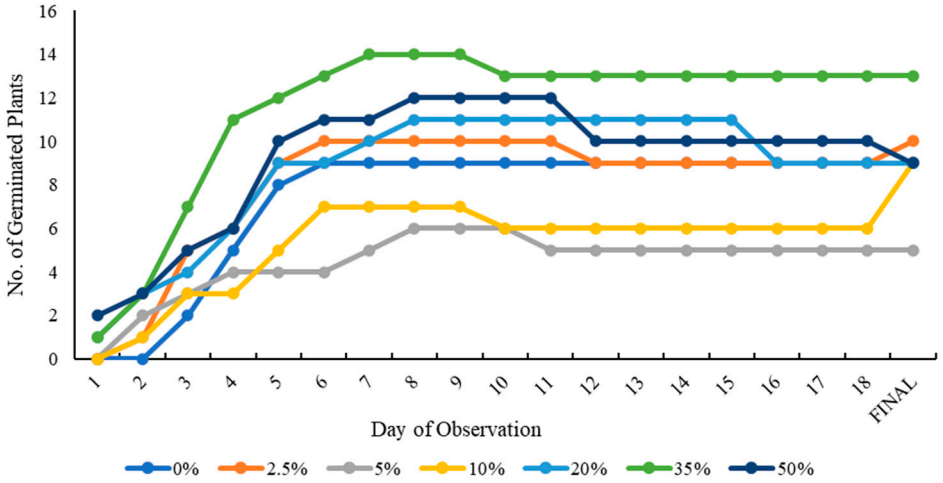

Figure 11. Number of sunflowers that germinated amongst different composted spent coffee ground concentrations. Germination reflects the process where a plant develops from a seed into a seedling. For each day of observation, a total count of all plants that exhibited this condition was taken for each concentration and, as such, error bars were not used for this graph. Reductions in germination reflect plants that died over the course of the experiment and were thus not included in future observations.

Composted SCG had no significant effects on seedling height during the 18-day growing period (Figure 12) but did affect cotyledon production.

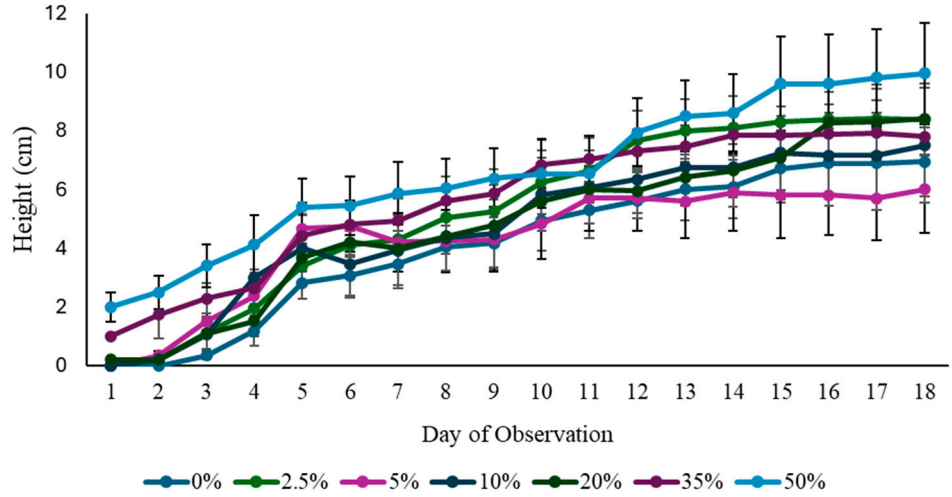

Figure 12. Plant height of sunflowers compared against different concentrations of composted spent coffee grounds (error bars are the SEM). Height improves a plant's access to sunlight, making it able to photosynthesise easier, but can also reduce stem maintenance, making it more vulnerable to weather conditions (e.g., wind and rain).

Differences in cotyledon emergence amongst composted SCG treatments were statistically significant at day 18 (ANOVA: $p = 0.000$). The highest values were achieved in the

35% concentration, while the lowest values were seen in the 5% and 10% concentrations (Figure 13). A similar pattern was recorded for true leaf production, with seedlings in the 35% treatment producing greater numbers of true leaves more rapidly than the other treatments (ANOVA $p = 0.000$; Figure 14). A similar pattern emerged for the production of the second and third true leaves, although in the case of the latter, the 50% composted SCG treatment outperformed the 35% treatment.

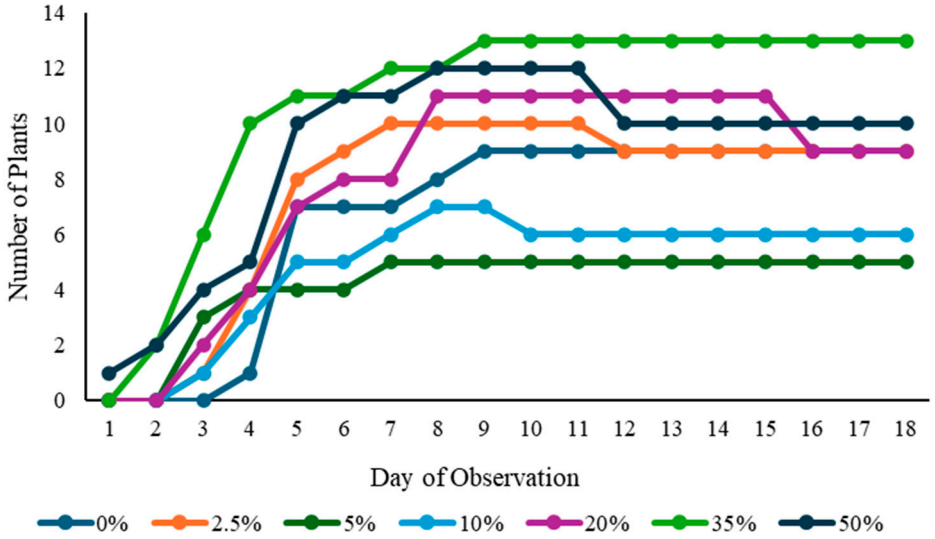

Figure 13. Cotyledon emergence of sunflowers in composted spent coffee ground soils at different concentrations. Cotyledon represents when plant leaves initially emerge from the seed, providing the plant with nutrients until it is able to photosynthesise. For each day of observation, a total count of all plants that exhibited this condition was taken for each concentration and, as such, error bars were not used for this graph. Reductions observed in the graph reflect plants that died over the course of the experiment and were not included in future observations.

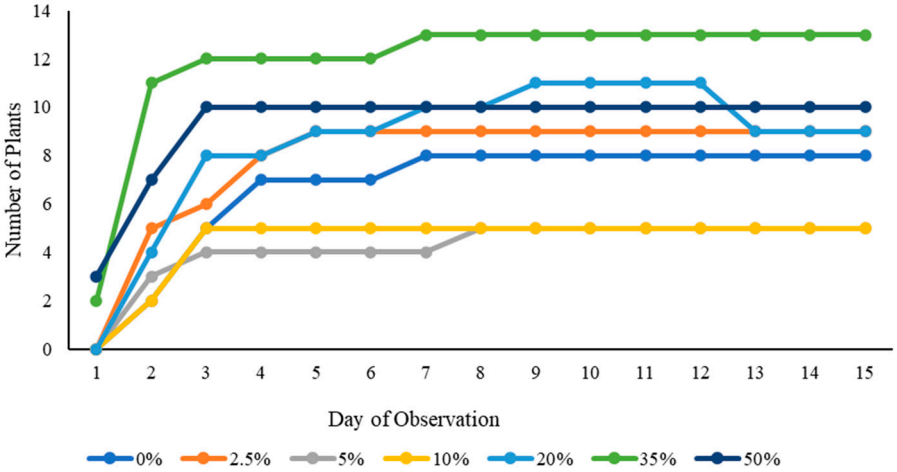

Figure 14. Sunflower first true leaf emergence in different spent coffee ground compost concentrations. First true leaves are counted as the second observed leaf set on plants (as the first leaves are the cotyledon leaf set) and are the first adult leaves on a plant, allowing the plant to become a photosynthetic organism. For each day of observation, a total count of all plants that exhibited this condition was taken for each concentration and, as such, error bars were not used for this graph. Reductions observed in the graph reflect plants that died over the course of the experiment and were not included in future observations.

Significant differences in total root length were observed amongst different SCG compost concentrations, with the 0% control treatments producing the highest average root

length (Figure 15). For the other concentrations, a pattern was observed where increasing concentrations of composted SCGs resulted in shorter total root lengths in the seedlings, with this pattern statistically significant for the 10%, 35% and 50% treatments (p = 0.016, 0.013 and 0.035, respectively).

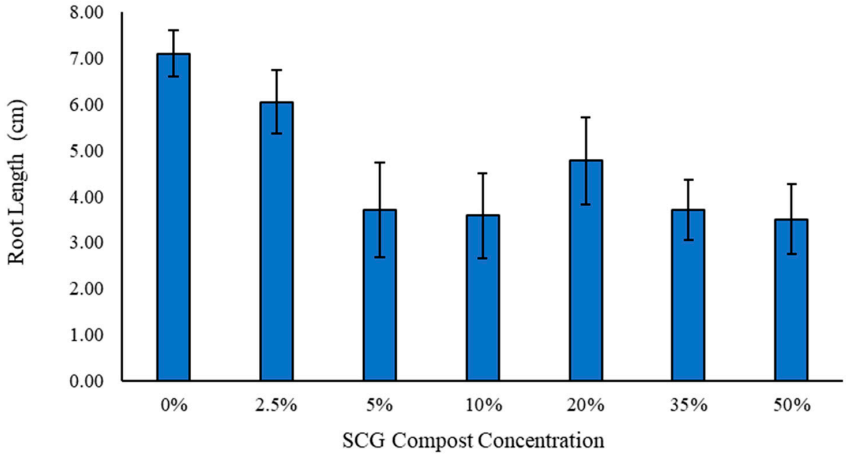

Figure 15. Differences in sunflower root length in composted spent coffee ground soils (error bars are the SEM). Root length allows plants to access and absorb higher amounts of nutrients and water from the soil.

In contrast to the findings for total root length, no significant differences were found amongst treatments for shoot length (p = 0.244).

There was no consistent pattern between average seedling fresh weight and the proportion of SCGC in the substrate: the only significant comparison observed was between the 50 and 35% treatments (p = 0.020; Figure 16). There were no significant differences in the dry weights of the seedlings amongst treatments.

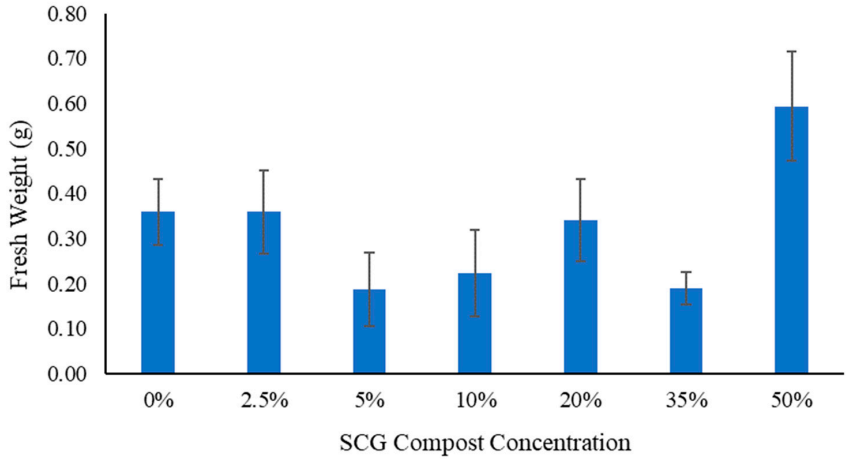

Figure 16. Comparisons of sunflower fresh weight in soils containing differing composted spent coffee ground concentrations (error bars are the SEM). Fresh weight is a useful measurement for evaluating the yield of plants.

No differences between treatments for average seedling root and shoot mass were observed, as was the case for raw SCG. There were, however, statistically significant differences ($p < 0.05$) amongst treatments for the root/shoot ratio, with the lower concentration treatments having higher root/shoot ratios (Figure 17). The relationship presented was thus similar to that shown for the raw SCG.

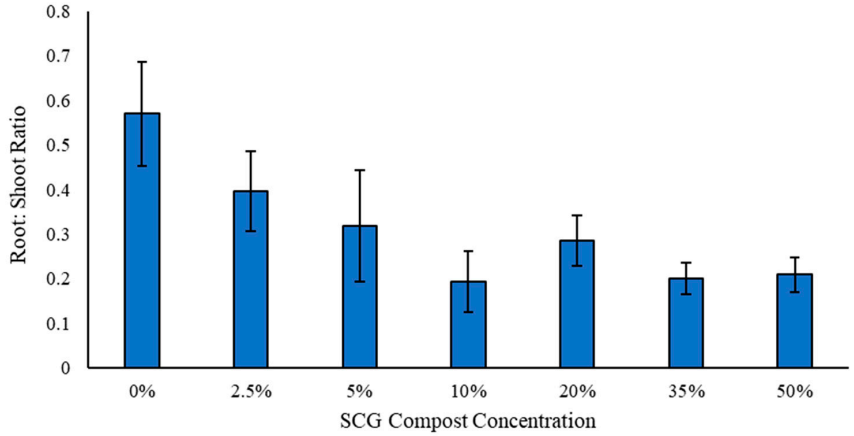

Figure 17. Sunflower root/shoot ratio in different concentrations of composted spent coffee ground soils (error bars are the SEM). The root/shoot ratio provides a ratio of belowground and aboveground biomass, providing a holistic assessment of how plants respond to the surrounding environmental conditions.

Composted SCGs demonstrated no pathological effects on sunflower seedlings, with seedlings benefiting the most from higher concentrations of composted SCGs, which were 50% and particularly 35%. Higher SCGC concentrations resulted in higher initial germination rates, increased cotyledon emergence and improvements in first, second and third true leaf emergence, with 35% performing the best in each all categories, excluding third true leaf emergence, where 50% was best. All other tested concentrations varied in performance amongst live plant variables. Destructive plant measurements showed that increased concentrations of composted SCGs reduced root lengths, and fresh weight results were inconsistent, aside from 50% having a much higher fresh weight than 35%. Like raw SCG findings, any addition of composted SCGs reduced the root/shoot ratio of plants, with lower concentrations resulting in a higher root/shoot ratio.

3.3. Soil Profile Measurements

Substrates that contained either raw or composted SCGs had a higher water-holding capacity than the reference soil (Figure 18), with a difference of 10% between soil (22.65%) and the 1-year-old SCG compost (33.45%). Raw SCGs had the highest water-holding capacity of 47.68% water content percentage (WC %), while composting 6 months or longer reduced SCG WC% significantly ($p = 0.000$).

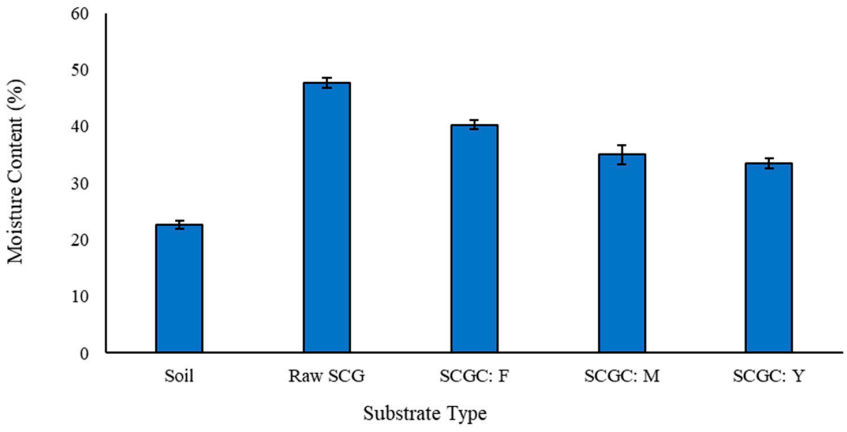

Figure 18. Moisture content percentage of differing substrates. Spent coffee ground compost (SCGC) is represented by three different decomposition states, fresh (SCGC: F), six months (SCGC: M) and >1 year (SCGC: Y) (error bars are the SEM).

Significant differences in pH amongst the substrates were observed (Figure 19), with the soil mix and raw SCGs having an acidic pH of ≈5, while composted SCGs had significantly ($p = 0.000$) higher pH values and were close to neutral. All composted treatments had statistically similar pH values ($p > 0.081$).

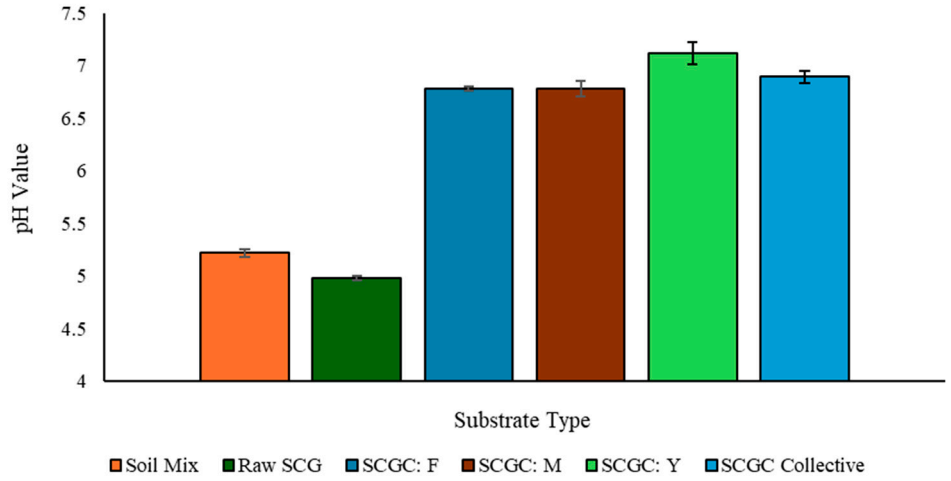

Figure 19. Differences in pH amongst different soil substrates. Spent coffee ground compost (SCGC) is rep-resented by three different decomposition states, fresh (SCGC: F), six months (SCGC: M) and >1 year (SCGC: Y) (error bars are the SEM).

The electrical conductivity of raw and composted SCGs was much higher than in the soil mix (Figure 20), with a difference of almost 500 µS between the soil mix and the SCG substrates. SCG compost at 6 months of age had the highest electrical conductivity of 1843 µS, with raw SCGs and fresh composted SCGs having similar EC levels, 1641.2 µS and 1664.4 µS, respectively. Significant differences ($p = 0.000$ in all cases) between the soil and all SCG treatments were observed.

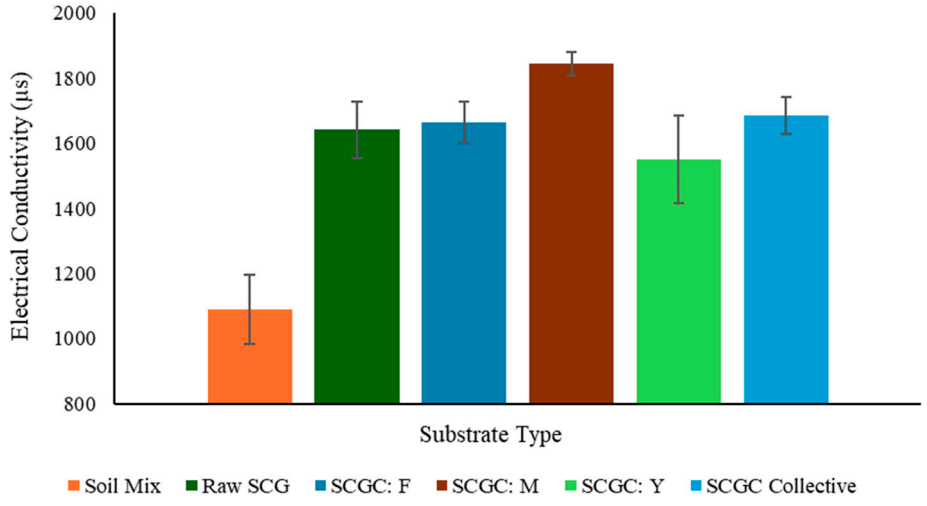

Figure 20. Comparisons of electrical conductivity between different soil substrates, measured in microsiemens (µS). Spent coffee ground compost (SCGC) is represented by three different decomposition states, fresh (SCGC: F), six months (SCGC: M) and >1 year (SCGC: Y) (error bars are the SEM).

Differences between total carbon values for the tested substrates and concentrations were significantly different ($p = 0.001$). However, post hoc testing revealed no consistent patterns related to either composting or SCG concentration and, as such, total carbon is considered unlikely to be the primary cause of the biological effects shown in the plants (Figure 21).

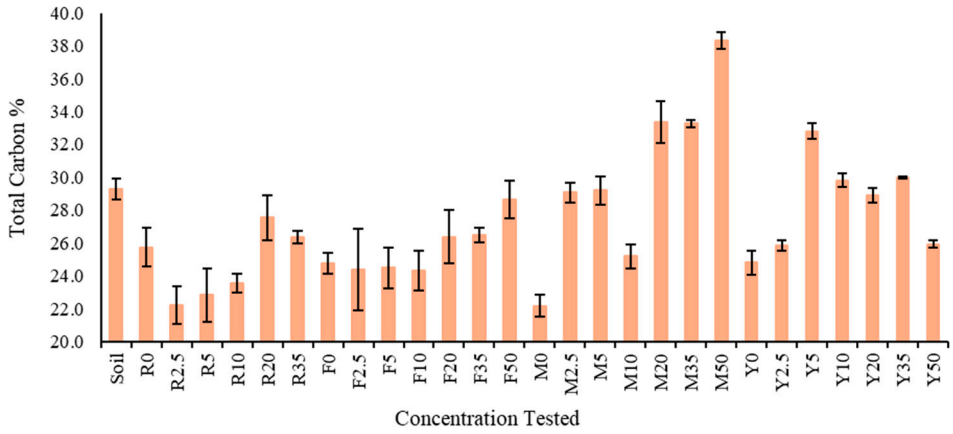

Figure 21. Total carbon measured between all substrates and concentrations. Each value is represented by a letter: raw spent coffee grounds (R) and composted spent coffee grounds at the decomposition stages of fresh (F), 6 months (M) and >1 year (Y). Each value's number represents the different tested concentrations: 0%, 2.5%, 5%, 10%, 20%, 35% and 50% (error bars are the SEM).

Total nitrogen showed significant differences amongst both substrate types and concentrations ($p = 0.001$; Figure 22). For all SCG types, comparisons between higher concentrations, in particular 35% and 50%, and lower concentrations were statistically significant ($p < 0.05$). Interestingly, the total N concentrations of the 6-month composted treatment were considerably higher than the raw and >1Y composted samples.

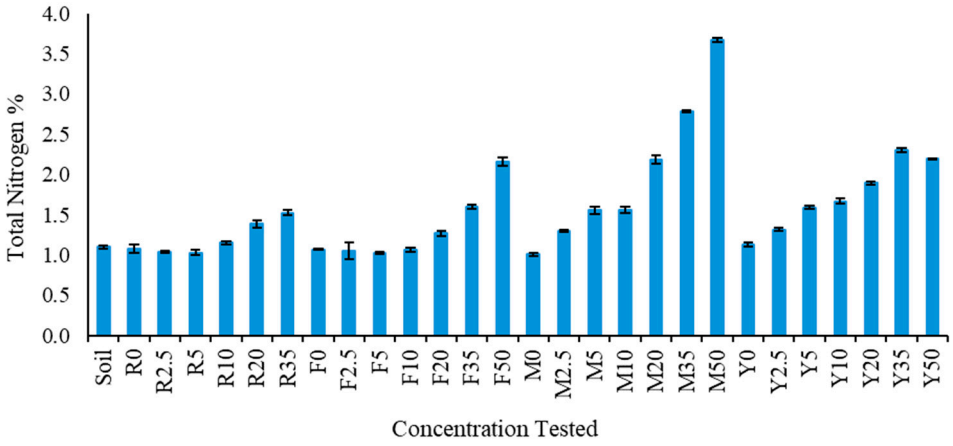

Figure 22. Total nitrogen measured all substrates and concentrations. Each value is represented by a letter: raw spent coffee grounds (R) and composted spent coffee grounds at the decomposition stages of fresh (F), 6 months (M) and >1 year (Y). Each value's number represents the different tested concentrations: 0%, 2.5%, 5%, 10%, 20%, 35% and 50% (error bars are the SEM).

Soil profiling of SCG substrates revealed that both raw and all composted SCG treatments contained a significantly higher water-holding capacity and electrical conductivity than commercial soil mix. Raw SCG pH had a similarly acidic pH to the commercial soil mix, being slightly more acidic than the soil mix. In contrast, the pH of all composted SCGs treatments was neutral. While total carbon analysis revealed no consistent patterns, total nitrogen analysis revealed that increasing concentrations within each SCG substrate increased the total nitrogen percentage of the substrate. Concentrations of 35% and 50% displayed the highest total nitrogen percentage, with the highest overall values being detected in SCGCM at concentrations of 35% and 50%.

4. Discussion

4.1. Spent Coffee Ground Substrates' Effects on Sunflower Growth

As expected a priori, raw spent coffee grounds (SCGs) were found to have a detrimental effect on plant development. All measured plant variables demonstrated that at a concentration of 35% raw SCGs, sunflowers performed significantly worse than the other tested concentrations. These findings were broadly consistent with the existing literature, showing that higher raw SCG concentrations typically result in plant growth reduction [33] with lower seed emergence [21], biomass reduction [21,23] and reduced leaf numbers [36] also being observed in the current study. Unlike other studies [28,33], which observed negative effects of raw SCGs at 10%, this study found that plants grown in the 10% and 20% concentrations performed similarly to lower concentrations, whilst in some cases outperforming them (plant height; Figure 3). Similarly, destructive plant metrics, while more variable, also indicated inferior plant performance at 35% raw SCGs. The reduced performance of plants grown in 35% raw SCGs is likely attributed to the toxic components present in raw SCGs, with both the current and previous research [37,38] finding that neither pH nor nitrogen availability were associated with negative plant health effects resulting from SCGs. The current study's scope precluded specific tests for the proposed toxic components in raw SCGs: caffeine, tannins and chlorogenic acid [39,40]. However, identifying these compounds, as well as the toxicity symptoms and concentrations caused by them, would prove beneficial towards further understanding raw SCGs' agricultural limitations. Furthermore, as sunflowers were the only plant species tested, the specific findings of this experiment may not be applicable to other plant species. As such, observing and recording live and destructive plant measurements of a variety of plant species would prove beneficial to future replications, providing a wider range of more applicable data.

Also as predicted, the current study found that higher concentrations of composted SCGs were the most effective at stimulating plant growth, with concentrations of 35% and 50% SCG compost associated with higher germination rates, improved cotyledon growth and increases in true leaf set emergence. This is consistent with previous findings that composted SCGs improved plant growth at only high concentrations [33]; however, the current findings indicate improved performance of 35% composted SCG relative to 50%, indicating that there may be a concentration limit to the useful rate at which composted SCGs can be used as a soil amendment. Composted SCGs at higher concentrations were found to reduce the root length of growing sunflowers, which indicates a growth retardation effect at higher SCG compost concentrations. This finding is novel, and it is thus recommended that further work with other crop species and longer plant growth times would be valuable for determining the scale of these effects.

A significant, novel finding of the current work was that any SCG application, either raw or composted, at any concentration affected the root/shoot ratios of the sunflower seedlings by restricting root growth without restricting aboveground phytomass. The observed effects may be attributed to the ion concentration in SCGs, as higher salinity is known to decrease root hair length and density [41]. It is not likely that the toxic components of raw SCGs are playing a role in these effects, as they were consistent between raw and composted SCGs. These effects of SCGs' effects on the root/shoot ratios of plants have not been previously documented, and so it is suggested that future research on this aspect be conducted, in particular to determine whether this is a useful or detrimental effect for agricultural purposes.

4.2. Soil Properties of Spent Coffee Ground Substrates

There were considerable differences in substrate properties amongst the substrate formulae tested within the current study. Both raw and composted SCGs had a higher water-holding capacity than the tested commercial soil product, which may be attributed to their higher levels of humic materials [42,43]. Due to water being limiting for plant growth [43], this offers potential for SCGs, particularly composted SCGs, to become a valuable soil supplement, especially for agricultural substrates that have low organic levels and low

water-holding capacity, such as sandy soils. The water-holding capacity of composted SCGs decreased the longer they were decomposed, which can be attributed to the enlargement of particles during decomposition, resulting in faster soil draining [44]. There may have been some association with this effect and the reductions in root length observed in the SCG compost treatments, as particle compaction has been shown to potentially restrict root growth under some circumstances [45].

Maintaining pH within a specific band is crucial for facilitating plant macronutrient and trace metal availability [46]. Soils that are too acidic or alkaline inhibit plant growth and decrease nutrient availability, make some otherwise essential elements phytotoxic and negatively affect useful microorganisms in the soil [47]. Most plants can grow in a pH range between 5 and 8, though there is considerable variability amongst plant species. Sunflowers require soil pH ranging from slightly acidic to alkaline, with peak growth from pH = 6–6.8 [48]. Within this study, both raw SCGs and commercial soil mix had an acidic pH of ~5, making them suboptimal for sunflower growth: sunflowers grown at pH = 4.7–5.3 typically show reduced yields [49]. All the composted SCG treatments had pH = 6.7–7.1, making them suitable for sunflower growth, again supporting the value of composted SCGs as an agricultural soil amendment. Whilst the low pH of raw SCGs likely contributes to the detrimental effects of this material on plant growth, as the pH of both control soil and raw SCG substrates was similar, pH can be eliminated as the primary cause of these effects.

A specific range of substrate electrical conductivity is required for crops to take up water and nutrients. Within Australia, general agricultural irrigation limits for salinity vary amongst crops, with salt-sensitive crops handling up to 650 µS and the upper limit for moderately salt-sensitive crops being 1300 µS [50]. The electrical conductivity values seen in the tested substrates showed that SCG substrates, both raw and composted, had noticeably higher electrical conductivity (>1500 µS) compared to the soil control (1100 µS). The 600 µS difference between soil and raw SCGs is equivalent to 3.84 mg/L of dissolved ions. This would make the SCG amendments unsuitable for salt-sensitive crops, although it would be suitable for use on salt-tolerant crops such as sunflowers, which have an upper tolerance threshold of 4000–5000 µS [50].

Total nitrogen (N) levels were found to correlate to SCG concentrations, which is consistent with the existing literature [22]. SCG compost had higher N levels than raw SCGs. Six-month composted SCG at 35% and 50% had the highest N levels of 3–4%. As total N is the limiting nutrient for plant growth and is a key requirement of all fertilisers used in agriculture [51], SCGs after composting for six months would be of significant benefit for total N amendment. Comparatively, SCGs composted for >1 year had much lower total N values of around 2% at 35% and 50%, providing evidence that 6 months is a preferable time for SCG composting operations.

In contrast, total carbon (C) levels showed little variation amongst tested substrates and are unlikely to have resulted in the observed biological effects within this study. This is evidence that the properties of SCGs, whether detrimental or beneficial for plant growth, are unlikely to be due to the outright quantity of organic material provided.

4.3. Study Limitations

It was beyond the scope of the present work to assay for the postulated toxic materials in raw SCGs (e.g., caffeine and chlorogenic acid), and the restriction of the work to a single plant species constrains our ability to generalise the findings to other agricultural crops. Different plant species are known to display varying effects when exposed to SCG application and have different optimum ranges for soil parameters such as pH and electrical conductivity. Furthermore, the nutrients assessed in this study, carbon and nitrogen, are not solely responsible for plant growth, as other nutrients, such as phosphorus and potassium, are also required in varying amounts to facilitate plant growth. As such, assessing the effects of other nutrients, as well as comparing a range of plant species, will provide a broader scope for agricultural SCG application.

5. Conclusions

This study assessed the effects of raw and composted spent coffee grounds (SCGs) on plant growth, alongside assessing the differences in several critical properties of the tested substrates. Raw SCGs were found to be toxic for sunflower seedling growth at a 35% by mass concentration, with notable decreases in germination, plant height, cotyledon and true leaf set emergence. Composted SCGs assisted plant development and were most effective at higher concentrations, with 35% producing improvements in sunflower germination rate and success, as well as cotyledon and true leaf set emergence. This study also determined that SCG substrates, both raw and composted, had higher water-holding capacities and electrical conductivity levels than a reference commercial soil mix, leading to restrictions on the crops for which SCG amendments will be suitable. It was also found that the initially acidic pH of raw SCGs was ameliorated by composting.

A significant finding of this study was that the addition of SCGs was found to affect the root/shoot ratio of plants, though the causes and broader consequences of these effects are currently unknown and will require further testing. This study also found that higher concentrations of total nitrogen were found in SCGs at six months of composting, at which stage, the composted SCGs performed effectively as a soil amendment. With current agricultural practises composting SCGs for 12 months or longer, this indicates that reductions in the current SCG composting time by 50% may be possible, improving the efficiency of mass agricultural SCG use. This finding opens up the possibility of a significant contribution to circular economy: it is known that coffee consumption is approximately 12% higher in the cool months compared to in summer [52]; thus, SCG availability has a seasonal cycle. With the adoption of the reduced 6-month composting time recommended in the current work, it is possible that a matching cycle could be developed, leading to an efficient cycle of SCG–crop movement. The findings of this study indicate that SCGs can act as a valuable soil amendment after 6 months of composting time. Given the limitations of the current work, we cannot recommend its use on crops other than sunflowers with confidence without specific testing, especially for salt-sensitive species.

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Conflicts of Interest: The authors declare no conflicts of interest.

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