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The Use of Organic and Mineral Amendments to Improve Zimbabwean Soil Health Utilising Plant Growth and Hydrocarbon Breakdown as Indicators

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Supervised by Professor Karen Johnson, Dr. Lynsay Blake, Dr. Stephen Chivasa and Dr. Angela Sherry

A thesis submitted in fulfilment of the requirements for the degree of Master of Science in Engineering in the Department of Engineering at Durham University.

School of Engineering and Computing Sciences

Durham University

April 2020

27 **The Use of Organic and Mineral Amendments to Improve Zimbabwean Soil**
28 **Health Utilising Plant Growth and Hydrocarbon Breakdown as Indicators**

29

30 **Summary**

31 Southern Africa faces a great problem threatening the sustainability of maize crops
32 because of the inability of African soils to grow said plants and their deficiency in many different
33 factors such as water holding capacity, nutrients availability, and aggregate stability, amongst
34 others. The hypothesis behind this study is that mineral and organic matter amendments can
35 improve soil health and thereby increase potential hydrocarbon bioremediation, since this is
36 only possible in a well structured, healthy soil. Soil health is measured in this study using plant
37 health itself as an indicator, as well as the soil's ability to bioremediate hydrocarbons. It is also
38 expected that with the addition of mineral and organic amendments not only the physical
39 characteristics of the soil change, but the chemical and biological ones as well. To date, the
40 use of both mineral and organic amendments for soil improvement has not been deeply
41 explored by many researchers. The study is composed of two growth trials in which
42 Zimbabwean soil is mixed with either compost, quartzite, water treatment residual (WTR), or
43 a combination of them, two identical sets of amendments were done with the difference that
44 one of the sets contained an addition of nutrients (plant food). The main findings are firstly that
45 for plant health a single compost amendment (10% of compost combined with soil) and a co-
46 amendment of compost and WTR (10% of each material in combination with soil) are both
47 statistically significantly ($P < 0.05$) better for plant height and for above ground and below
48 ground biomass. Nutrients addition (plant food), improves the co-amendment's biomass but
49 does not have the same effect in the single compost amendment's biomass. Secondly, it was
50 found that of all soil types the single amendment of compost had the highest CO₂ emissions,
51 after 30 days of oil contamination. This implies that the addition of nutrients (NPK) negatively
52 affected CO₂ emissions rates when soil was contaminated with oil, potentially suggesting that
53 NPK addition has a negative effect in the soil microbiome of this Zimbabwean sandy soil.

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62 acknowledged”.

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99 and sharing this incredible experience with me. We will forever be research twins.

100

101

102 **Dedication**

103 I would like to dedicate this work to my mom and dad, Beatriz and Julio, who shaped me into
104 the person that I am today and made me strong enough to carry out this experience. I would
105 also like to dedicate this to my grandparents Agustin Valdez and Josefina Borboa, and my
106 sisters Mei Ling and An Zhilei, as well as to the rest of my family.

107

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148 **1. Introduction**

149

150 1.1 Soil Health

151 Soil health is defined as the “capacity of soil to function as a vital living system to
152 sustain biological productivity, maintain environment quality and promote plant, animal and
153 human health” [1]. The degradation of soil presents a global problem and sandy soils are
154 arguably of most concern because of their low water holding capacity and low fertility [2]. In
155 Southern Africa, sustainability of maize-based cropping systems is a big challenge, yet the
156 demand for it continues to increase [3].

157 Approximately 70% of the arable land area in Zimbabwe is covered by granite-derived sandy
158 soils which are known for their low nutrient capital and susceptibility to degradation [4]. These
159 low levels of nutrients, particularly nitrogen, account for continued declines in the production
160 of maize in small holder farms in Zimbabwe [5]. Many African sandy soils, such as
161 Zimbabwean soil, have low organic carbon contents which leads to a vicious cycle of low
162 capacity to accumulate and protect soil organic matter (SOM) [6]. According to Lal (2016)
163 SOM has three main components: plant and animal residues and living microbial biomass,
164 active or labile soil organic matter, and relatively stable soil organic matter. It is largely made
165 up of humic acids which are composed of carbon, nitrogen, hydrogen, oxygen, phosphorus,
166 and sulphur, with Soil Organic Carbon (SOC) being the largest component, and usually around
167 50% of SOM [11]. SOM both retains and supplies plant nutrients, improves soil aggregation,
168 reduces soil erosion and enhances water holding capacity [7]. Furthermore, SOM increases
169 “structural stability, resistance to rainfall impact, rate of infiltration and faunal activities” [8].
170 The application of organic matter to soil can potentially increase SOM and it also influences
171 the distribution of SOC and Total Organic Nitrogen (TON) between SOC fractions [9]. SOC
172 improves aggregate stability by providing a food source to microorganisms that can then
173 produce extrapolymeric substances that bind together soil particles creating microaggregates
174 [10].

175 SOC is at the heart of soil health as it underpins all soil functions, chemical, biological, and
176 physical. It has a strong impact on soil quality (“capacity of the soil to function”, Karlen et al.
177 1997), functionality and health (“capacity of soil to function as a vital living system to sustain
178 biological productivity, maintain environment quality and promote plant, animal and human
179 health”, Doran and Zeiss 2000) [11]. SOC supplies plant nutrients (chemical attributes),
180 enhances cation exchange capacity, improves soil aggregation and water retention, and
181 supports soil biological activity (biological attributes) [8]. It has been proven that when changes

182 in the organic carbon content of soils occur, there is a correlation with changes in the structural
183 form (physical attributes) and stability of soils, this change can be of great magnitude
184 depending on the soil texture [12].

185 Inorganic minerals, such as iron and aluminium hydroxides, play an important role in binding
186 soil particles together [13]. This because Fe and Al oxide surfaces are positively charged
187 which helps adsorb negatively charged organic molecules creating strong bonds which allow
188 soil aggregates to form. For this reason, this thesis explores the relatively unexplored topic of
189 soil improvement technologies combining both organic and inorganic mineral amendments.

190 There are many indicators of soil health and this is still very much a topic of debate in the soil
191 science and agricultural world, with everyone agreeing that it encompasses chemical,
192 biological and physical parameters. Parameters include pH, bulk density and water holding
193 capacity (physical attributes), Soil Organic Carbon (biological attribute) and total nitrogen and
194 total phosphorus (chemical attributes) [11]. In this thesis plant growth, which is dependent on
195 all of these interrelated physical, chemical and biological attributes is used as a soil health
196 indicator. As Lal (2016) stated, “soil health is represented by plant health itself” [11]. In
197 addition, soil’s ability to bioremediate hydrocarbon reflects on a well structured soil, for this
198 can only occur when, amongst other things, a soil presents a well balanced C:N:P ratio, a
199 good structure, and a well preserved microbiome. In order to explore this second hypothesis,
200 that improving soil health improves hydrocarbon remediation, basal respiration was used to
201 monitor hydrocarbon breakdown and microbial activity in our amended soils.

202

203 1.2 Effect of amendments on soil physical attributes

204 “Soil structure refers to the size, shape and arrangement of solids and voids, continuity
205 of pores and voids, their capacity to retain and transmit fluids and organic and inorganic
206 substances, and ability to support vigorous root growth and development” [11]. Physical
207 attributes of soil health are all dependent on soil’s structure which is dependent on soil
208 aggregation and related to soil strength (the ability to withstand erosion). Soil structure affects
209 plant growth by influencing root distribution and the ability to take up water and nutrients, which
210 is important due to the fact that aggregation also tends to increase with increasing root length
211 density [13].

212

213

214

215 1.2.1 Organic matter amendments on physical attributes

216 Organic matter amendments can improve soil structure and water holding capacity of
217 soil through increasing aggregation and improving the pore structure. The type and
218 characteristics of the organic matter added to the soil are important, as well as type and
219 characteristics, the amount of organic matter also influences soil aggregation [14]. Leroy et al.
220 (2008), stated that “not only the quantity but also the quality of the organic matter applied has
221 a significant influence” [9]. Furthermore, Abiven et al. (2008) showed that formation of stable
222 aggregates can be modelled in relation to the biochemical nature of the organic matter used
223 [15]. In contrast, Mtangadura et al. (2017) conducted an experiment in which they evaluated
224 the input of organic resources in Southern Africa on maize productivity in a long-term
225 experiment [6]. In said experiment, organic matter of low and high quality was evaluated, and
226 their results suggested that both, high and low quality, organic resources can “practically”
227 increase SOC in the medium to long term. In terms of soil strength, Davies (1985) found a
228 strong increase in soil shear strength due to organic matter, and Spivey et al. (1986) found a
229 strong correlation coefficient of $r= 0.88$ between shear strength and organic content [45][46].

230

231 1.2.2 Inorganic amendments on physical attributes

232 It has been widely agreed that Fe and Al oxides interact with organic matter in macro-
233 aggregate stability and, therefore, stabilise aggregates [16]. Inorganic minerals, as WTR, have
234 the ability to also change particle size distribution which may allow for more water movement
235 and aeration [17].

236 Furthermore, WTR has been found to improve soil properties as water retention and pH [18].

237

238 1.3 Effect of amendments on soil chemical attributes

239 Organic matter amendments can increase SOC and carbon storage in soils. This
240 increase takes place via two means: directly (via carbon inputs) and indirectly (by increasing
241 plant production) [7]. However, some researchers still focus the attention on the uncertainty
242 that surrounds the subject. According to Gregorich et al. (2005), organic matter amendments
243 increase carbon and nitrogen pools increasing the potential for carbon dioxide (CO₂), nitrous
244 oxide (N₂O) and methane (CH₄) emissions, these are greenhouse gasses and they may even
245 alter some environmental conditions of the soil, such as its moisture, temperature and pH
246 [19][47].

247

248 1.3.1 Organic amendments on chemical attributes

249 The addition of organic matter to soils generally increases the nutrient holding capacity
250 by increasing the macronutrient availability (NPK) which is why this is a common practice in
251 cropping systems to enhance net primary productivity [19]. Due to the high cost of chemical
252 NPK fertiliser, smallholder farmers in Africa have used locally available organic nutrient
253 resources, such as, livestock manure, compost, woodland litter, cereal and legume crop
254 residues [6]. Furthermore, organic amendments have been reported to have a high
255 effectiveness in the immobilisation of various types of contaminants [20].

256

257 1.3.2 Inorganic amendments on chemical attributes

258 Due to the high cation exchange capacity generally associated with WTRs, it has been
259 found that these materials have the ability to provide soil with cationic nutrients [18]. However,
260 it has also been found that minerals like Fe and Al oxides, contained in WTR, immobilise
261 elements such as P, Pb and As [20].

262

263 1.4 Effect of amendments on soil biological attributes

264 Microbe-mediated processes are highly sensitive to perturbations in soil, therefore, the
265 capacity of the soil to recover from perturbations can be assessed by monitoring microbial
266 activities [21]. Soil microorganisms have been shown to play critical roles in the regulation of
267 many soil related factors, such as soil fertility, plant health, and the cycling of carbon, nitrogen
268 and other nutrients [22]. More than 90% of the total soil microbial biomass are constituted of
269 bacteria and fungi, which are responsible for the majority of soil organic matter decomposition
270 [23]. Bacterial and fungal communities can be influenced by plants as well as vice versa [24].
271 Plants have a strong effect on the composition of microbial communities in soil through
272 rhizodeposition and decay of litter and roots [21]. Rhizodeposition is the continuous flow of
273 carbon-containing compounds from roots to soil [25] which then continues to interact with
274 inorganic minerals releasing micronutrients. The environment where complex microbial
275 communities and plant roots interact via nutrients released by the plant is called rhizosphere
276 [26]. This part of the soil is thought to be of great importance to both plant health and soil
277 fertility [19].

278

279

280 1.4.1 Organic amendments on biological attributes

281 Organic amendments result in an increase in nutrient availability and aside from the
282 remediation of soil fertility, can help in balancing C:N:P ratio in soil for when hydrocarbon
283 contamination occurs this ratio tends to get unbalanced for the increase on carbon, thus
284 increasing nitrogen and phosphorus availability in soil via organic matter inputs has been
285 proven to be an effective tool for biodegradation activity [24]. It is due to the central role of
286 microorganisms in the cycling of N and C, and their sensitivity to change, that microbial and
287 biochemical characteristics are used as soil quality indicators [21]. Therefore, it is of great
288 importance to find eco-friendly amendments such as organic amendments [27].

289 Soil microbial respiration can be considered as a useful indicator of the total microbial activity
290 since basal respiration is used to measure said activity in soil [21][28]. Soil microbial activities
291 and communities play an important role on petroleum hydrocarbon degradation with bacterial
292 communities being responsible for the degradation of the saturated and partially aromatic
293 hydrocarbons [29]. Even though CO₂ production is not directly related to oil carbon
294 biotransformation, changes in microbial activity indirectly reflect oil microbial breakdown [28].
295 Furthermore, it has been suggested that CO₂ evolution is considered “vital evidence” linked to
296 transformations of organic carbon and characteristics of soil quality [27].

297

298 1.4.2 Inorganic amendments on biological attributes

299 Most of the research on the biological effects of inorganic amendments focuses on the
300 use of inorganic chemical fertilisers (NPK) to improve either crop productivity or as agents for
301 enhanced bioremediation helping microbes to increase the degrading ability of the indigenous
302 community [29].

303

304 1.5 Organic and inorganic amendments combined

305 The objective of this project is to measure changes in soil health when organic and
306 inorganic amendments are added to Zimbabwean soil. The hypothesis is that organic
307 amendments such as compost and inorganic mineral amendments like water treatment
308 residual will provide organic matter, nutrients and carbon to the soil, therefore having a positive
309 effect on soil health, not only on nutrient holding capacity but also on soil structure. Plant
310 growth (height and biomass) is used a soil health indicator and measured over 10 weeks. The
311 second hypothesis is that the improvement in soil health, the ability of the soil to bioremediate
312 hydrocarbons could be enhanced, and therefore, reflect on the impacts of the soil

313 amendments on soil health once they are put under contamination stress. Hydrocarbon
314 breakdown and microbial respiration were measured through microcosm experiments over
315 the course of 32 days.

316

317 **2. Materials and Methodology**

318 The following sections include data on the four materials (soil, compost, WTR and
319 quartzite) used to produce different 'soil types'. As well as of other elements, such as lime and
320 two types of nutrients, which were used during the plant growth trials soil preparation. Data on
321 the four main materials was obtained from analyses performed by the Geography
322 Department's laboratories at Durham University and is reported as it was obtained from the
323 spreadsheet sent by them.

324 In addition, the experimental methodologies which were used to prepare the soil types and to
325 measure improvements on soil health are explained. These methods include two plant growth
326 trials and microcosm experiments, which served as a way to test soils' ability to biodegrade
327 hydrocarbons, and the preparation for them. It should be noted that the first plant growth trial
328 was carried out as part of the method development process, allowing to perfect the
329 methodology for the second trial.

330

331 2.1 Materials characterisation

332 Moisture content was measured for all materials. These calculations were made by
333 oven drying at 105°C for 4 hours. The pH was measured using the pH meter Hanna H18424
334 in a calibration range between 4.01 and 7.01 units. The extraction was made using 50 ml of
335 deionised water and 20 g of 'as received' soil. The exchangeable bases (extractable Ca, K
336 and Mg) were calculated, for an extract of the soil, using the instrument Agilent 5100 ICP-OES
337 and 5 grams of sample. Total carbon and nitrogen were calculated by combustion method
338 using Flash 2000 Organic elemental Analyser. Extractable phosphorus (plant available) was
339 obtained using the instrument Agilent 5100 ICP-OES with 2.5 g of sample.

340

341 2.1.1 Soil

342 Zimbabwean soil was sourced from Domboshava Training Centre (17°36' S; 31°08'
343 E; 1542 m a.s.l) located 30 km North East of Harare Zimbabwe. The site is characterised by
344 having a sub-humid climate and receiving an annual rainfall of <750 mm [28]. The soil was
345 sampled in October 2018 from a plot measuring approximately 300 m² using hand hoes and
346 shovels to a 20 cm depth. Soil was sieved to <2mm and air-dried before transportation and
347 usage. This soil is broadly classified as Paraferallitic 6G soil according to Zimbabwe soil
348 classification or as Lixosol according to the World Reference Base (2006), it has a
349 concentration of 0 ppm of calcium, 39.1 ppm of potassium and 36.5 ppm of magnesium. It has

350 an Effective Cation Exchange Capacity (ECEC) of 6.5 (meq/100g). It is composed of granitic
351 parent material fused with dolerite intrusions [5]. The soil can be described as a sandy-clay
352 loam with 220 g clay kg⁻¹, 50 g silt kg⁻¹ and 730 g sand kg⁻¹. The mean density is 2.6788 (ρ ,
353 g/cm³), a pH of 4.63 units, which makes it an acidic soil, and a total carbon of 0.47% (w/w), a
354 total nitrogen of 0.03% (w/w) and .00039% (w/w) of extractable phosphorus.

355

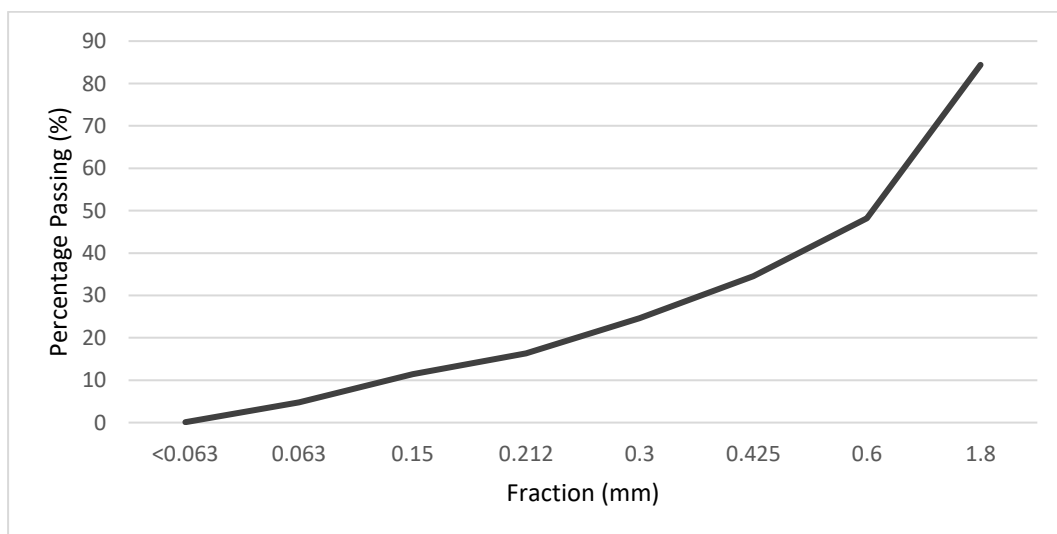
356 2.1.2 Compost

357 Compost containing 50% peat named “Gro-sure, All-Purpose Compost” was
358 purchased from a local Durham, England store. The moisture content of the compost was
359 found to be 58.2%. The pH was found to be of 4.9 pH units, with 46.90% (w/w) of total carbon
360 and 1.28% (w/w) of total nitrogen. Its density was of 1.56 (ρ , g/cm³). The compost was sieved
361 to <2mm. Compost was gently compacted using hands while filling up the pots.

362

363 2.1.3 Water Treatment Residual (WTR)

364 WTR is the final product of water purification, the WTR used for this project was
365 obtained from Mosswood Water Treatment Works in Durham and was stored already sieved
366 to <2mm. This material contains iron and manganese oxides, which are expected to increase
367 aggregate stability. It has a moisture content of 38.1% (w/w), a pH of 4.2 units, a concentration
368 of 2.7 (meq/100g) of Ca, 0.1 (meq/100g) of K and 0.6 (meq/100g) of Mg. A total carbon of
369 19.98% (w/w) and total nitrogen of 5.06% (w/w).



370

371 *Figure 1. Particle size distribution curve for dry WTR after sieving <2mm.*

372

373 2.1.4 Quartzite

374 The material was purchased as Natural White Aquarium Gravel from a local Durham,
375 England pet store with the purpose of it being chemically inert and pure. Quartzite was ground
376 to replicate the particle size distribution (PSD) of the WTR (Fig. 1) by two means, through a
377 mechanical grinder and manually using a mortar and pestle, the latter to try to obtain more
378 material from the particle sizes 0.425-0.6 mm, 0.3-0.425 mm, 0.212-0.3 mm and 0.15-0.212
379 mm.

380 The purpose of the use of quartzite on the amendments is to highlight the differences between
381 the chemical and physical influences of WTR on soil as an amendment, this under the
382 speculation that quartzite may provide a control for the physical attributes of WTR but with no
383 chemical influence. Calculations were made with the same volume of quartzite as WTR, and
384 although the particle size distribution was controlled, it was not possible to control for pore size
385 distribution or microbiome. Therefore, variations between soil types might not reflect only
386 chemical differences.

387

388 2.1.5 Maize

389 Seeds of maize from Zimbabwe were used as a way of measuring the soil's ability to grow
390 plants. The seeds were purchased and imported to England in a sealed bag. Previous to
391 planting, the seeds were germinated for 3 days, the processes of planting and germination in
392 more detail are explained in the methodologies section 2.2.2.

393

394 2.1.6 Nutrients

395 During the plant growth trials and due to the soil's specifications, the addition of two
396 types of fertilisers was thought to be the best approach to ensure that nutrient limitation
397 (chemical characteristic) was not a factor for maize growth, thus giving the possibility to assess
398 the impact of the different amendments on soil with respect to biological and physical
399 characteristics.

400 One of these was an All-purpose Liquid Plant Food purchased from a local (Durham, England)
401 shop. It is an NPK Fertiliser solution 6-3-6 with macro and micro-nutrients such as, nitrogen,
402 phosphorus, potassium, copper, and iron (Table 1). This fertiliser was added in the middle of
403 the first trial at week 5, and in the middle of the second one, at day 33 (Table 2). The calculation
404 followed the recommendations on the container using one cap (50 ml) diluted into 4.5 L of
405 water. In the first trial, the liquid plant food was distributed across the amendments with a

406 double dosage to the ones that lacked organic matter input, such as soil and soil and quartzite.
 407 However, during the second trial the dosage was one litre of diluted plant food per tray
 408 containing 20 pots, without distinction among the NPK amended soil types.

409 The second fertiliser used, was added at the beginning of the second trial (Table 2), it was a
 410 compound D exported from Zimbabwe, it was purchased from a company called Zimbabwe
 411 Fertiliser Company (ZFC) and its contents can be seen on Table 1. The calculations of the
 412 amount of compound D that was to be added to each individual pot were made by targeting
 413 26 kg P ha⁻¹ for soil types containing non-organic materials or base soil, and targeting 14 kg
 414 P ha⁻¹ for those soil types containing organic materials. This was done under the assumption
 415 that the organic materials would already provide an input of nutrients to the soil type.

416 The calculation then was made by using the relation of 26 kg of fertiliser to 10 000 m² (1 ha)
 417 of soil or soil type, therefore the surface area of the pot was multiplied by 26 kg and then
 418 divided by 10 000 m². The result being the kilograms of fertiliser would then be converted into
 419 grams and used in each of the pots. The same process was done to calculate the amount of
 420 fertiliser for organic materials containing pots only changing the relation to 14 kg of fertiliser
 421 for 10 000 m².

422 *Table 1. Table showing the composition of All-purpose Liquid Plant Food and Compound D in percentages, fertiliser*
 423 *called All-purpose Liquid Plant Food was used for the two plant growth trials, whereas compound D was only used*
 424 *for the plant growth second trial see Table 2 for further information on this.*

Component	Quantity (%)	Compound d (%)
NITROGEN (TOTAL)	6.00	7.00
Urea Nitrogen	3.30	
Nitric Nitrogen	1.70	
Ammoniacal Nitrogen	1.00	
Phosphorus Pentoxide	3.00	14.00
Potassium Oxide	6.00	7.00
Copper	0.002	
Iron	0.01	
Molybdenum	0.001	
Zinc	0.002	

425

426 *Table 2. Fertiliser addition showed for both trials. For the first growth trial (12 weeks duration), plant food was added on*
 427 *week 5 to all soil types with second dose only given to those lacking organic matter (soil, soil-quartzite). For the second trial*
 428 *(7 weeks duration), compound D fertiliser was added on the day of planting with a double dosage to those amendments*
 429 *lacking organic matter (soil, soil-quartzite) and plant food was added to NPK amended soil types on week 5, same dose.*

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
First Growth Trial					Plant Food							
Second Growth Trial	Compound D Fertiliser				Plant Food							

430

431 2.1.7 Lime

432 The lime used was purchased from a local (Durham, England) shop and contained
433 50% calcium carbonate (CaCO_3) and 50% dolomite ($\text{CaMg}(\text{CO}_3)_2$). The calculations for the
434 amount of lime needed were done by targeting a pH of 5.5 units. Sandy soils require 600 kg
435 ha^{-1} of lime to raise the pH by 0.1 unit.

436 The pH of the soil needed to be risen by 0.9 units this number was divided by a constant 0.3
437 and then multiplied by 600 (kg of lime required per ha). The final number was the amount of
438 kg required per ha of soil which was then just calculated considering the surface area of each
439 pot in grams.

440

441 2.2 Experimental methods

442 2.2.1 Soil preparation

443 Soil preparation presents a difference from the first growth trial to the second one, said
444 difference is further explained in the following sections where the adjustments made to the
445 methodology under which the plant growth trials developed are supported by literature found
446 on similar projects.

447

448 2.2.1.1 Plant growth trial 1

449 Fifteen different combinations across all the materials previously described were put
450 together with seven replicates ($n=7$) per soil type. The effect of the amendments was
451 evaluated through a period of time of 12 weeks.

452 The soil type ratios were calculated by taking into consideration the dry mass of each material
453 and the pot size (9 x 9 x 9.5 cm). However, the ratios were created with field moist materials,
454 meaning that no drying was done prior to the mixing of the materials. In order to calculate the
455 amount of dry mass needed of each material, the water content of all materials was calculated
456 by the standard method for soil and rock by mass [30].

457

458 *Table 3. Soil types and composition of materials for plant growth trial 1, the letters on the name code*
 459 *state the following, S= Soil, C= Compost, Q= Quartzite and WTR= Water treatment residual. The*
 460 *numbers correspond to the percentage of each material in the mixture. **Highlighted soil types***
 461 ***represent those that received a double dosage of nutrients.** Quartzite by mass refers to the one*
 462 *that was applied as it was purchased. While, for quartzite addition by volume, quartzite that was*
 463 *grounded to the same PSD as in WTR was added.*

Soil type	Materials			Quartzite (%)
	Soil (%)	Compost (%)	WTR (%)	
1 S100	100			
2 SC 9010	90	10		
3 SC 8020	80	20		
4 SWTR 9010	90		10	
5 SWTR 8020	80		20	
6 SQ 9010 (mass)	90			10
7 SQ 8020 (mass)	80			20
8 SQ 9010 (volume)	90			10
9 SQ 8020 (volume)	80			20
10 SCWTR 801010	80	10	10	
11 SCWTR 602020	60	20	20	
12 SCQ 801010 (mass)	80	10		10
13 SCQ 801010 (volume)	80	10		10
14 SCQ 602020 (mass)	60	20		20
15 SCQ 602020 (volume)	60	20		20

464

465

466 2.2.1.2 Plant growth trial 2

467 The second growth trial, was run for 7 weeks due to concerns that the pot size was too
 468 small to run for 10 weeks. In the first trial, the root system of the plants seemed to have
 469 reached a stage of growth at 10 weeks where they filled the pots and could not develop any
 470 further because of the limited space. Therefore if shoot and root systems all reached the same
 471 stage, comparison amongst soil types would become difficult. For the previously stated
 472 reasons it was believed that the biomass data for this first trial was compromised. Thus, the
 473 second trial lasted 7 weeks, six soil types were made with combinations of the materials (Table
 474 4), however, this second time there was a control set of amendments which had no added
 475 nutrients to them, making up two lots of six soil types each in total, one lot with nutrient addition
 476 and one without.

477 Of the six soil types, three were single amendments (2, 3 and 4 in Table 4), meaning soil and
 478 one of the materials combined. Two other combinations were co-amendments (5 and 6 in
 479 Table 4), meaning a combination of two materials and soil, and one acted as a control of only
 480 soil (1 in Table 4). The other six combinations were the same as the previously described with
 481 the difference that once the trial started nutrients were not added to them.

482 *Table 4. Composition, in percentages, of the different soil types for the second growth trial. Highlighted*
 483 *soil types represent the ones that received double dosage of compound D at week 5. Quartzite addition*
 484 *in this second trial was only by volume (with similar PSD of WTR).*

Soil type	Materials			
	Soil (%)	Compost (%)	WTR (%)	Quartzite (%)
1 S100	100			
2 SC 9010	90	10		
3 SWTR 9010	90		10	
4 SQ 9010	90			10
5 SCWTR 801010	80	10	10	
6 SCQ 801010	80	10		10

485

486 As in the first trial (see section 2.2.1.2) the calculation of materials was done by dry mass.

487 A further amendment done to the soil preparation for the second trial involved the application
 488 of a method of equilibration of materials [31][32]. This method allowed materials to
 489 homogenise by settling and interacting before planting. This ‘settling’ period in open trays in
 490 the greenhouse was of four weeks. During these four weeks, the soil types were mixed, wetted
 491 and dried twice a week.

492

493 2.2.2 Germination and planting

494 2.2.2.1 Plant Growth Trial 1

495 Maize seeds were germinated prior to planting. This process was done in a growth room
 496 at 30 °C during three days. All pots were labelled and to avoid losing material through the
 497 bottom holes of the pots, tissue paper was placed at the bottom of each pot before adding the
 498 mixtures. While placing the material into the pots it was important to maintain a similar volume
 499 amongst all of them, this with the purpose of all pots having similar volume of material for roots
 500 to occupy.

501 Once all the material was placed in each pot, a hole of approximately 2 cm was made and the
 502 seedling was placed inside. Then, it was covered with the topsoil of the pot avoiding covering
 503 the growing stem. Pots were arranged inside trays that fitted fourteen pots in each tray,
 504 meaning two soil types were placed in each tray. The pots were initially watered from the top
 505 until the water reached the top edge of the pots, this was done for the first four weeks, and
 506 afterwards, watering was done by adding water to the tray and leaving the roots to take it up
 507 from the bottom of the pots. The amount of water added to each tray depended on different
 508 factors, these being, the position of the trays in the greenhouse, the soil type and the level of
 509 growth of the plants. Thus, the amount of water added to the trays was calculated by taking
 510 into consideration their needs and with the purpose of maintaining a healthy environment so
 511 that water deficiency was not a factor. Watering was carried out every three days.

512 Plants were grown for twelve weeks in a greenhouse under controlled temperature of 23°C
513 during the day and 18°C during the night. These changes in temperature were dependent on
514 the lights which were on for 16 hours and off for 8 hours. During week 5 of the trial, it was
515 noted that plants across the soil types that lacked organic matter were starting to die, it was
516 then that the addition of plant food took place. As explained on section 2.1.6, double dosage
517 of the plant food was added to those amendments that lacked organic matter and one dose
518 was applied to the ones that had organic matter input.

519

520 2.2.2.2 Plant growth trial 2

521 During the second growth trial, and due to the ineffectiveness of tissue paper to contain
522 material and roots inside the pots, frost cloth was placed instead, at the bottom of each pot
523 before the addition of the mixtures. Once all the material was placed inside the pots, a hole of
524 approximately 5 cm was made into them and the compound D fertiliser was placed inside and
525 slightly mixed with the topsoil of the pot. For the soil types containing materials that were rich
526 in organic matter (such as compost) 0.89 grams of compound D were added to each pot,
527 whilst, for the pots containing only soil and soil and quartzite, 1.79 grams were added (Table
528 4). The seed was then placed in the hole and partially covered by the soil without
529 compromising the growing stem.

530 The pots were arranged on trays that fitted twenty pots and the watering process was the
531 same as the one described for the first growth trial. Similarly to the first trial, plants were grown
532 in the same greenhouse with the difference that this second trial was done for 7 weeks. The
533 second NPK addition was added on week 5, this was the same plant food used for the first
534 trial, however, the same amount of plant food was added to all of the soil types.

535

536 2.2.3 Plant height and weight measurements

537 During the trial, plant height was measured and recorded twice a week as it was used
538 as an indicator to compare the effect of different amendment materials on soil health. The
539 measurements were done with a plastic 30 cm ruler which was placed at the base of the plant,
540 the longest leaf was used as the reference point to measure height. Height measurements
541 were recorded once a week starting at week 3, once the plants had developed leaves.

542 Once the trial was finished, the plants were harvested. This was done by cutting off the stem
543 of the plant at the point in which it intersected the soil, this was later stored in a paper bag.
544 The root system was removed from the soil and stored in a separate paper bag. The roots of

545 each plant were removed by shaking off the soil attached to them and then washing them with
546 tap water to remove the remaining soil particles, after that the roots were washed with de-
547 ionised water to further eliminate unwanted materials attached to the roots.

548 All of the bags were taken to be oven-dried for 48 hours at 65°C to remove all moisture, and,
549 once the weigh was constant, they were taken out of the oven and placed on a scale where the
550 weights were recorded to obtain dry biomass of both the plant and its root system.

551

552 2.2.4 Hydrocarbon biodegradation microcosm experiment

553 Soil types which facilitated the most plant growth were selected to undergo a
554 hydrocarbon biodegradation experiment with the purpose of testing their ability to breakdown
555 hydrocarbon pollution. The soils used for this experiment were sampled the day of harvesting.
556 All of the pots of each individual soil type were recombined together into a tray and soil was
557 then homogenised, 100 grams of sample were collected into a plastic sealed bag and labelled
558 with the name of the soil type (Table 5). Quartzite and compost amendments had resulted in
559 high relative plant growth but due to the significant plant die off (n=7 at time zero became n=2
560 at week 7) this soil type was not selected for microcosm experiments.

561 Aside from soil types that had gone through the plant growth trial, the original materials were
562 selected to act as controls in the microcosm experiments and are referred to as 'raw materials'.

563 *Table 5. Soil types and raw materials selected for hydrocarbon biodegradation experiments.*

<i>No. of Soil type</i>	<i>Amendment</i>
1	100% Zimbabwean Soil (raw material)
2	100% Compost (raw material)
3	100% Water Treatment Residual (raw material)
4	90% Soil – 10% Compost
5	90% Soil – 10% WTR
6	80% Soil – 10% Compost – 10% WTR

564

565 The microcosm experiments were carried out at Newcastle University, Newcastle, UK. To set
566 up the microcosms, autoclaved glass serum bottles were filled with 10 grams of raw material
567 or soil type. Two sets of bottles were created, one set was contaminated with 128.2 µl of an
568 un-degraded North Sea crude oil (known as 'oil contaminated') and the other one was not
569 (known as 'uncontaminated'). 100 µl of a mixture of NPK was added to all of the bottles,
570 without any exceptions. The NPK consisted of 2% NaNO₃ and 0.1% of KH₂PO₄, so the C:N:P
571 ratio was 100:2:0.1. The addition of nutrients was made to ensure the microorganisms in all
572 of the microcosms had enough essential nutrients to grow. It is important to note that soil types
573 labelled as "+NPK" in the Results and Discussion section refer to the nutrient addition during

574 the plant growth trial (not to the nutrient addition during the microcosm experiment which was
575 applied to all microcosms).

576 After the set up, they were placed in a cold room with a temperature of 4°C to slow down
577 microbial activity until the first GCMS measurement, which took place the next day.

578 The gas chromatography-mass spectrometry (GC-MS) method was performed with Fissons
579 Trio 1000 fitted with Pora Plot Q GC column. The calibration of the GCMS had to be done at
580 the beginning of every run and after 30 minutes of injections of microcosms. This calibration
581 started with an injection of air of 100 µl, followed by sequential injections, every minute, of
582 100, 80, 60, 40 and 20 µl of two gases, one containing 1% CO₂ and the other one containing
583 10% CO₂ respectively. After the 11 injections of standard gas were made, 100 µL of sample
584 were injected into the septum, this was repeated for every microcosm at intervals of one
585 minute until the CO₂ in all of the bottles was measured.

586 Peak area measurements were obtained from m/z 44 and m/z 32 mass spectra to determine
587 CO₂ and O₂ concentrations in the microcosms. The CO₂ indicated the extent of oil
588 biodegradation whereas the O₂ served to monitor that the microcosms did not become anoxic.
589 Once the monitored levels of oxygen declined below 75%, air injections were made to the
590 microcosm bottles to replenish them with enough oxygen. This was done by opening the bottle
591 and flushing five times air with the help of a syringe. On the day where there was air flushing,
592 a measurement of CO₂ was done before and after said air flushing and then taken into
593 consideration in the calculation of cumulative CO₂ for the rest of the days of the experiment.

594

595 2.2.5 Statistical Analyses

596 Statistics packages Minitab 18 and SPSS 22 were used in order to perform the statistical
597 analyses of height and weight of the plants, and biodegradation rates of CO₂ emissions.

598 One-way ANOVA tests were performed for both plant growth results, and biodegradation rates
599 of CO₂ results. Tukey tests as post-hoc analysis was performed on Minitab 18 with a p-value
600 of <0.05, in order to determine statistical significance amongst plant growth results. For the
601 biodegradation results, LSD post-hoc analysis was performed on SPSS 22 using a p-value of
602 <0.05 to determine statistical significance amongst the results. Standard error was used for
603 variation of the data.

604

605 **3. Results and discussion**

606 3.1 Plant Growth Trial 1

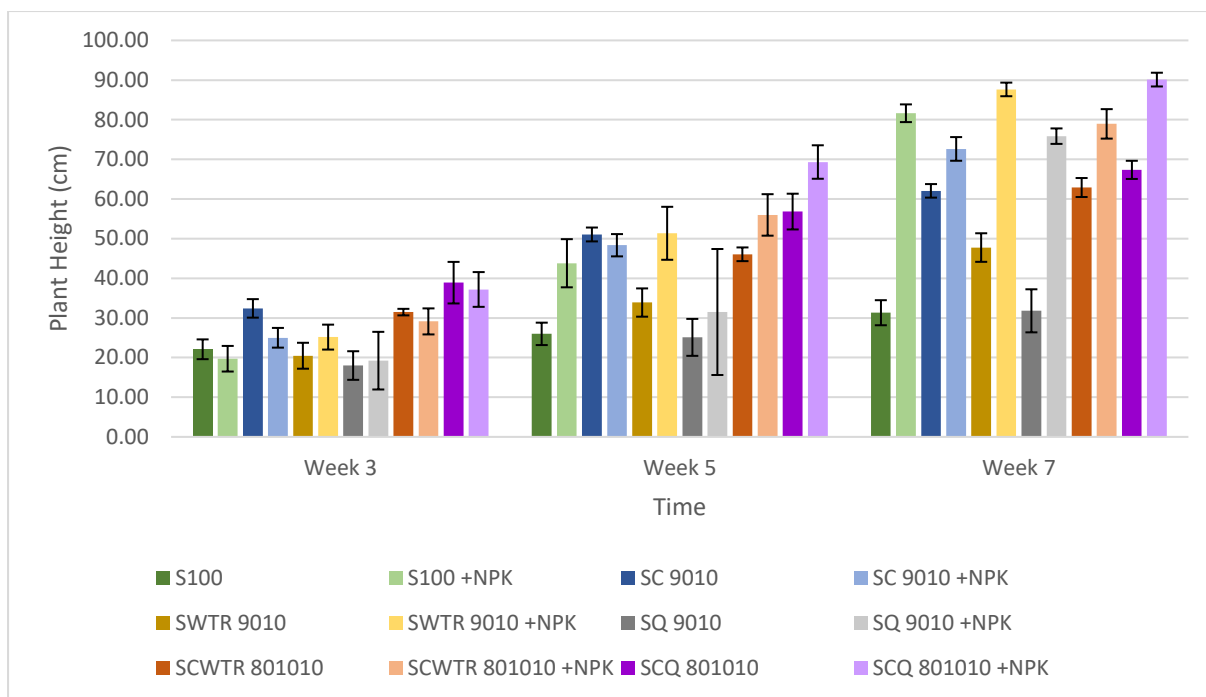
607 As previously stated, the results obtained from the first growth trial were formative and
608 allowed us to perfect the second trial methodology. The biomass data obtained from this trial
609 is believed to be compromised because of the pot size and the duration of the experiment
610 (2.2.1.1), the data is not presented in this section of discussion. However, the measurements
611 of the different soil types plant height, and plant development throughout the weeks, were
612 beneficial to select a smaller number of soil types to run for the second trial.

613

614 3.2 Plant Growth Trial 2

615 During the growing stage of the trial, measuring the height of the plants was considered
616 an acceptable way of knowing which amendment was working best besides looking at different
617 plant factors, such as colour, texture of leaves and stem size.

618 Plant height developed throughout the weeks differently in each soil type, but said difference
619 was not noticeable until week 5, that is when the plants started to grow at different rates (Fig.
620 2). Throughout the weeks, some plants behaved differently to the rest of them in the same soil
621 type, which in some cases ended with them dying. Plants that stopped growing and started
622 drying out were kept in the trays so that watering did not differ during the trial. Plants that were
623 growing in soil without any amendments (S100) started off growing similarly to the rest of the
624 soil types. However whilst the amended soil types showed a growth of 3-11 cm every week
625 without NPK addition and 2-19 cm every week with NPK addition, maize growing on
626 unamended soil (S100) presented only 1-3 cm of growth on average between measurements
627 confirming its poor quality.



628

629 *Figure 2. Height progress of all amendments from week 3 to week 7 of the trial. Each bar represents*
 630 *the mean of height measurements of each soil type. Error bars show standard error.*

631 When organic matter or chemical fertiliser was applied to the soil, a difference was visible
 632 throughout the trial, this difference also extended to what was able to be seen qualitatively in
 633 the colour and apparent strength of the plants (Fig. 3). Furthermore, nutrients in Zimbabwean
 634 soil were found to be low in concentration (section 2.1.1), this can also be confirmed by other
 635 authors, as an example Mapfumo et al. (2007), and Mtangadura et al. (2017) [6][48].

636 Plants growing on S100 +NPK started differentiating from the ones growing on soil without
 637 NPK amendment (S100) from week 5 of the trial, whereas, plants growing on SC 9010 showed
 638 a statistically significant ($p < 0.001$) difference from S100 right from week 3 (Fig. 5 and 6). Plant
 639 growth mostly depends on the availability of N and P, this availability is influenced by the level
 640 of organic matter in soil, compost is known to increase soil's organic matter content
 641 [33][34][35]. Therefore, the earlier increase in plant height in compost amended plants could
 642 be explained by the availability of its nutrients for plant uptake.

643 Statistical difference ($P \leq 0.05$) was found on week three (first plant height measurement)
 644 between S100 +NPK and SC 9010. S100 +NPK had an average height of 19.71 ± 8.55 cm
 645 whereas SC 9010 had an average height of 32.41 ± 6.18 cm. To explain this, it has been found
 646 that in soils with low organic matter, applied nutrients are easily lost from the root zone,
 647 moreover, crop plants feed on nutrients by root uptake [36][34]. The unavailability of nutrients
 648 on NPK amended soils may be a reason to explain their late development.

649

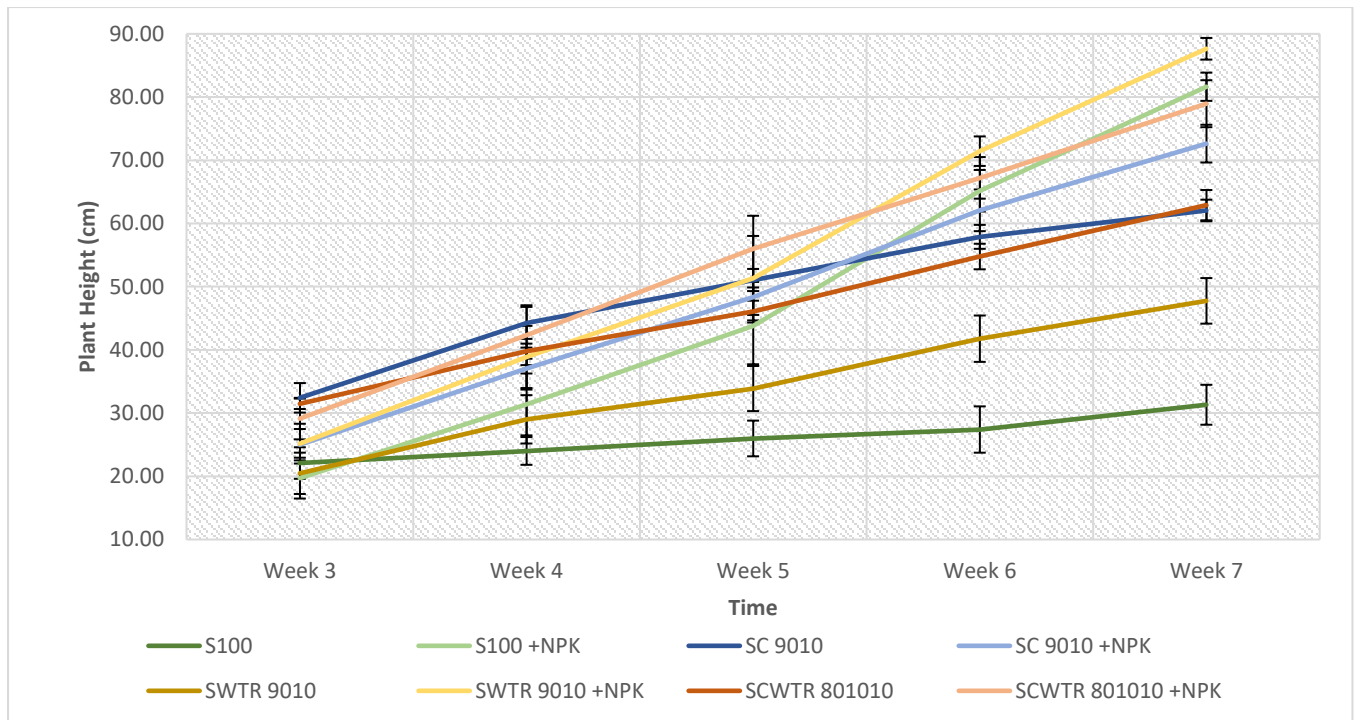


650

651 *Figure 3. Plant comparison (week 6) of S100+NPK (three pots on the left)*
652 *and S100 (three pots on the right). Fertiliser addition to the S100 soil type considerably increased plant height and plant appearance*
653 *with a better colour and stronger looking stem.*

654 All soils with an organic matter input showed a difference in trend of growth when compared
655 to the base soil (Fig. 4). However, SWTR 9010 did not behave as the rest of the amendments
656 when compared to the soil. This can be easily explained by the characteristics of WTR (see
657 section 2.1.3) and the fact that it has been found to immobilise P, making it unavailable for
658 plant uptake [18].

659

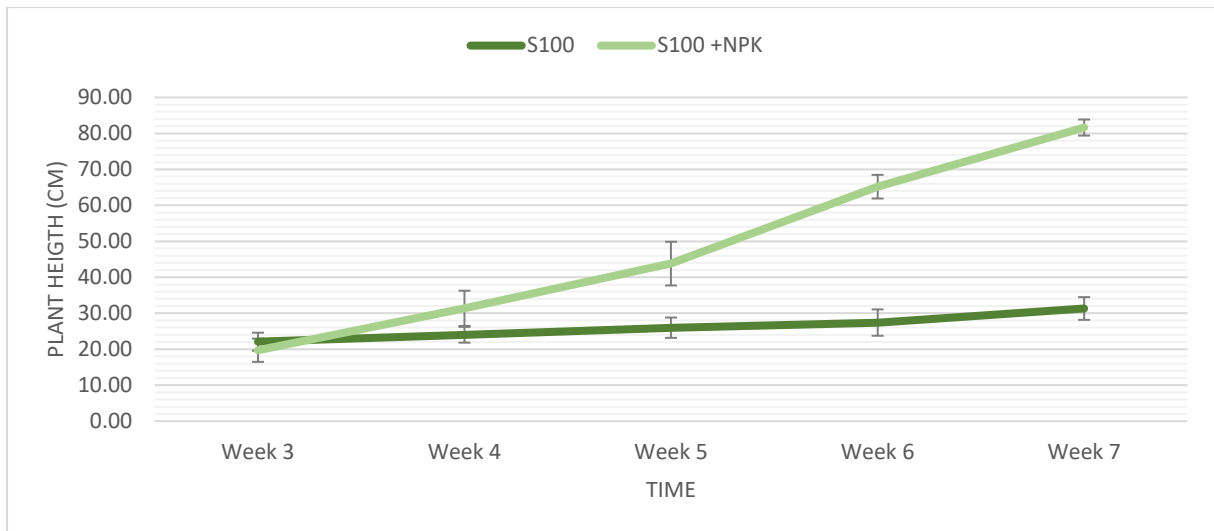


660

661 *Figure 4. Comparison between soil types with and without nutrients addition throughout the last 5 weeks*
 662 *of the experiment. Note that by the end of the trial (week 7) n=7 for SC 9010, SWTR 9010 and SCWTR*
 663 *801010, and n=5 for Soil100. And for the soil types with nutrients addition, n=6 for Soil100, SC 9010,*
 664 *SCWTR 801010, and n=5 for SWTR 9010.*

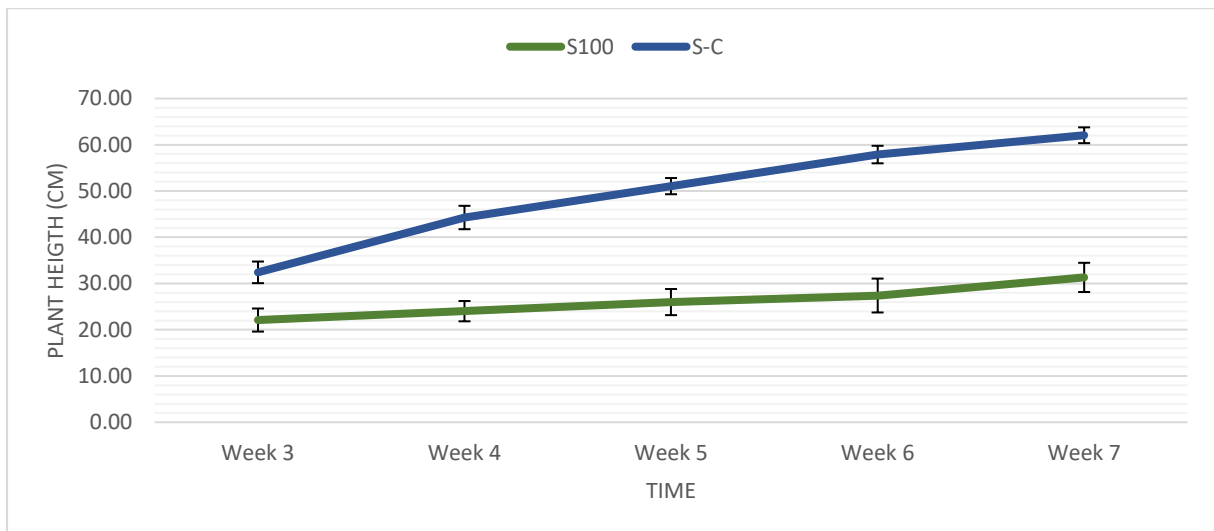
665 The second addition of fertiliser to the NPK amended soil types showed a bigger increase
 666 from week 5 to week 6 on the height of plants than the one seen on the weeks prior. This
 667 increase is particularly seen on S100 +NPK and SWTR 9010 +NPK. In S100 +NPK, plant
 668 height increased an average of 21 cm from week 5 to week 6, if compared to the increase
 669 from week 4 to week 5, which was of 12.43 cm on average, almost double. Similarly, on
 670 SWTR9010 +NPK, the increase from week 4 to 5 was of 12.47 cm on average whereas the
 671 one from week 5 to week 6 was of 20 cm.

672



673

674 *Figure 5. Comparison of plant growth between S100 and S100 +NPK. The data points in each week*
 675 *were taken from the average growth of the n plants in each soil type. Note that n=5 for the last two*
 676 *weeks of S100 and n=6 for the last two weeks of S100 +NPK. Error bars show standard error.*



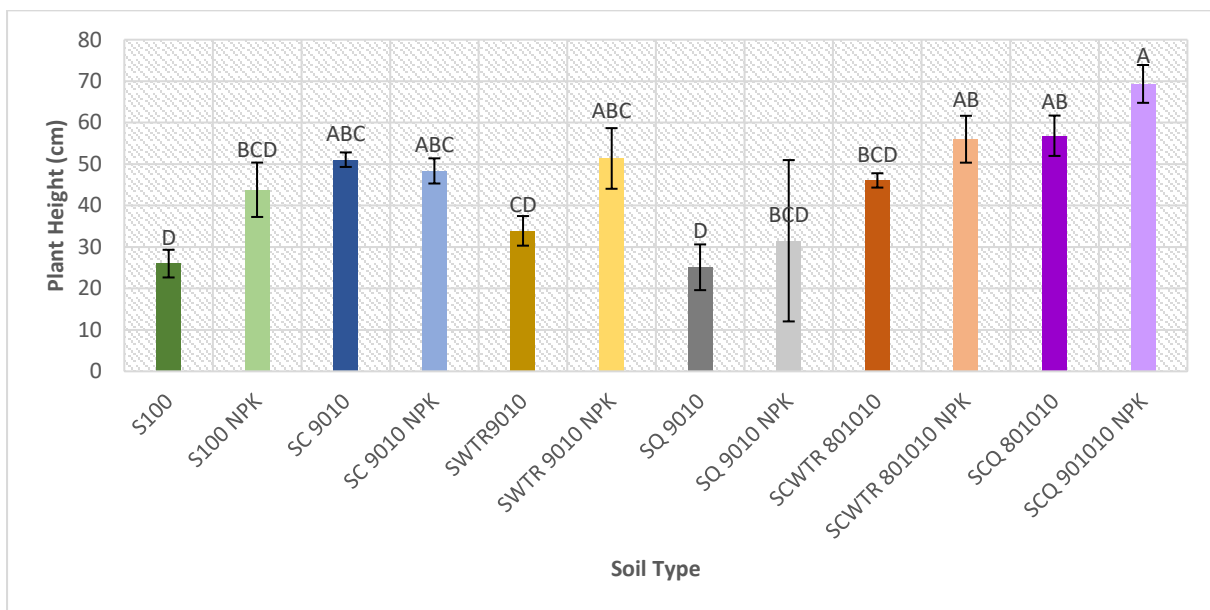
677

678 *Figure 6. Comparison of plant growth between S100 and SC 9010. The data points in each week*
 679 *were taken from the average growth of the n plants in each soil type. Note that n=5 for the last two weeks of*
 680 *S100 and n=7 for all weeks of SC 9010. Error bars standard error.*

681 Soils that lack nutrients tend to cause a restriction of crop growth which is why the addition of
 682 organic matter (Fig. 6) and fertiliser (Fig. 5) to the soil has made a significant difference on
 683 growth (height) of these soil amendments compared to the soil by itself (S100) [32]. On granitic
 684 sandy soils, as the one used, nitrogen is a limiting nutrient on crop productivity and the addition
 685 of it has been proven to boost both above and below ground maize biomass (Fig. 9 a-b) [6].

686 As previously mentioned, at week three statistical difference ($P < 0.05$) was found between
 687 S100 +NPK and SC9010. However, no significant difference was found between soil types
 688 with and without nutrient addition, which could go on to indicate that either the NPK added at
 689 the beginning of the trial did not make any contribution to the soil or that it needed further time
 690 to create an impact on plant development.

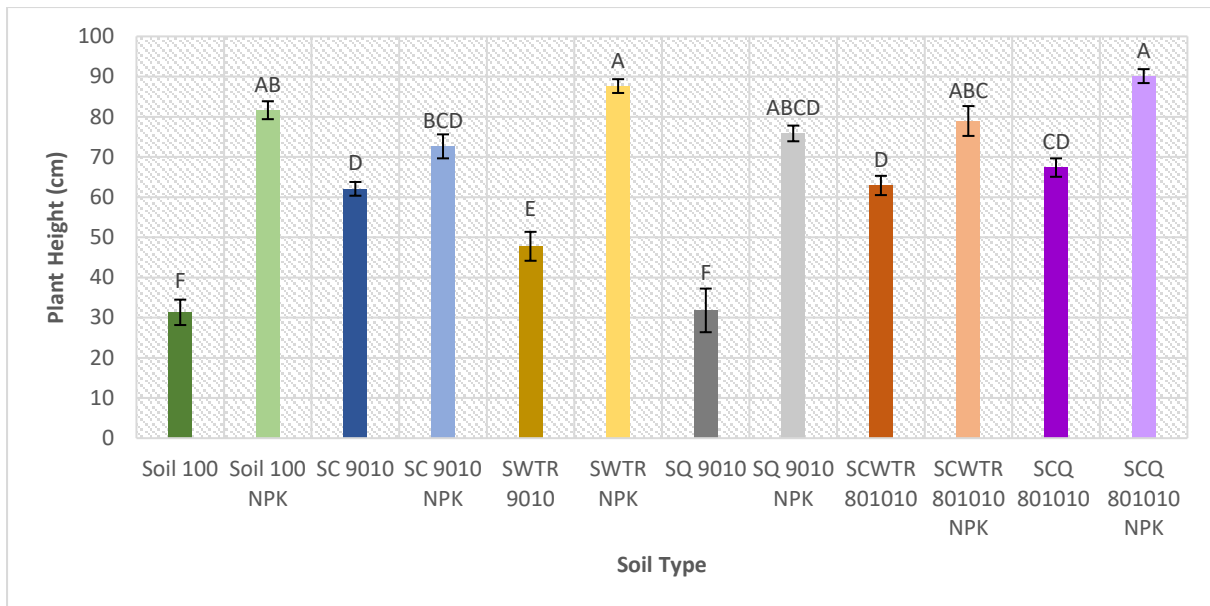
691 At week 5, a significant difference was found across all soil types on plant height ($P \leq 0.05$). At
 692 this stage of the growing period, the base soil did not represent a significant difference against
 693 S100 +NPK, this could be due to the reaction time of the compound D or maybe it was washed
 694 off due to lack of nutrient holding capacity while watering, since this was done from the bottom
 695 of each pot at the beginning of the trial but started to be done from the top of the pots after
 696 week 3. However, S100 did present a significant difference when compared against SC 9010
 697 (Fig. 7), which goes further on to explain how organic matter addition had an earlier impact on
 698 plant growth than fertiliser addition.



699
 700 *Figure 7. Week 5 plant height average of growth. Significant difference ($P < 0.05$) was found and is*
 701 *represented with a lettering system. Bars that do not share a letter are significantly different, and error*
 702 *bars show standard error. It is important to note that $n=3$ for SQ 9010 NPK, and one of the plants was*
 703 *starting to dry out thus the error bar being greater than for the rest of the soil types.*

704 Both SWTR 9010 +NPK and SCWTR 801010 +NPK showed a significant difference ($p < 0.05$)
 705 with S100 at week 5 (Fig. 7), the co-amendment had taller plants with an average of
 706 55.99 ± 16.36 cm while the single amendment had an average of 33.89 ± 9.44 cm. Since P
 707 available for plants on compost was found to be high (261 mg/kg) and it is known that WTR
 708 fixes P, it can be hypothesised that even with the P fixation of WTR, there was enough
 709 remaining P available for plant uptake from the further addition of this on the fertiliser [18].

710 The second addition of nutrients (the All-Purpose Liquid Plant Food) was applied to all NPK
 711 soil types on week 5, therefore measurements after this week were affected by this addition.
 712 As previously mentioned, a bigger increase on average centimetres of height was seen on
 713 some of the soil types after the NPK second addition. This impact can be seen on further
 714 significant differences found on week 7, last measurement.



715

716 *Figure 8. Week 7 plant height average of growth. Statistical difference ($p \leq 0.05$) is shown with a lettering*
 717 *system, bars that do not share a letter are significantly different. Error bars show standard error of the*
 718 *measurements. Note that $n=2$ for SQ 9010 +NPK.*

719 Since nutrients in compost were found to be low in concentrations (1.28% N, 0.026% P), it is
 720 believed that this may have caused plants growing with NPK addition (S100 +NPK) to grow
 721 significantly higher than the ones with a 10% compost addition (SC 9010) by the end of a
 722 seven week trial (Fig. 8). S100 +NPK had an average of 81.63 ± 5.47 cm on height whilst SC
 723 9010 had an average of 62.05 ± 4.52 cm. And, although no significant difference was found
 724 between SC 9010 with and without NPK addition, SC9010 +NPK had a higher average of
 725 plant height and no significant difference when compared against S100 +NPK. Sikora and
 726 Enriki (2001) stated that the combination of compost with a chemical fertiliser is considered
 727 as an “appealing alternative” to compensate for the fact of the unavailability of nutrients of
 728 compost compared to chemical fertilisers [49]. This coincides with what was found on the
 729 growth trial where, although no significant difference was found, SC 9010 +NPK had taller
 730 plants on average than the same soil type without fertiliser addition. It is also hypothesised
 731 that in a longer trial, statistical difference could be found between these two soil types.

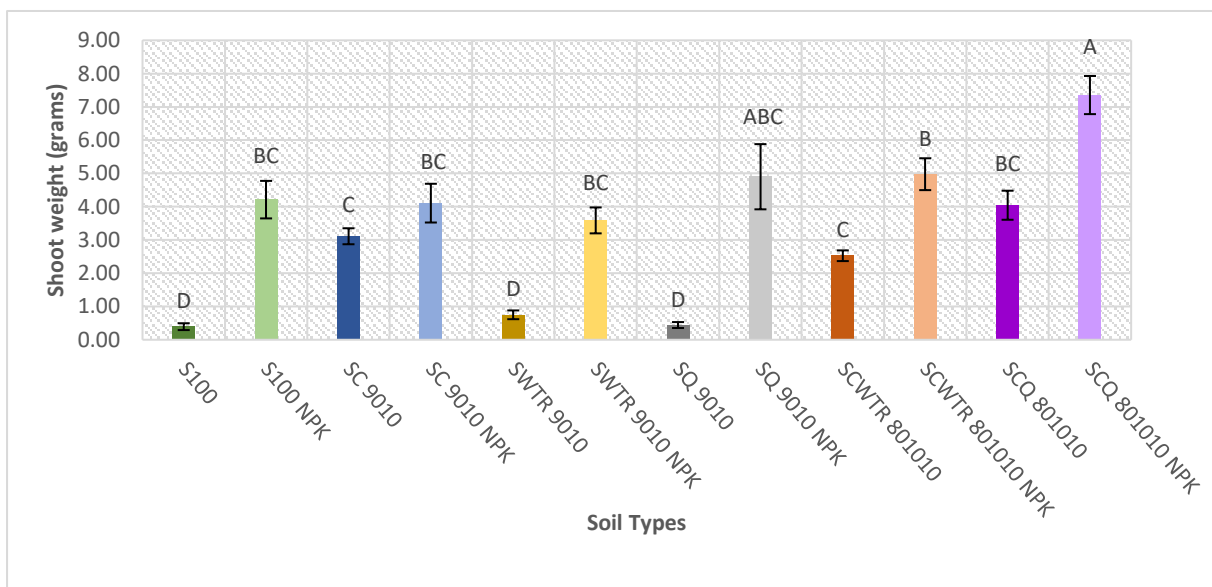
732 SWTR 9010, 47.75 ± 6.33 cm, was significantly higher than S100, 31.32 ± 7.06 cm, at the end
 733 of the trial, whereas SQ 9010, 31.8 ± 12.14 cm, was not significantly higher than S100. This
 734 statistical analysis of comparison between WTR and quartzite, could indicate that the addition
 735 of WTR to the soil goes further than just a physical contribution, and might be a chemical one.
 736 However, since as previously stated (section 2.1.4), quartzite PSD did not fully imitate that of
 737 WTR, the physical characteristics of WTR cannot be completely ruled out by these results.

738 However, no significant difference was found between SCWTR 801010 and SCQ 801010,
 739 neither with the same co-amendments and NPK addition. This can also be observed on shoot

740 biomass weight for the non-NPK co-amendments (Fig. 9-a), however when comparing
 741 SCWTR +NPK against SCQ +NPK on shoot biomass, significant difference was found
 742 SCQ +NPK having an average weight of 7.36 ± 1.4 grams, and SCWTR +NPK 4.98 ± 1.17
 743 grams. This meaning that even when in height they were similar, on shoot biomass weight a
 744 difference was found. Furthermore, the same difference was found on root weight, where SCQ
 745 +NPK had an average root weight of 6.8 ± 0.63 grams, and for SCWTR +NPK it was of
 746 4.87 ± 2.07 grams (Fig. 9-b). Meaning that on plant health, SCQ provided a better soil
 747 combination for maize growth than the one SCWTR did, with nutrient addition. Further
 748 research on the effects of quartzite with NPK addition could be used to explain the effect the
 749 mineral has on sandy soil.

750 It is also important to note that the number of plants on SQ9010 +NPK significantly decreased
 751 during the trial with four of them dying during the first couple of weeks and one more drying
 752 out by week 4. By the end of the trial, the soil type had only 2 plants, however these two plants
 753 presented high levels of height and biomass.

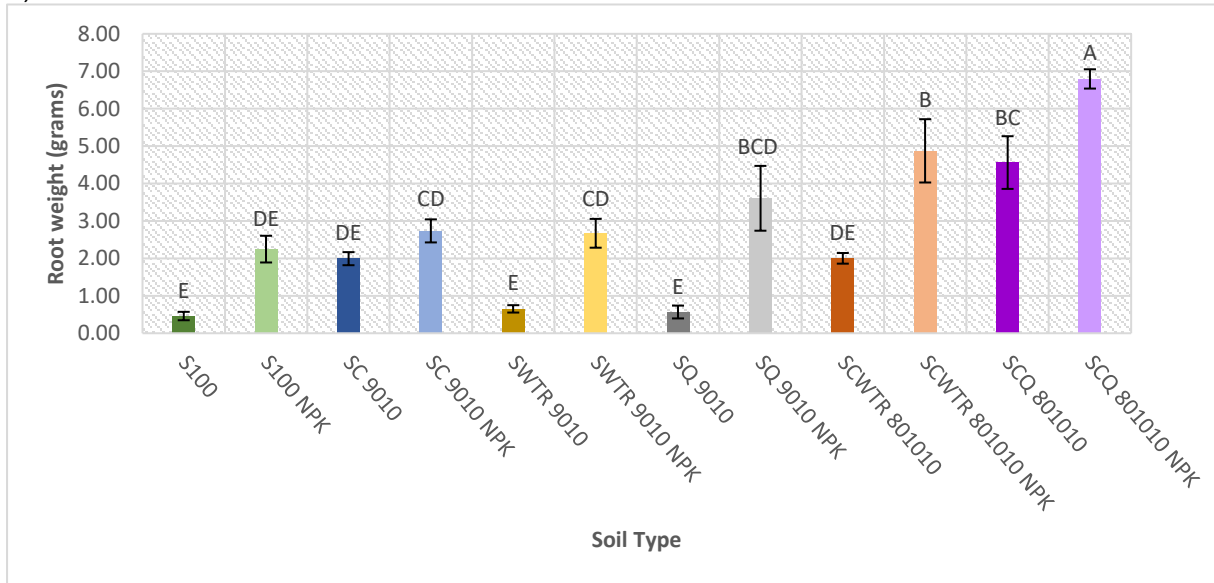
754 a)



755

756

757 b)



758

759 *Figure 9. Week 7 measurements of shoot (a) and root (b) biomass weights of all soil types in grams of*
 760 *dry biomass. Labels show statistical difference ($p \leq 0.05$), bars that do not share a letter are significantly*
 761 *different. Error bars show standard error of the measurements.*

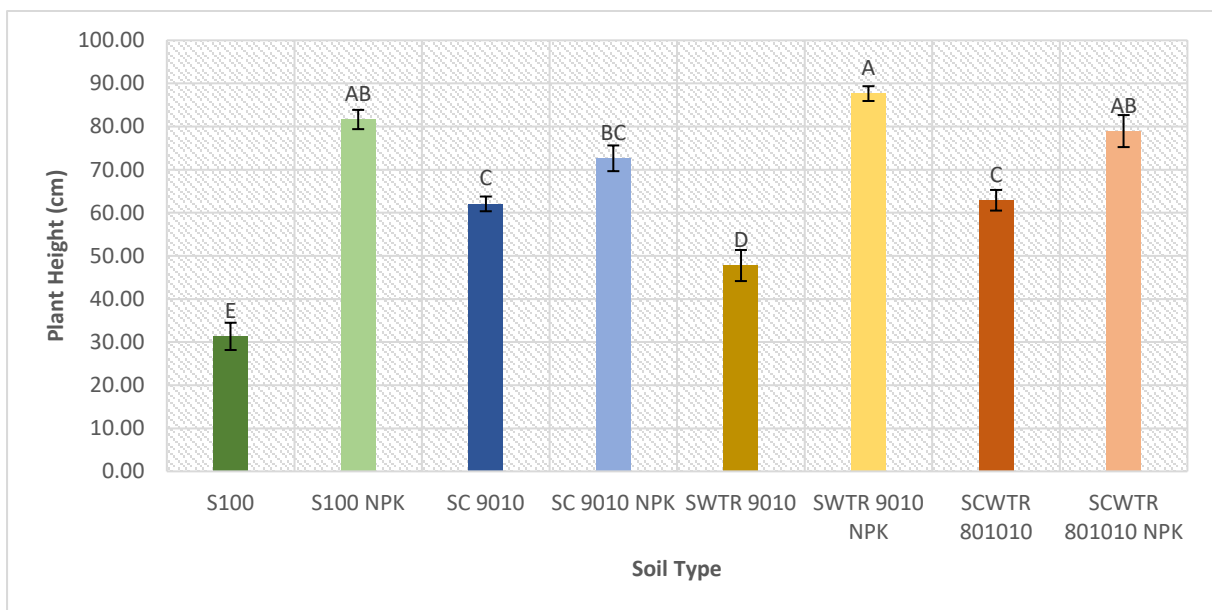
762 Dry biomass measurements followed a similar trend to that followed by plant height, soil was
 763 statistically lower in both shoot and root biomass than the organic amendments. It is important
 764 to note that in soils where $pH < 5.5$, root elongation is inhibited [37] but the $pH > 5.5$ in all
 765 cases due to lime addition to the raw Zimbabwean soil at soil type preparation stage. Root
 766 and shoot weights on S100 +NPK and SC 9010 had no significant difference, however it is
 767 important to note that the correlation (r) of plant height and shoot weight of S100 +NPK was
 768 $r = -0.02$, similarly, the relation between plant height and root mass was $r = -0.5$. Whereas for
 769 SC 9010, for plant height and shoot weight $r = 0.35$, for plant height and root weight $r = 0.31$.
 770 Meaning that, although S100 +NPK had significantly taller plants, they were not as strong-
 771 looking as the ones growing on SC9010 which had a consistent co-relation between height
 772 and weight of shoot.

773 *Table 6. Average shoot and root biomass (grams) of all amendments. Note that $n=2$ for SQ 9010 NPK*
 774 *soil type.*

n	SOIL TYPE	SHOOT BIOMASS	ROOT BIOMASS
5	Soil 100	0.39	0.46
6	Soil 100 NPK	4.21	2.24
7	SC 9010	3.11	1.99
6	SC 9010 NPK	4.10	2.73
7	SWTR 9010	0.75	0.65
5	SWTR 9010 NPK	3.58	2.67
5	SQ 9010	0.44	0.56
2	SQ 9010 NPK	4.90	3.60
7	SCWTR 801010	2.53	2.00
6	SCWTR 801010 NPK	4.98	4.87
6	SCQ 801010	4.04	4.56
6	SCQ 801010 NPK	7.36	6.80

775 No significant difference was found on plant height or shoot biomass when comparing SCWTR
 776 +NPK against S +NPK, SC +NPK and SWTR +NPK. However, when comparing root biomass,
 777 SCWTR +NPK had a significant heavier root system than the rest of the amendments, perhaps
 778 this can be explained by drainage of water due to the physical contribution of WTR to the soil
 779 in combination with compost.

780 Further statistical analysis was performed to compare soil types without taking into
 781 consideration quartzite soil types. This with the purpose of narrowing down to the hypothesis
 782 of organic matter and inorganic mineral amendments addition to the soil having an effect when
 783 compared against base soil. This analysis showed a significant difference on plant height
 784 when comparing the single amendments of compost and WTR against the base soil where
 785 SC 9010 provided the highest average, SWTR 9010 following and S100 with the smallest
 786 average of height (Fig. 10), further significant difference was found when comparing S100
 787 against SC 9010 on shoot biomass, but no difference comparing S100 against SWTR 9010
 788 (Fig. 11) (Table 6). In addition, on root biomass, no significant difference was found in any of
 789 the soil types.

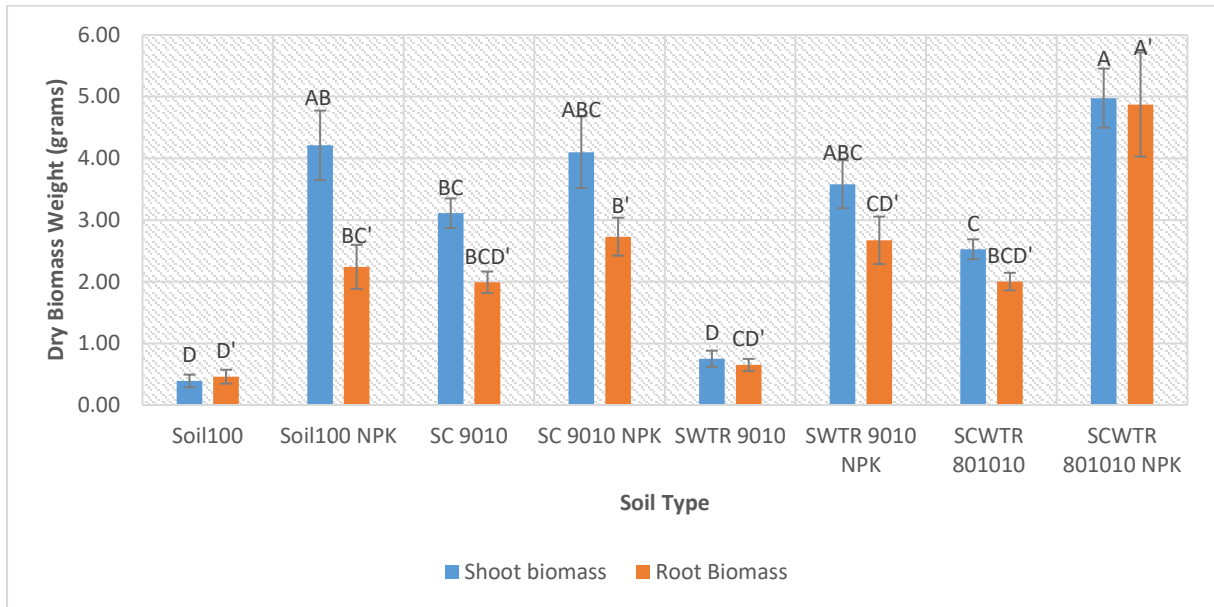


790

791 *Figure 10. Plant height measurements in centimetres recorded on week 7. Labels show significant differences, bars that do*
 792 *not share a letter are significantly different. Error bars show standard error.*

793 In 2017, Mtangadura et al. experimented with high and low quality organic resources in
 794 Zimbabwean soil and found that their results suggested an increase on soil organic carbon in
 795 the medium to long term (5-9 years) [6]. Therefore, it is hypothesized that due to the small
 796 period of time in which the plant trial was done, and the fact that plant growth does not seem
 797 to be slowing down (Fig. 4), longer time is needed to assess the differences between the
 798 addition of NPK fertiliser to the soil and the addition of an organic material for it to be able to
 799 conclude which one might be more beneficial for Zimbabwean soil. This is further backed up

800 by the fact that no significant difference was found on either shoot or root biomass between
 801 S100 +NPK and SC 9010 (Fig. 11).



802

803 *Figure 11. Biomass (shoot and root) dry weights recorded on grams. Labels show significant difference*
 804 *between bars. Bars that do not share a letter are significantly different. Different ANOVA tests were*
 805 *performed, one for shoot biomass ($p < 0.05$) and another for root biomass ($p < 0.05$).*

806 SWTR 9010 was significantly higher than Soil 100 by the end of the trial. In 1980, Rengasamy
 807 et al. found that the addition of WTR to a pot trial of maize increased yield of dry matter of the
 808 crop in pots grown with and without a fertiliser addition. However, in this trial, it did not increase
 809 shoot biomass significantly (Fig. 11) [50]. Furthermore, on the same experiment it was also
 810 found that the P uptake of plants was reduced at the high rate of 10 g of WTR per kg of soil.

811 According to literature, the optimal rate of WTR addition to improve corn yields is of 0.1 to 10
 812 g of WTR per kg of soil [34]. However, the addition to the single amendment SWTR 9010 was
 813 of 300 g of WTR per kg of soil (approximately). Thus, it can be hypothesized that the addition
 814 of WTR was too high and, therefore, plants grew in an unsuitable environment. However,
 815 when fertiliser was added to the single amendment, P limitations were 'compensated' and
 816 available for plant uptake.

817 Although not significantly different, the co-amendment with NPK addition (SCWTR 801010
 818 +NPK) provided with more shoot biomass. In addition, significantly higher root biomass was
 819 found in the same co-amendment (SCWTR 801010) than in the rest of the soil types which is
 820 showed on figure 10. This could be an indication of the good development of the root system
 821 provided by the co-addition of compost and WTR to a sandy soil. The hypothesis being that
 822 the addition of WTR could be improving soil properties like soil structure and water holding
 823 capacity [34]. Furthermore, in the co-amendment the rate of WTR application lowered to 192

824 grams of WTR per kg of soil, meaning that the impact of WTR on P was different than the one
825 in the single amendment.

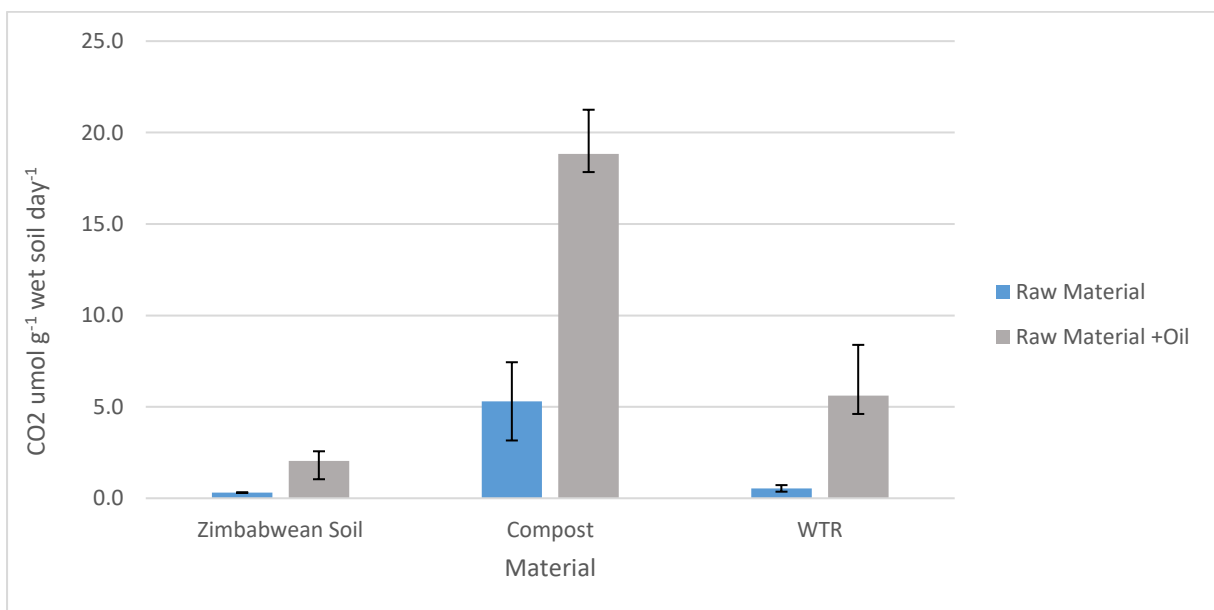
826 Plant development as well as shoot and root biomass might suggest that the co-amendment
827 compost and WTR could be improving soil properties, as suggested by the literature, and the
828 addition of a fertiliser with targeted nutrients for maize growth might increase crop fertility of
829 Zimbabwean sandy soil.

830

831 3.3 CO₂ production as a proxy for hydrocarbon biodegradation

832 Microbiological parameters measurements, such as soil respiration, provides
833 information on the presence and activity of microorganisms as well as on the intensity,
834 duration, and type of the effects of hydrocarbon pollution. Therefore, CO₂ emissions serve as
835 a good index of the impact of soil pollution [38].

836 During the preparation of the soil types for the plant growth trial, as previously described in
837 the methodology section, lime was added to the soils to raise the pH. The application of lime
838 to soils has been shown to enhance soil carbon loss by increasing C solubility, microbial
839 activity, and thus the rates of C decomposition [39]. Because all soils had lime in them, the
840 CO₂ emissions represented in the graphs, might show C losses or increased microbial activity
841 when comparing against the raw material Zimbabwean Soil, for this 'raw material' represents
842 soil as it was imported from Africa, with no lime addition.



843

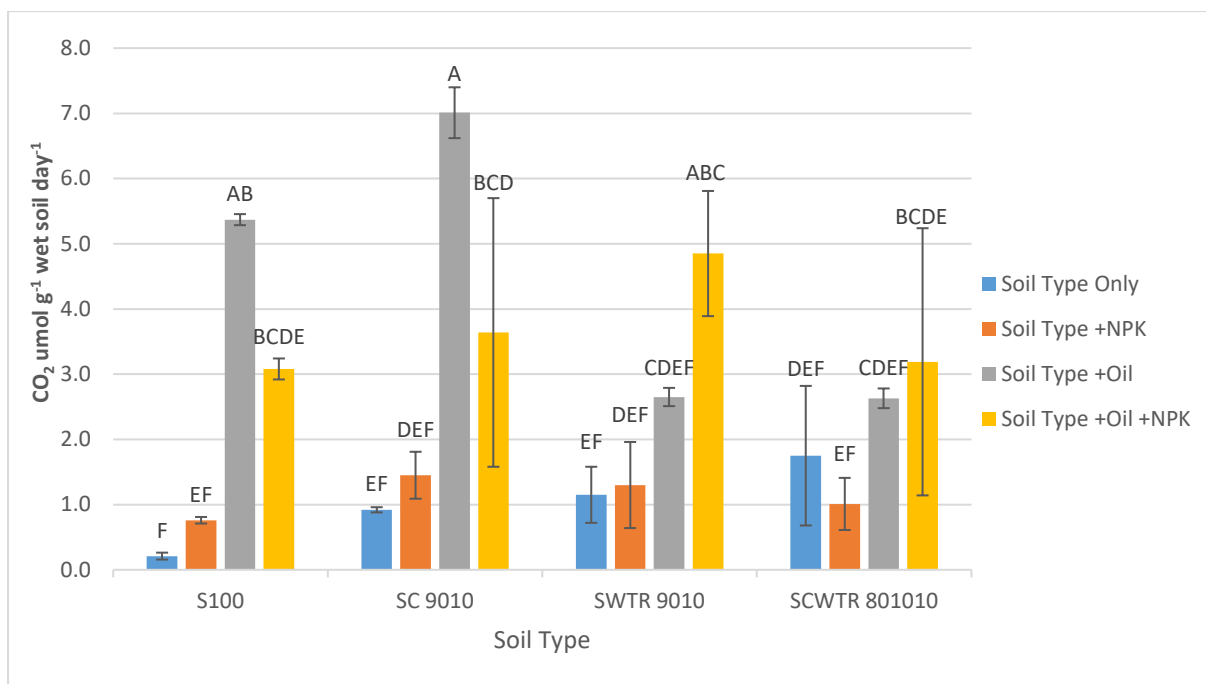
844 *Figure 12. Rate of raw materials CO₂ emissions. Error bars show standard error. Important to note that*
845 *these materials categorized as raw still have had a nutrient addition during the set-up of the*
846 *experiments.*

847 Statistical difference ($P < 0.05$) was found between oil-contaminated and uncontaminated raw
848 material in compost and WTR, meaning hydrocarbon mineralization was occurring on those
849 raw materials. However, no significant difference was found between Zimbabwean soil with
850 and without oil added to it. Even though WTR and compost are both mineralising
851 hydrocarbons, compost is significantly better than WTR, meaning that out of the three raw
852 materials used, compost provided the most CO_2 emissions for both uncontaminated and oil-
853 contaminated materials.

854 It has been found that compost provides a high diversity of microorganisms and that those
855 degrading hydrocarbons can be found amongst them [40]. Furthermore, the organic matter
856 contained in compost influences sorption/desorption processes of hydrophobic organic
857 contaminants [41].

858 WTR CO_2 emissions compared to Zimbabwean soil emissions did not present a significant
859 difference, however, on contaminated materials the difference was significant. An increase in
860 CO_2 emissions on contaminated raw materials compared against uncontaminated raw
861 materials can be seen (Fig. 12), this rate can also represent microbial activity. Hydrocarbon
862 contaminants serve as organic carbon sources for microorganisms, thus increasing microbial
863 activity, which can also explain why contaminated rates are higher than uncontaminated rates
864 [38].

865 Although organic amendments have been found to enhance soil respiration rates on different
866 soils, no significant differences were found when comparing basal respiration of S100 against
867 the rest of the soil types (Figure 13) [27]. The low levels of Zimbabwean soil CO_2 emissions
868 could be a consequence of its low organic matter content for it has been suggested that soils
869 with a higher organic matter and clay content are less affected by hydrocarbon contamination
870 [38].



871

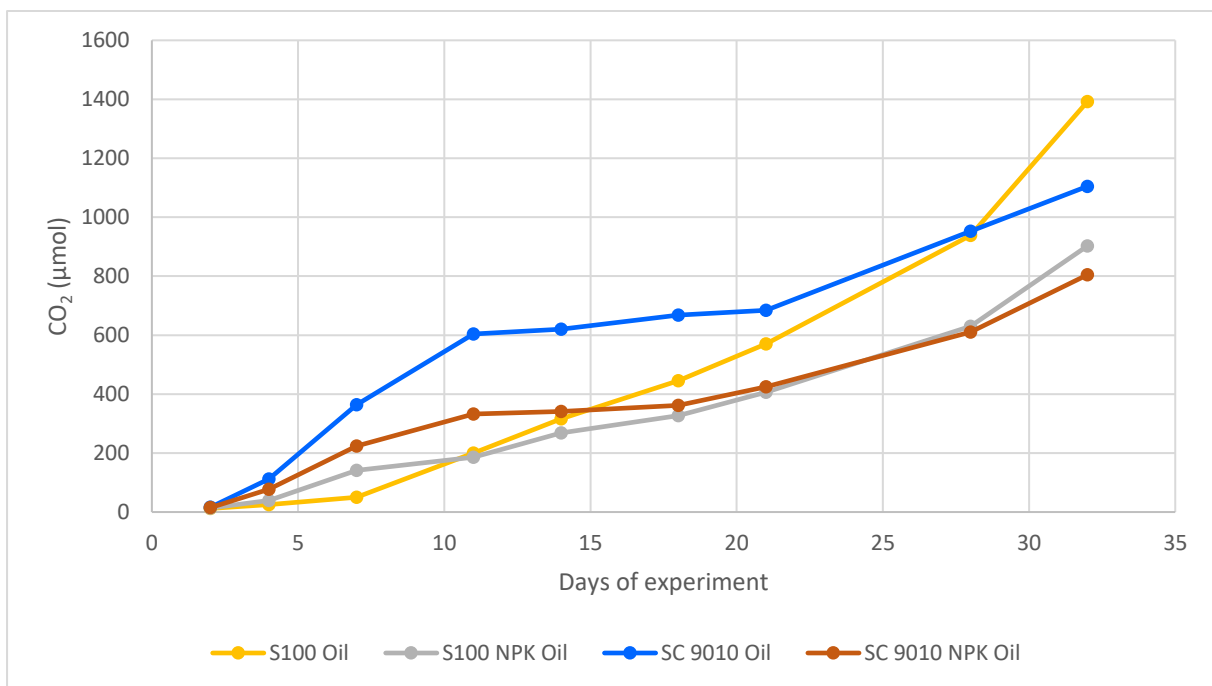
872 *Figure 13. Rate of CO₂ emissions on all soil types. Labels show significant difference, bars that do*
 873 *not share a letter are significantly different. Error bars show standard error. Soil types that include*
 874 *“+NPK” refer to the NPK added during the plant growth trial.*

875 Out of the four soil types assessed, S100 and SC 9010 showed significant difference in basal
 876 respiration with the addition of oil, whereas SWTR 9010 and SCWTR 801010 did not show a
 877 significant difference.

878 From the results obtained of the 32-day microcosms set up, no significant difference was found
 879 between non-NPK amended soil types and NPK amended ones in all but one soil type, this
 880 being SC9010. Fertiliser addition to soil type SC 9010 produced less CO₂ emissions. The lack
 881 of effectiveness of nutrient addition to the rates of soil types could be explained by the fact
 882 that soil types catalogued as “+NPK” received fertiliser during the growth trial and a second
 883 addition was made before starting the microcosm experiments. Plants grown in SC9010+NPK
 884 had no statistically significant difference in either shoot or root biomass at 7 weeks and so we
 885 can assume that root exudates were not different in either soil type. Therefore we might
 886 hypothesise that an ‘overdose’ of nutrients could explain an inhibitory effect on hydrocarbon
 887 biodegradation and that by extension possibly NPK has a deleterious effect on the ability of
 888 the soil microbiome to degrade hydrocarbons. Furthermore, it has been suggested that
 889 organic and inorganic fertilisers are needed to maintain chemical, physical and biological
 890 characteristics of soil quality in order to achieve a high removal of hydrocarbon compounds
 891 [27]. However, studies have shown that fertiliser amendments produce no increase in
 892 biodegradation rates, and this can be attributed to the complex composition of soils and other
 893 factors [42].

894 According to Fig. 14, cumulative CO₂ showed that soil types with oil contamination were not
 895 entering into a lag phase by the end of the experiments. It also showed no significant difference
 896 when comparing polluted soil types S100 +NPK and SC9010, however SC9010's rate is
 897 higher than S100 +NPK (although not significantly), a longer experiment could provide enough
 898 time for compost to have a greater effect on soil microorganisms and a greater rate of CO₂
 899 emissions. Rolling et al. (2004), found that polluted soils treated with compost amendments
 900 can produce higher bacteria community structures which leads to higher degradation rates
 901 when compared to liquid nutrient sources, and that the addition of compost increases microbial
 902 diversity and has been found to take less time during bioremediation than a fertile, productive
 903 soil [40].

904 Soil respiration was significantly increased by hydrocarbon pollution, therefore the effect seen
 905 in the rate of S100 +NPK might be that of the hydrocarbon introduction to the soil
 906 microorganisms rather than the biodegradation of said hydrocarbon. In 2006, Labud et al.
 907 reported that the contaminants used increased soil respiration in a clayey and sandy soil but
 908 particularly in the latter.



909

910 *Figure 14. Cumulative CO₂ for selected soil types throughout the 32-day experiments.*

911 As it is observed on figure 14, soil types suffered a lag phase during the first measurements,
 912 this is consistent with what was found by Labud et al., 2006. They found an initial lag phase
 913 in soil respiration in polluted sandy soils and suggested that this could be due to a period of
 914 adaptation needed by microorganisms.

915 Among many different physical, chemical, and biological factors that can affect microbial
916 community composition and activities, temperature is listed as one [21][39]. The microcosm
917 bottles were not stored under controlled conditions which means they were set at room
918 temperature, on wintertime through the duration of the experiment. As a result, it is
919 hypothesized that further statistical differences could be found in a longer experiment.

920

921

922 **4. Conclusions**

923 Zimbabwean soil suffers from poor structure, as expressed by many studies [3][4][5][6].
924 It can be concluded that organic matter input and nutrient addition to this soil creates a
925 significant increase in plant development, which at the same time, helps improve soil
926 properties, as reflected on plant growth and microbial respiration.

927 The results show that compost amendment created a fit environment for plant growth and
928 proved to be the best material to promote microbial activity in the soil. However, short-term
929 experiments can only provide with enough data to set up a long-term experiment, and it is for
930 that reason that it is concluded in this work that compost amendment (with NPK making no
931 statistically significant improvement) and compost-WTR co-amendments both provided
932 evidence of soil improvement in terms of plant biomass (with NPK making a statistically
933 significant improvement) and no signs of these improvement to be slowing down. It is believed
934 that in longer experiments these organic and inorganic amendments would show significant
935 differences when compared against fertiliser addition with the last one having a less beneficial
936 effect on soil health (plant development) and hydrocarbon breakdown.

937 Further analyses to microbial communities are also needed in order to explain hydrocarbon
938 breakdown activity and how each material changed microbial communities and their activities
939 in each soil type.

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