Analysis of the effects of organic matter using plant growth and microbial activity as soil health indicators

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Analysis of the effects of organic matter using plant growth and microbial activity as soil health indicators – Sachary Victoria Luna Mandujan.

Abstract

Soil organic matter (SOM) contributes to the stabilisation of soil structure by aggregating mineral particles together. SOM also provides nutrients for plant growth and carbon for microorganisms. To identify what the effects of SOM on soil function such as plant growth and microbial diversity and function are, three different waste materials, compost, water treatment residual (WTR) and anaerobic digestate (AD), were mixed in various combinations with agricultural soil to make several 'soil types'. Maize was grown for 7 weeks in these soil types. The first hypothesis was that organic matter/inorganic mineral co-amendments can improve soil health and thereby its capacity to remove pollutants such as hydrocarbons. The second hypothesis was that organic matter has important biological and physical roles in improving soil structure as well as the already well understood chemical role of providing nutrients.

Analysis of biomass shoot measurements showed that without NPK AD only and AD/WTR soil types provided the best medium for plant growth giving 52.32% increased shoot biomass with AD only soil type compared to soil alone, and 47.59% with AD/WTR soil type compared to soil alone. NPK addition to AD/WTR soil type provided the best medium for plant growth with statistically significantly increased biomass shoot compared to all soil types with NPK. In oil contaminated soils, the effect of residual NPK in soil was to reduce CO_2 concentration rates used as a proxy for oil biodegradation, and this was statistically significant for AD as a single amendment.

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Dedication

I would like to dedicate this work to my mum Lupita, my dad Rafael, and my sisters Anayanci and Valeria. Thank you for always making me feel your love and support even from far away.

1 Introduction and aims

1.1 Introduction

The UN have stated that soil health underpins all 17 Sustainable Development Goals (SDGs) launched in 2015. Soil health (SDG15) is essential for food security (SDG3), climate change mitigation and adaptation (SDG13) including resilience to climate events [1]. One of the main problems to overcome in regards of soil health is poor soil structure which leads to soil erosion [2] and resultant loss in soil organic matter (SOM). Increasing levels of SOM is considered an effective way to improve soil health and using organic waste materials is a sustainable way to achieve this whilst simultaneously addressing SDG12, responsible use of resources.

The UK Government's 'Renewable Heat Incentive' scheme encourages waste organic matter to be anaerobically digested for biogas production [3]. However, as these practices increase, waste material from anaerobic digestion will increase as well. This thesis explored the opportunity to use the residual product from anaerobic digestion as a soil improvement technology both with and without inorganic minerals [3].

1.2 Aims

It is well-known that the study of physical and chemical characteristics of soil is necessary to measure soil quality, to keep its functionality to develop anthropogenic activities; however, biological characteristics, although less well researched have great importance too. In fact, a recent study demonstrates that physical characteristics of soil can be improved by changing its biology using soil amendments with high organic matter content [4]. The aim of this research was to consider the biological and physical role organic matter additions play in soil health whilst trying to keep the chemical (nutrient availability) factors the same. This analysis is inherently difficult since chemical, physical and biological parameters are all interrelated. Soil provides a source of nutrients, water and oxygen dynamics, and physical support to plants [5]. Therefore, plant growth was studied since the capacity of soil to grow plants is an indicator of its overall health [6]. Additionally, the capacity of the soil to remove pollutants through the soil microbial basal respiration activity [7] is another facet of soil health [1]. Basal respiration of the soil microbial community was analysed as a way of identifying any

changes to soil health relating to hydrocarbon contamination, as these communities are sensible to changes in the environment [8] [9] [10].

2 Soil health

As well as the interrelated nature of biological, physical and chemical properties of soil it is also important to point out the intrarelationships within soil biology, such as the relationships between the microbial community and plants in soil, which also encompass physical and chemical processes. For instance, microbes and fungi have a symbiotic relationship with plants, exchanging nutrients which they can extract from minerals [11] for carbon with plants through their roots [5]. The root system develops dependent on chemical availability of nutrients as well as the physical structure of the soil [12]. The microbial community around the root systems of plants is called the rhizosphere and has an important role to play in this exchange process which involves inorganic minerals, organic matter and microbial communities [13]. Consequently, it is important to consider that all soil biology processes are dependent on the physical and chemical environment in which soil is operating.

The term soil health integrates the biological processes occurring in soil, in addition to the physical and chemical processes [1]. Soil health is defined as the 'capacity of soil to function as a vital living system to sustain biological productivity, maintain environment quality and promote plant, animal and human health' [14]. The determination of soil health, can be achieved through the analyses of some soil health indicators such as pH which indicates the acidity and alkalinity of soil, the capacity of soil to keep nutrients measured by cation exchange capacity (CEC), the arrangement between soil particles measured by aggregate stability, water infiltration rate and soil organic carbon (SOC) [14]. Arguably the most important component of soil health is the SOC which is a component of SOM; however, as previously mentioned, it must be noted that physical, chemical and biological properties of soil are all interrelated, thus, any changes in one may also have an impact on the rest of them.

2.1 Using organic and mineral materials as soil co-amendments to improve soil health

External factors, such as management, can disturb soil health either in a positive or negative way. Land use and practices such as tillage often negatively impact on SOM by exposing previously physically trapped SOC which can then be microbially respired. The resultant reduction in aggregate stability negatively affects the soil structure [15] [16]. Even so, the fact that soil management practises such as tillage can also be helpful due to the stimulation of bacterial respiration must be acknowledged [17]. Ultimately SOM levels however must be maintained or enhanced if soil health is to be maintained.

Aggregate stability of soil can be increased mainly through the cationic bridges between organic matter and minerals [18]. The binding of such materials can be caused by hydrophobic sections within organic matter and the mineral surface [12]. It has been suggested that amendments rich in minerals have positive influence in the organic matter content and nutrient concentrations in the long term, as well as in microbial activities involving carbon, nitrogen and phosphorous mineralization [19] [20].

A previous research using amendments and co-amendments of materials with high organic matter content such as compost and water treatment residual (WTR) demonstrated positive results for aggregate stability [4]. Compost is a product usually rich in nitrogen and phosphorus, obtained from a biological process of oxidation and the main component is organic matter [16]. WTR is the sludge material generated from clean water treatment processes, which is usually disposed of in landfill [21]. Depending on the coagulant used during the process, WTR can be iron or aluminium rich [22]. Waste products such as anaerobic digestate (AD) have revealed to influence carbon and nitrogen soil content in a positive way, in addition to the increase of microbial abundance [23]. AD is an organic material rich in nutrients [22], depending on the feedstock, along with the process used to obtain it, which can be chemical or biological [24]. During anaerobic digestion, organic and inorganic matter is decomposed in the absence of oxygen [25]. This process reduces odours and pathogens through fermentation [24], which is why anaerobic digestion has been used for decades as a stabilization method for potentially contaminant materials such as sewage sludge [25]. Besides containing nitrogen and phosphorus, AD from sewage sludge contains microorganisms, EPS (extracellular polymeric substances), colloids, mineral particles, and ionic components [24]. Due to its high nutrient content, once the sewage sludge has been stabilised, the solid residue is dried or dewatered and can be used for land application [25]. It has been suggested that AD has great benefits used as soil amendment [26] [27]. However, it is important to highlight the fact that the use of AD organic amendments and the biological and physical effects on soil health needs further investigation [17] [28].

The rationale behind this thesis was that adding organic matter and minerals together, components described in the following sections, might improve soil health and in doing so, potentially improve hydrocarbon degradation capability. Results were presented comparing both plant growth and hydrocarbon breakdown in a typical agricultural soil amended with combinations of AD, compost and WTR.

2.2 Soil organic matter properties

Although it represents 1 to 6% of the components of a typical soil [13], the organic substances and compounds, and chemical elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur in SOM have great influence on soil properties and functions. All soil functions are underpinned by SOM. SOC is generally around 50% of SOM. It is important to note that there is not just one type of SOC – it has recently been agreed that SOC can be split into 3 pools, one which is very easily respired by microorganisms (turnover weeks to a year), one which is less easily respired (turnover a few years) and one which is tightly bound to minerals (turnover tens to hundreds of years) [1]. The type of organic matter will relate to its capacity to help improve soil structure; however, this topic needs further research. In addition, the biological, chemical, and physical properties of organic matter may vary depending on whether the organic matter is the end product of aerobic (like compost) or anaerobic (like AD) processes [29].

2.2.1 Chemical properties

Organic matter has an important chemical role in soil which is balanced by inputs from plant roots and manures and outputs such as C decomposition to CO_2 through microbial activity [12]. The SOC found in SOM has a major effect on pore water chemistry by regulating many chemical exchange reactions [30]. Such processes occur due to the negative charge of SOM, which results in interactions with inorganic minerals which are often positively charged [13] resulting in soil aggregation [17]. Cation exchange capacity (CEC) can be defined as the sum of cations neutralising the negative charge per unit mass of soil. CEC has been broadly studied for years, as it examines the distribution of positively charged ions, which provides a better understanding of processes such as soil acidity [31], and the contribution to mineral and nutrient distribution and retention [17]. Importantly some macronutrients are available as positively charged forms such as K and N, therefore SOM has an important role in controlling availability of these important nutrients [17].

Organic matter is also important in controlling how easily organic contaminants such as hydrocarbons are remediated. For example, hydrocarbon spills onto organic rich soils may be more difficult to remove through pump and treat systems than spills onto sandy soils [32]. However, bioremediation of the spill through volatilisation and mineralisation may be easier in the organic rich soils than in the sandy soil as there is more organic matter available as a

food source for microbial respiration [7]. Accordingly, as a method to analyse the influence of SOM on soil, this thesis explored the potential improvement of soil bioremediation by increasing the food source (nutrients) through the SOM added.

2.2.2 Biological properties

Through the analysis of soil microbial communities, it has been found that, although communities also include Archaea, fungi and viruses [33], bacteria are the most abundant organisms in soil with an estimated number of 2.5×10^{29} cells of all biomes [33]. Research has also shown that the most common bacterial groups found in different types of soils are Alpha, Beta and Gamma groups of *Proteobacteria, Actinobacteria, Cytophagales, Acidobacteria, Planctomycetes and Verrucomicrobia* [34] through rRNA genes analysis. SOM affects soil properties through its important impact on microbial life. Microorganisms, for instance, have a regulating role in nutrient cycles. *Nitrosomonas* bacteria is an example of an ammonia oxidizing microorganism during the nitrogen cycle. In the interest of understanding microbial processes affecting soil properties, microbial community function has been generally studied using an approach of microbial activity, such as microbial respiration [35]. Through microbial respiration the breaking down of organic molecules and conversion to elements such as CO_2 occur [36]. Thus, microbial respiration can be measured by CO_2 emissions produced from decomposition of SOM [37]. Consequently, measurement of CO_2 was used in this research to compare amended and unamended soil and discuss the potential differences within microbial communities.

2.2.3 Physical properties

Bacterial in soil produce extracellular polysaccharides (EPS) and other by-products, which influence microbial community growth on soil through the protection that binding agents provide [17]. Consequently, as microorganisms bind particles together and create aggregates, it is known that soil physical properties are influenced by SOM. SOM role on soil structure was explored in this thesis through the comparison between amended and unamended soil effect on plant growth, which represent soil health, as it was previously mentioned. Soil structure is essentially the arrangement of the solid and the void space [13]. Pores represent the void space arranged in soil structure and play different roles in soil, depending on the

size. Macropores, formed by the space between the particles in soil, allow water and air to move through the soil and provide space for plant roots to grow and for some soil fauna to live in [13] [38]. Micropores hold bacteria, water, and organic compounds [13]; although, fluid is considered immobile within these pores because their size is too small, fluid is consumed by plant roots through suction [5]. Solid space arranged in soil structure is built by microaggregates, which are made of plant and microbial cells, and by-products as previously mentioned, such as polysaccharides [17], and by macroaggregates from plant roots and branched, tubular filaments in fungi known as fungal hyphae [32].

2.3 Inorganic mineral properties

Soil is largely made up of (up to 40% by volume) inorganic minerals such as clays, carbonates, iron oxides and aluminium oxides. Minerals play a key regulating role in controlling pore water chemistry where the pH and redox potential will determine which ones are dissolving and which ones are precipitating at any one time [13]. The mineral assemblage present in soil is dependent on the underlying geology. The main mineral source used in this project was WTR to analyse the effect and potential improvement to the previously mentioned regulating role in soil.

2.3.1 Chemical properties

Some nutrients are available to plants in negatively charged forms, hence P and N [16]. Consequently, the ability of soil to hold nutrients is determined by the amount and type of inorganic minerals such as iron and aluminium oxides and clays as well as the organic matter present in the soil [17].

The chemistry of the soil is also dependent on what contaminants are present and both inorganic contaminants, for example lead (Pb) or arsenic (As), can be potentially immobilised by minerals such as Fe oxides [39] [40]. Fe oxides and Mn oxides are also capable of transforming some organic contaminants like hydrocarbons into CO_2 and water [41] [42]. Accordingly, this project explored the effect of inorganic minerals through hydrocarbon degradation, in combination with organic matter.

2.3.2 Biological properties

Bacterial cells produce a sticky substance called mucilage, which attaches to clay particles and in doing so provides protection to bacteria from predators [17]. Minerals influence the soil microbial community as well, as it has been suggested that a greater ratio of clay particles prevents bacterial desiccation due to higher CEC and surface area [11]. Minerals also provide an important source of micronutrients for microorganisms as well as potential attachment surfaces for their growth. These biological effects of minerals in soil were analysed through this thesis by comparing amended and unamended soil performance of plant growth and hydrocarbon degradation.

2.3.3 Physical properties

The texture of the soil (otherwise known as the particle size distribution) is an important factor in governing the macro and micro pore distribution within a soil. Texture is determined by the mineral composition since some minerals are more easily weathered than others, and some minerals (such as clays) can shrink and swell dependent on water content [43]. Texture therefore determines how a soil transmits and holds water, and therefore, what its redox potential will be, which is clearly then related to what microbial processes can take place, aerobic or anaerobic [43]. This thesis analysed the effects of minerals on soil by changing the mineral composition through the addition of inorganic minerals and organic matter and comparing amended and unamended soil.

3 Materials and methods

3.1 Characterisation of materials

The characterisation of all the materials used for the trial was performed by the laboratories in the Geography Department at Durham University. Analysis of all materials included total carbon and total nitrogen by combustion method using Flash 2000 Organic Elemental analyser in five repetitions as quality control, furnace temperature of 950°C, oven temperature of 50°C, helium flow 130ml/min and oxygen flow 250ml/min. Effective cation exchange capacity (ECEC) in meq/100g was performed as well, by calculation of exchangeable K^+ + exchangeable Ca^{2+} + exchangeable Mg^{2+} + exchangeable acidity, and Al^{3+} saturation (%) = (exchangeable Al^{3+}/ Effective CEC)*100. Using pH meter Hanna H18424 pH from materials was obtained firstly through 1:2.5 $CaCl_2$ extraction, where 50ml 0.01M $CaCl_2$ solution were added to 20.00g of 'as received' material, stirred and left to settle for one hour, then stirred again immediately before measurement. Secondly, 1:2.5 deionised water extraction was performed where 50ml deionised water (18.2M) were added to 20.00g of 'as received' material, stirred and left to settle for one hour, then stirred again immediately before measurement. Bicarbonate extractable (plant available) phosphorus by ICP-OES in mg/kg was obtained as well, using Agilent 5100 ICP-OES with wavelength of 178nm where 2.5g of 'as received' sample were tested adding 50ml of 0.5M $NaHCO_3$ (pH 8.5), shaken for 30 minutes, and filtered through Whatman 42 filter paper. The information obtained from each material through the previously described methods performed by the Geography Department and Durham University is detailed in the following sections.

3.1.1 Soil

Approximately 85kg of agricultural soil was collected from Nafferton Farm (+54.9857, -1.899) in June of 2019, with pH of 5.9, total carbon 2.67% w/w, total nitrogen 0.25% w/w, extractable phosphorus 5mg/kg, and ECEC 13.9 meq/100g when analysed. As this site was used for previous experimental trials by Kerr [4], all large stones and non-organic material had been removed by the time the soil was utilised for this trial. The soil was stored in sealed plastic bags keeping field moist under ambient conditions until use.

3.1.2 Compost

Two 50L bags of Westland Horticulture Gro-Sure All-Purpose compost, with a medium level of peat were used, and stored indoors at 20°C, until use. The pH was 4.9, total carbon 46.90% w/w, total nitrogen 1.28% w/w, extractable phosphorus 261mg/kg, and ECEC 84.3 meq/100g when analysed.

3.1.3 Water treatment residual (WTR)

This trial used iron rich WTR which was provided by Northumbrian Water's Mosswood Water Treatment Works in County Durham. As it was previously used for amended soil experimental trials [4], it had already been air-dried, broken down by hand and sieved to <2mm. The pH was 4.2, total carbon 19.98% w/w, total nitrogen 0.81% w/w, extractable phosphorus 1mg/kg, and ECEC 13.4 meq/100g when analysed and it was kept in plastic containers indoors at 20°C.

3.1.4 Anaerobic digestate (AD)

Anaerobic digestate, sourced by Northumbrian Water Limited, was produced via a thermal hydrolysis process (THP) by treating the raw sludge with steam at 6 bar pressure and 165°C for 30 minutes to remove pathogens. During this process, once the sludge has been passed to a flash tank and its temperature has been reduced, it is passed to an anaerobic digester with an average retention time of 19 days. The digested sludge is then centrifuged as the final step. The material had pH 6.2, total carbon 33.48% w/w, total nitrogen 5.06% w/w, extractable phosphorus 289mg/kg, and ECEC 85.7 meq/100g when analysed. It remained in plastic containers and covered with plastic bags, as it was stored outdoors.

3.2 Preparation of soil and soil mixtures for use in the plant trial

The various amendment materials were added to the Nafferton farm soil as laid out in Table 1; each combination is referred from here onwards as 'soil type'. Five different soil types were tested, including unamended soil as control, prepared by calculating ratios based on dry mass but in reality, mixed using field moist materials in most cases. To obtain such ratios, water content was calculated for each material according to BS1377 (1990), by oven-drying a

small sample at 105°C for 24 hours. The mixtures were then prepared, considering the preparation of ~20 pots (9 x 9 x 9.5 cm) per soil type, by pouring the calculated material in trays and mixing by hand. Later, they were left to be wetted and air-dried constantly for four weeks to settle, to achieve homogenisation of the soil [44] [45]. The larger clumps of soil were broken gently by hand to avoid breaking natural soil aggregates. After this time, a layer of frost cloth was placed in the bottom of each pot to allow water infiltration as well as prevent soil loss through the drainage holes. The pots were all filled to the same volume, ~ 2 cm below top of the pot, and gently compacted by pressing by hand.

Table 1. Combinations of materials prepared by percentage.

Soil type	Soil (%)	Compost (%)	AD (%)	WTR (%)
S100	100			
SC9010	90	10		
SAD9010	90		10	
SADWTR801010	80		10	10
SCWTR801010	80	10		10

3.3 Nutrients

Fertiliser (Miracle-Gro All Purpose Concentrated Liquid Plant Food) was added at the beginning of the trial (time zero) with the aim of removing NPK as a rate-limiting variable for those NPK amended soil types. This was important since each soil type contains different materials which have differing nutrient contents. The concentrated liquid plant food contained nitrogen (N) total 6.0%, phosphorus pentoxide (P_2O_5) soluble in water 3.0%, potassium oxide (K_2O) soluble in water 6.0% and less than 0.05% of nutrients such as copper and manganese. Fertiliser was diluted in tap water, following manufacturers' indications. According to crop's requirements, 50ml of fertiliser were diluted in 8L of tap water, and a full dose of the preparation (100ml) was poured into pots without organic matter amendment, and a half dose (50ml) into organic matter amended pots, as organic matter already provides high nutrient content.

3.4 Plant growth trial

Maize seeds were incubated for three days at 30°C to ensure the germination of all the seeds by the time of planting. Once the seeds had germinated, they were selected randomly, and one shoot was planted per pot. Following the planting, liquid fertiliser was added to one set of seven replicates per soil type, and one set of seven replicates was prepared without fertiliser addition. A total of 70 pots with one plant each were placed in trays in a greenhouse receiving 16 hours of light, 8 hours of darkness, 30°C over day and 18°C over night for seven weeks. The watering regime entailed watering each pot individually during the first week from the top. After this time and during the rest of the trial, the roots had grown enough to take up water from the bottom of the trays, so it was poured into the containers to prevent nutrient wash out.

3.4.1 Plant height and weight

A week after planting, measurements from top of the soil to the tallest leaf of each plant were recorded twice a week using a 30cm±0.01 cm ruler. At the end of the trial all the plants were harvested and above and below biomass were separated and stored in paper bags to weigh them. To remove soil particles stuck on below biomass, as was the case in soil types with WTR, roots were gently washed with tap water. According to the dry weight method, all biomass was oven dried to 65°C for 48 hours. After this time, weight was recorded using an analytical balance with 0.1mg of weighing accuracy. The process was repeated until constant weight was achieved, and the final weight recorded.

3.5 Oil biodegradation experiments

The determination of oil biodegradation rates was achieved following the guidelines at the School of Natural and Environmental Sciences in Newcastle University. This analysis was performed on soil types at the end of the plant growth trial. Microcosms were prepared in serum bottles filled with 10g of each soil type sample (n=3) for aerobic biodegradation experiments. Gas chromatography–mass spectrometry (GC-MS) was achieved using Fisons Trio 1000 fitted with Pora Plot Q GC column. To determine the rates of oil biodegradation and compare CO_2 production, two sets of each soil type were arranged, one with the addition of crude oil and one without, and sodium nitrate 2% ($NaNO_3$) and potassium dihydrogen

phosphate 0.1% (KH_2PO_4) were added to all microcosms to prevent the decline of microbial activity through the experiments. This meant that all soil types had N and P added – this was to ensure that NPK was not rate limiting for oil biodegradation or basal respiration without oil. In the results section the difference between NPK amended soil types and non-NPK amended soil types is referred to whether or not plants had been grown with NPK added.

The bottles were sealed with butyl rubber stopper. For 32 days, CO_2 was monitored on the headspace of the microcosms, where injections using a 100µl push-lock syringe were sequentially introduced in an established GC-MS run of 180 minutes. Calibrations with 1 and 10% CO_2 gas standard were made at the beginning of the run and between every 30 samples. The data was obtained from m/z 44 and 32 mass spectra corresponding to CO_2 and O_2. Concentration rates were calculated in µmole CO_2 g-1 wet soil day-1 from the initial linear phase of CO_2 accumulation with time, after the lag phase.

3.6 Statistical analyses

The analysis of height and weight of the plants, as well as the biodegradation rates of CO_2 emissions were performed using statistics packages Minitab 18 and SPSS 22 based on the accessibility of each software depending on the analysis. To determine statistically significant difference between plant growth considering height and weight of all soil types at the end point of the trial, treatments were analysed using one-way ANOVA and Tukey test as post-hoc analysis through Minitab 18 using p-value < 0.05. To determine statistically significant difference between biodegradation rates of CO_2 results analysed at the same time following the plant growth trial, one-way ANOVA and LSD post-hoc analysis was performed on SPSS 22 using p-value < 0.05. Standard deviation was used for variation on plant height and weight, and standard error was used for variation on biodegradation rates of CO_2.

4 Results

4.1 Plant growth trial

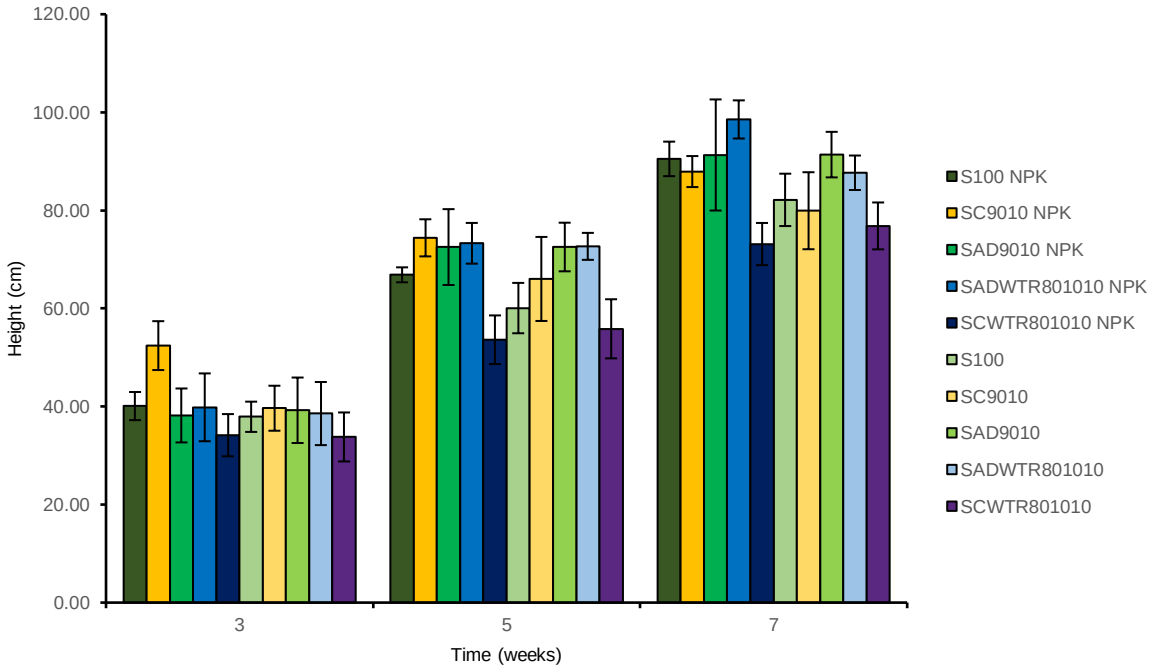

Figure 1. Evolution of mean plant height of all soil types over 7 week trial with NPK added to the indicated soil types at time zero only; 100% unamended soil (S100), 90% soil amended with 10% compost (SC9010), 90% soil amended with 10% AD (SAD9010), 80% soil amended with 10% AD and 10% WTR (SADWTR801010), 80% soil amended with 10% compost and 10% WTR (SCWTR801010). Error bars represent standard deviation (n=7).

Recorded plant height measurements showed no statistical significance ($p < 0.05$) when comparing the same soil types with and without NPK addition through the trial (Fig. 1), except for SC9010 at week 3 and SADWTR801010 at week 7. Whereas SC9010 height at week 3 was statistically significantly higher (52.41±4.98 cm) with NPK addition than without (39.70±4.59 cm without NPK addition). SC9010+NPK was also statistically significantly higher than S100 or S100+NPK at week 3 (Fig. 2). At the end of the trial SADWTR801010 was the only soil type demonstrating significant difference ($p < 0.05$), with plant height of 98.55±3.98 cm for SADWTR801010 with NPK, and 87.35±3.52 cm for SADWTR801010 without NPK (Fig. 3).

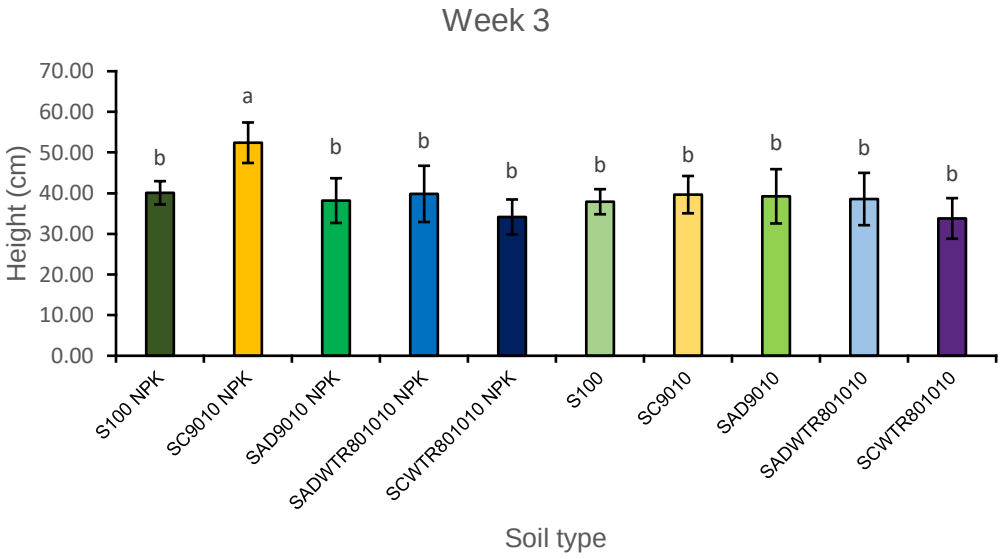

Figure 2. Effect of NPK addition compared to no NPK addition at time zero on plant height at week 3 for 5 soil types: 100% unamended soil (S100), 90% soil amended with 10% compost (SC9010), 90% soil amended with 10% AD (SAD9010), 80% soil amended with 10% AD and 10% WTR (SADWTR801010), 80% soil amended with 10% compost and 10% WTR (SCWTR801010). Bars that do not share a letter are significantly different ($p < 0.05$). Error bars represent standard deviation (n=7).

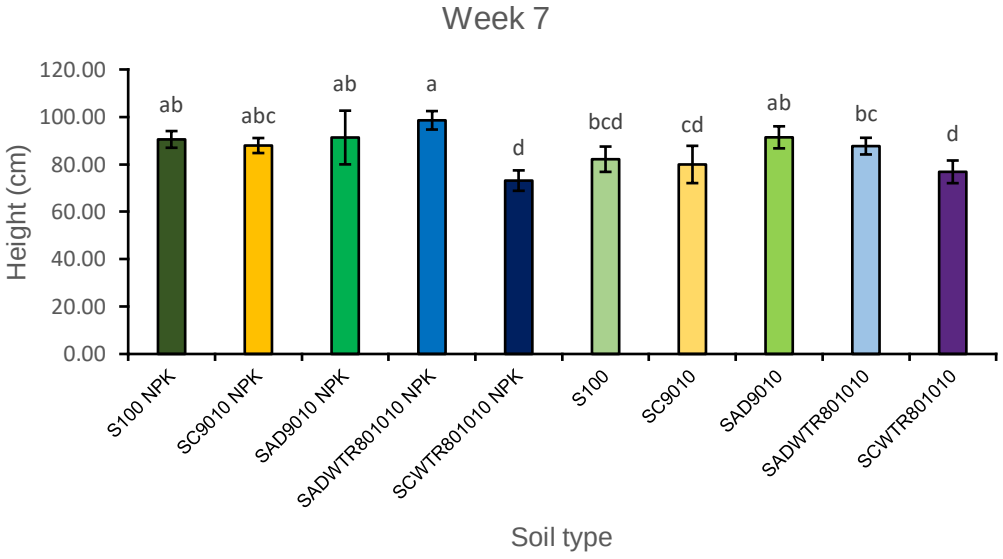

Figure 3. *Effect of* NPK addition compared to no NPK addition at time zero on plant height at week 7 for 5 soil types: 100% unamended soil (S100), 90% soil amended with 10% compost (SC9010), 90% soil amended with 10% AD (SAD9010), 80% soil amended with 10% AD and 10% WTR (SADWTR801010), 80% soil amended with 10% compost and 10% WTR (SCWTR801010). Bars that do not share a letter are significantly different ($p < 0.05$). Error bars represent standard deviation (n=7).

Analysis of final plant height (week 7) between soil types with NPK and without NPK addition, indicated SCWTR801010 was the least favourable soil type for maize plant height during this trial, with a difference of 19.19% less compared to S100 (both soil types with NPK addition), and a difference of 6.46% less compared to S100 (both soil types without NPK addition).

Providing a second dimension to the results, above ground dried biomass analysis indicated larger differences between the effects of the soil types on maize plants during this trial (see Fig. 5); however, when comparing either height results or above ground dried biomass, it should be noted that the previously mentioned negative effects on plants grown with both compost and WTR (soil type SCWTR801010), are evident in practise (see Fig. 4). The plants which were grown in SCWTR801010 weighted less on average than those grown with AD and WTR (soil type SADWTR801010) despite of the height, as can be observed in Fig. 4.

Figure 4. Three (from n=7) plants grown on 80% soil amended with 10% AD and 10% WTR (SADWTR801010) with NPK on the left and three (from n=7) plants grown on 80% soil amended with 10% compost and 10% WTR (SCWTR801010) with NPK on the right, at the end of the 7 week trial.

Turning now to results from a different soil type, it was observed when analysing S100 with NPK with above ground dried biomass weight of 4.97±0.68 g, an increase of 40.04% (and statistically significant) when using soil type SADWTR801010 with NPK (Fig. 5); in addition, S100 without NPK above ground dried biomass weight was 3.49±0.96 g, increased by 47.59% (statistically significant) when compared to SADWTR801010 without NPK addition. Statistically significant difference ($p < 0.05$) was also found when comparing SAD9010 without NPK (7.32±1.12 g) to S100 with NPK addition (4.97±0.68 g) (Fig. 5).

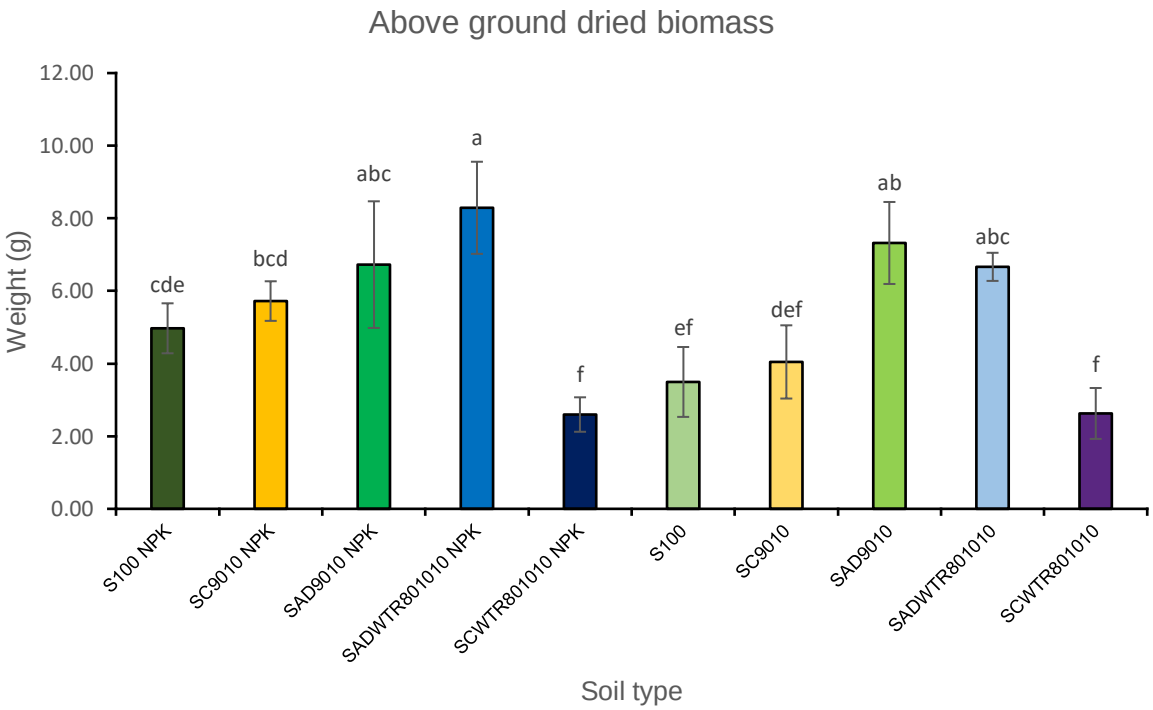

Figure 5. Above ground dried biomass (g) measured at the end of the trial of 5 soil types with and without NPK addition; 100% unamended soil (S100), 90% soil amended with 10% compost (SC9010), 90% soil amended with 10% AD (SAD9010), 80% soil amended with 10% AD and 10% WTR (SADWTR801010), 80% soil amended with 10% compost and 10% WTR (SCWTR801010). Bars that do not share a letter are significantly different ($p < 0.05$). Error bars represent standard deviation (n=7).

Another improvement was observed when comparing SC9010 which had a weight of 4.05±1.00 g, increased by 44.6% when compared to SAD9010 above ground dried biomass weight; however, when adding NPK to both soil types, no statistically significant difference ($p < 0.05$) was observed, since the difference is only of 15.00% increase in biomass weight in SAD9010 with NPK compared to SC9010 with NPK (Fig. 6 and Fig. 7).

Figure 6. Three (from n=7) plants grown with 90% soil amended with 10% AD (SAD9010) without NPK addition on the left and three (from n=7) plants grown with 90% soil amended with 10% compost (SC9010) without NPK addition at the end of the trial (week 7).

Figure 7. Three (from n=7) plants grown with 90% soil amended with 10% AD (SAD9010) with NPK addition on the left and three (from n=7) plants grown with 90% soil amended with 10% compost (SC9010) with NPK addition at the end of the trial (week 7).

Weight of below ground dried biomass, similarly to plant height, revealed no significant differences ($p < 0.05$) between any of the soil types without NPK addition, as observed in Fig. 8. However, it is interesting to note the statistical significance observed in SC9010 with NPK (weight of 5.65±2.93 g), compared to all the soil types without NPK addition, (except for SC9010 without NPK), when analysing below ground dried biomass.

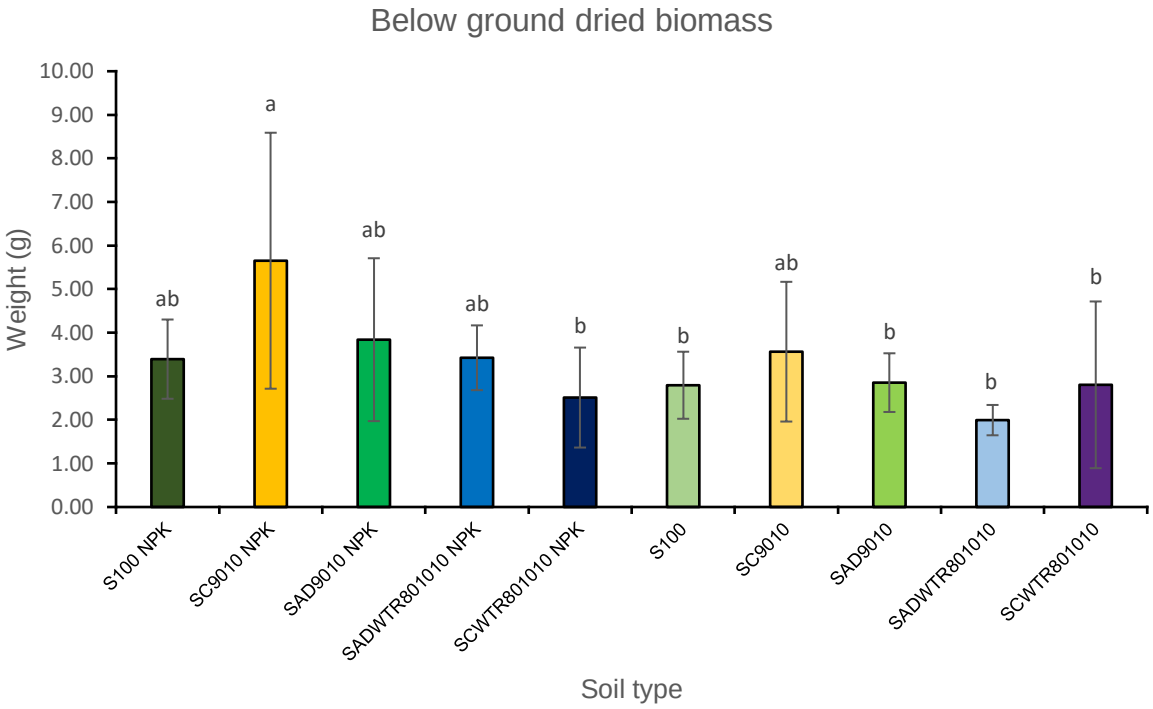

Figure 8. Below ground dried biomass of plants from all soil types at week 7; 100% unamended soil (S100), 90% soil amended with 10% compost (SC9010), 90% soil amended with 10% AD (SAD9010), 80% soil amended with 10% AD and 10% WTR (SADWTR801010), 80% soil amended with 10% compost and 10% WTR (SCWTR801010). Bars that do not share a letter are significantly different ($p < 0.05$). Error bars represent standard deviation (n=7).

4.2 Determination of CO_2

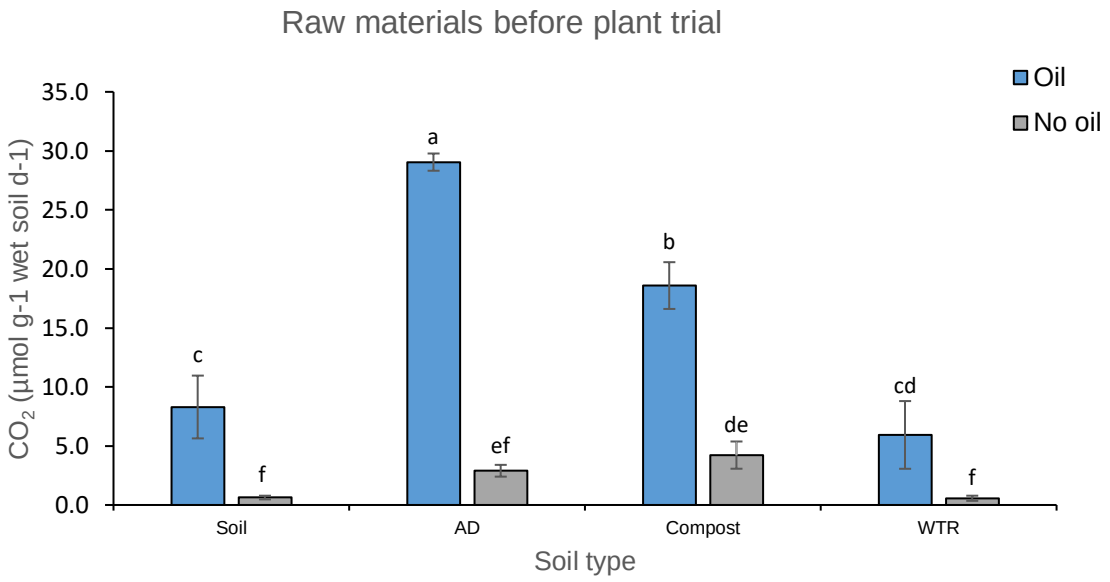

Figure 9. Comparison of CO_2 concentration rates from raw materials before 7 weeks plant growth trial soil, AD, compost and WTR with and without oil contamination. Bars that do not share a letter are significantly different ($p < 0.05$). Error bars represent standard deviation (n=3).

Statistical difference was observed when comparing each material with and without oil (Fig. 9). Analysing CO_2 concentration rates from all raw materials with oil contamination, AD stimulated oil biodegradation more than the rest of the materials. The basal respiration of the uncontaminated materials (no oil) showed that AD and compost stimulate CO_2 production the most.

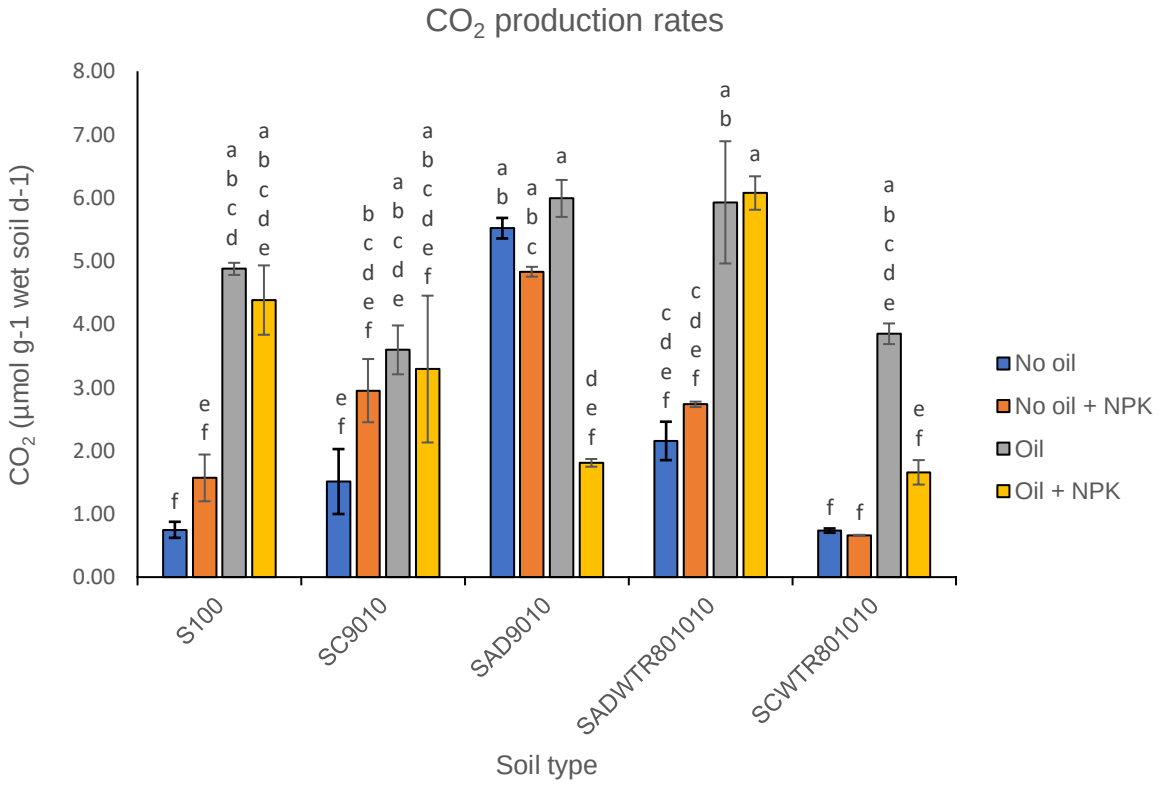

Figure 10. CO_2 concentration rates measured over 32 days from samples with and without crude oil contamination on all 5 soil types with and without NPK addition during plant growth trial; 100% unamended soil (S100), 90% soil amended with 10% compost, 90% soil amended with 10% AD, 80% soil amended with 10% AD and 10% WTR, 80% soil amended with 10% compost and 10% WTR. Bars that do not share a letter are significantly different ($p < 0.05$). Error bars represent standard error (n=3).

Basal respiration measured through samples without oil contamination was not statistically significantly stimulated by NPK added at the beginning of the plant growth trial in any soil type (Fig. 10). Oil biodegradation, using CO_2 evolution as a proxy for oil biodegradation, appeared to be statistically significantly inhibited by double NPK addition (residual from plant trial and from the addition at the beginning of microcosms experiments) for SAD9010.

Comparing oil and no oil rates of CO_2 in each soil type, it was observed that oil mineralisation without NPK occurred in all soil types, except SC9010 and SAD9010. In the case of samples where NPK addition was performed at the beginning of the plant trial, oil mineralisation occurred in SADWTR801010.

Analysing soil types with NPK, SADWTR801010 stimulated oil biodegradation more with a statistically significant difference compared to SAD9010 and SCWTR801010.

5 Discussion

Analysing height on week 3 of the trial, the only differences on soil types with and without NPK addition were observed in soil type SC9010. Although compost is usually added to soil to help with nutrient availability, it is often required to increase the amount of compost added to meet nutrient requirements for crops as well [17]. Plant nutrient availability in compost is reduced in many cases through the thermophilic phase of its production, which could make this material not as good a source of nutrients as chemical NPK [17] [46]. Thus, the addition of fertilisers to soil amended with compost has been recommended to counteract the low plant nutrient availability [46] [47] [48], especially mineral N fertilisers [49]. Accordingly, it could be suggested that the positive effects on plant height during the first 3 weeks of the trial observed in soil type SC9010+NPK, were due to the nutrient availability obtained from the fertiliser. Nevertheless, such effects were not significant at the end of the trial, which could be a result of the single addition of NPK at the beginning of the trial. Therefore, the plant height results from plants grown on soil type SC9010 with and without NPK during this experiment cannot represent just the biological and physical roles of SOM on soil health, since the effect could also be chemical.

Conversely, although SADWTR801010 was the only soil type presenting statistically significant differences in height when adding NPK (SADWTR801010+NPK) at the end of the trial (week 7), there were no statistically significant differences in either above ground or below ground biomass weight between SADWTR801010 and SADWTR801010+NPK. Thus, it could be suggested that NPK was not a rate-limiting variable for this soil type, which parallels with previous studies stating that nutrients are immediately plant-available in AD amended soils [22] [50] [51]. Additionally, considering the combination of AD and WTR, it could be suggested that the results from soil type SADWTR801010 were obtain due to a physical effect such as improvement in soil structure from the organo-mineral co-amendment.

Results from plant height of plants grown on soil type SCWTR801010+NPK were the only results that exposed statistically significantly less height compared to unamended soil (S100+NPK). According to the previously discussed results from SC9010, it could be suggested that the amount of compost added in this work, which was 10%, was not enough to provide nutrients for maize growth. In addition, the negative effect produced due to WTR and compost combination was likely caused by the fact that the Fe and Al oxides present in WTR strongly bind N and P, which probably limited the nutrient availability for plants and

microorganisms [12]. Both the initial deficiency and the bind of nutrients may have created an environment where microorganisms had to compete with plants to get enough nutrients, leading to a lessening in plant height and above ground dried biomass in soil type SCWTR801010 compared to the unamended soil.

Comparing the performance of AD and compost in this trial, SAD9010 had statistically significantly ($p < 0.05$) greater above ground biomass than SC9010, which suggests that the nutrients provided by the AD were more beneficial for shoot biomass than nutrients from compost. Nevertheless, this statistical significance was no longer observed when NPK was added to both the AD and compost amended soil. This suggests that when NPK was removed or at least reduced as a rate-limiting variable, AD results of shoot biomass were not statistically significantly greater than compost results. However, the SAD9010 results suggested that adding AD to this soil was better for shoot biomass than adding NPK to unamended soil. These SAD9010 and S100+NPK results agree to findings from a previous study where it was suggested the use of a combination of compost and AD with NPK fertiliser addition [49].

According to analysis of CO_2 rates on raw materials before plant growth trial, oil mineralisation occurred in all four materials. The highest rates were observed in AD and compost, which was probably due to high microbial activity in the biomass which was available to degrade the hydrocarbons [52]; an analysis of cell count could be performed to confirm this in future work.

Since shoot and root biomass were not statistically different between any of the soil types with and without NPK, it could be suggested that soil microbiome was negatively affected by the addition of NPK. There is research that shows when mineral fertilisers are added to soil, microbial biomass abundance is suppressed in the short-term [53]. Such abundance suppression potentially affected the oil degradation performance of NPK addition. Additionally, it has been stated that hydrocarbon degradation processes can be affected by high concentrations of nutrients [54] [55]. This finding has important implications for remediation of amended soil contaminated with hydrocarbons suggesting that both quality and quantity of nutrients on SOM added to soil must be taken into account to improve soil's capacity to remove pollutants, which is an indicator of soil health. Moreover, an analysis of nutrient concentration on previously amended soil is recommended for future studies before

performing oil biodegradation rates. This analysis would help to indicate the amount of nutrients added to microcosms to prevent microbial activity decline.

It is important to mention that the generalisability of this results is limited by the number of replicates (n=7) and size of the pots, which were constrained by the space on the greenhouse used for the experiment. By using bigger pots, the duration of the trial could be increased, and a greater evolution of the results could be observed since plant growth would not be as limited. However, for the purpose of this project, which was analysing the biological and physical role of SOM on soil structure, the results obtained are considered valid.

Further studies on this project may consider microbial analysis on soil samples such as illumine sequencing of the V4 region of the 16S rRNA gene. This study would provide identification and classification of the microbial community within each soil, thus lead to more specific conclusions of the biological role of SOM on soil structure.

6 Conclusions

By analysing the biological and physical role of SOM in soil health this thesis showed how different organic and mineral materials affected soil through plant growth and hydrocarbon contamination in a seven weeks pot trial. The results showed the benefits of using AD to grow plants and that the effects can be improved by combining with WTR. Additionally, in consideration of maintaining carbon in soil to prevent it from becoming a CO_2 source, SADWTR801010 with added NPK might be a more carbon neutral option for enhanced plant growth. The apparent negative effects of NPK on hydrocarbon degradation need further exploration but may have important implications for current enhanced bioremediation practices where NPK is used to improve degradation rates, by considering the quality and quantity of nutrients added. While the chemical factor was expected to be reduced by adding nutrients to the pots at the beginning of the trial, the level of plant-available nutrients already in the materials was out of the characterization of the materials range. It is also important to note that for all results, longer term trials should be performed in the future to draw any firm conclusions.

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