School of Biological, Earth and Environmental Sciences

Biochar as a Plant Growth Substrate Amendment

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2. ABSTRACT

Biochar (BC) is an alternative growth media, a potential soil health ameliorant and climate change mitigation strategy. The effects of an oak BC (OBC) with peat-free compost (PFC) and a rush BC (RBC) with PFC treatments were tested on plant growth of perennial ryegrass (*Lolium perenne L.*) and oilseed rape (*Brassica napus*) in potted glasshouse trials. Overall, plant height and yield of *L. perenne* and *B. napus* were not affected by OBC+PFC. RBC+PFC had no effect on plant height and yield of *B. napus*, but did have significant positive height and yield effects on *L. perenne.* OBC+PFC and RBC+PFC were 'charged' over 6-months of outdoor incubation and re-tested as charged OBC (COBC) + PFC and charged RBC (CRBC) +PFC on the same plants concurrently in the presence of abiotic stressors. The effects of both charged treatments and stressor combinations on both plants were shown to be individually statistically different with varying results between group interactions. The control (PFC) treatment had the most positive effect on plant height and yield for both plant species. Soil Plant Analysis Development (SPAD) measurements on *B. napus* plant health showed that COBC+PFC was most effective at alleviating abiotic stress. The structural and elemental components of OBC and RBC were analysed using a scanning electron microscope (SEM). The vastly porous nature of a hardwood OBC and its elemental constituents compared to a RBC was confirmed, strengthening other findings in the scientific literature.

3. AN INTRODUCTION AND BACKGROUND TO BIOCHAR

3.1 Global Pressures for Alternative Growth Media

Accelerated human population growth in the 20th and 21st centuries has put relentless pressure on the most diverse and complex ecosystem in the world - the soil (DeLong et al., 2015). Monocropping and other unsustainable, intensive agricultural practices such as excessive fertilizer and pesticide applications are some of the main anthropogenic causes of soil nutrient depletion and degradation (Nearing et al., 2017). Global soil degradation endangers soil as an immeasurably important ecosystem services, from carbon sequestration and storage (CSS) to global food security (DeLong et al., 2015). With estimates for the global population set to reach over 9 billion people by 2050, there has never been a greater need for research into and implementation of sustainable agricultural practices to improve and maintain soil health (Tahat et al., 2020).

3.2 Definition, Production and Interest of Biochar

Biochar (BC) is a carbon rich and vastly porous substance, generated as a co-product of pyrolysis (see *fig.1*), along with syngas and bio-oils (Basu, 2018). The substance is principally used as an environmental and agronomic tool for soil amendment (Huber et al., 2006) as well as for a wide range of other potentially sustainable industry uses. Uses like wastewater treatment (Xiang et al,. 2020), immobilization of soil contaminants (Jeffrey et al., 2015), thermal and mechanical insulation applications (Lazzari et al., 2019), adsorption of toxic chemicals in functional clothing (Hanoğlu et al., 2019), energy storage via BC electrodes in supercapacitors (Caguiat et al., 2018), biodegradable food-packaging (Diaz et al., 2020), media in air pollution control systems (Gwenzi et al., 2020) or as an animal health food supplement which subsequently produces an enriched BC manure after excretion (Schmidt et al., 2019) to name a few. The pyrolytic production of biochar from biomass and its various industrial uses can be observed in Figure 1.

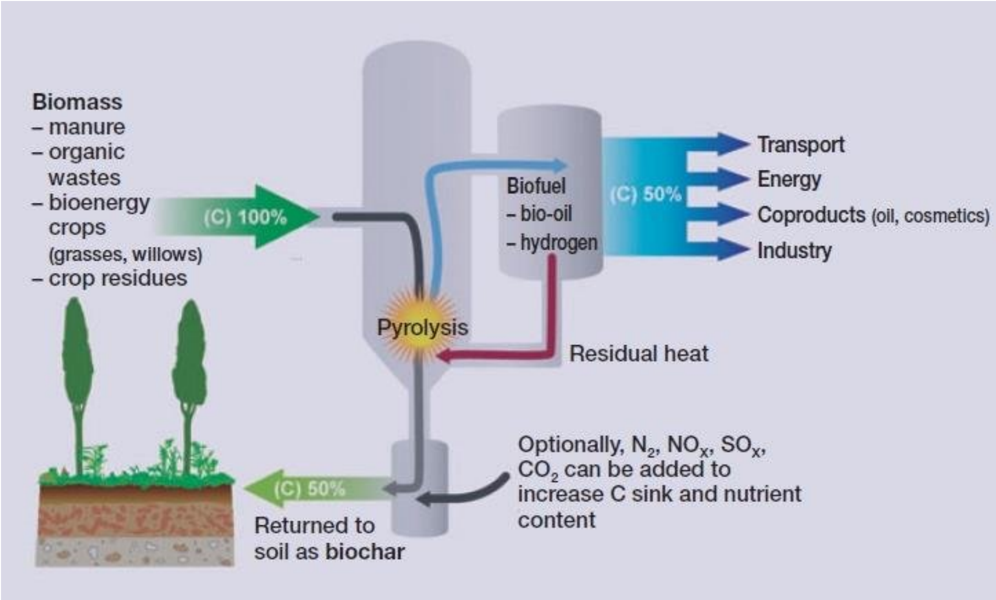

Figure 1: A diagram showing the production of BC (and other by-products) via pyrolysis and their potential uses. Source: Lehmann, 2007.

Global interest in BC has increased due to its carbon capture and sequestration (CCS) capabilities (Lee et al., 2017). Climate change is recognised as a global problem (Hanna et al., 2021) bringing with it increased instances of extreme weather with increasing intensity (Droste et al., 2020). BC becomes carbon negative when applied to soils and thus represents a potential climate change mitigation strategy for greenhouse gas (GHG) emission reduction (Sagrilo et al., 2015).

By 2050, the global population is estimated to be about 9 billion, thus increasing global pressure on energy and food production (Strange, 2015). Of these, 300 million will be living on floodplains due to global sea level rise (Kulp and Strauss, 2019). BC can help mitigate greenhouse gas (GHG) emissions by reducing GHGs emitted from agricultural soils (Case et al., 2014). BC made from sustainable feedstocks could reduce the net emissions of CO_2, N_20 and CH_2 by 1.8 Gt CO_2 eq at maximum, equalling 12% of total CO_2 emissions annually (Woolf et al., 2010). Gu and Bergmann (2015) estimated that syngas, used for energy production had a much lower global warming potential of 0.142 kg CO_2-eq /kWh, as opposed to natural gas (0.72 kg CO_2-eq /kWh) and coal (1.08 kg CO_2-eq /kWh).

BC's capacity to sequester carbon is a process involving the deceleration of the carbon cycle (Harvey et al., 2012). Once atmospheric carbon is fixed as plant biomass through photosynthesis and later pyrolysed into BC, it produces a more chemically and structurally robust material with a greater capacity to resist microbial degradation (Thomazini et al., 2015). Information in the literature regarding carbon emissions during pyrolysis is scarce and what data can be found is very limited. This highlights the need for more research into BC production for sustainability purposes.

Upon soil application, BC's physical occlusion from saprophytic soil microbes allow these microbes to interact with soil clay mineral deposits (Lehmann et al., 2015). This has been hypothesized to also promote the recalcitrant nature of BC in soils (Lehmann et al., 2015). Thus, the persistent BC can last from hundreds to thousands of years in soil (Kuzyakov et al., 2014). However, the timescale of BC humification and degradation within a soil depends on the heterogeneous nature of the BC (Ippolito et al., 2020; Hilscher and Knicker, 2011). This is determined by the influence of the types of feedstock, temperature and duration of pyrolysis of a BCs characteristic (Ippolito et al., 2020; Hilscher and Knicker, 2011). Ippolito et al (2020) extensive meta-analysis showed that pyrolysis temperatures greater than 500 ^{o}C together with hardwood as the feedstock, led to BC with half-lives greater than 1000 years, thus improving C sequestration and storage.

3.3 Biochar as a Substrate Amendment: an Ancient Agricultural Tool

BC was first described in 1966 in the literature as "terra preta" (Sombroek, 1966) or "Amazonian dark earth" within ancient central Amazonian soils, with evidence of its use as far back as 6000 B.C. (Glaser et al., 2001). Terra preta appears to have been formed by Amerindian cultures who practiced agricultural methods of burning crop residues and other biomasses which lead to a charring of the soils (Mann, 2002). Since then, there have been many studies (Gwenzi et al., 2020; Lee et al., 2017; Stavi and Lal., 2013; Huber et al., 2006) into the potential benefits and uses for such an archaic agricultural method with much greater amounts of research yet to be done.

BC also suppresses other GHGs including N_2O (Laird, 2008). A potential mechanism for this is the absorption of nitrate and ammonia to or by BC within the soil, thus suppressing nitrate leaching and N_20 emissions (Thomazini et al., 2015). Using a 15 N gas flux method Cayuela et al (2013) observed the influence of BC on denitrification, which decreased N_20 emissions from 10 to 90% between 14 individual agricultural soils. Other research has also shown an increase in genes associated with denitrification and N-fixation in the rhizosphere by soil microbial communities (Ducey et al., 2013).

In a study by Thomazini et al (2015), soil CO_2 and N_20 levels emitted to the atmosphere were found to be impacted significantly ($P = 0.04$ & $P = .0.3$, respectively) by BC application. Furthermore, the study found that BC addition suppressed N^20 emissions by an average of 63 % across all soils.

Estimates show that BC, as a negative emissions technology (NET), applied at a rate of 50 t ha yr^{-1} over 14 Mha could potentially remove 0.7 GtC eq. yr^{-1} (Smith, 2016; Genesio et al., 2012). Applied at the same rate over 26 Mha, BC could remove 1.3 GtC eq. yr^{-1} (Smith, 2016; Genesio et al., 2012) with projected figures for C sequestration between 0.5-2 GtC eq. yr^{-1} by 2050 (Fuss et al., 2018; Smith, 2016). Furthermore, Smith (2016) demonstrated BC's added advantages of lowering impacts on water use, land, nutrients, energy needs and costs as well as surface albedo compared to other NETs afforestation/reforestation or bioenergy production with carbon capture and storage (BECCS).

In comparison to BC the average individual mature tree removes 22 kgC eq. yr^{-1} (Schröder et al., 2013). Also contrasted is the projections for allowing the regrowth of global forests potentially sequestering 8.9 GtC eq. yr^{-1} up to 2050, while sustaining global food production and grassland ecosystems (Cook-Patton et al., 2020). This, together with C sequestration delivered by present global forests would remove 53% of atmospheric carbon per annum.

The application of BC as a soil amendment tool was shown to have a beneficial effect on soil fertility and crop productivity (Stavi and Lal., 2013). However, the ability of BC to ameliorate soil health and plant productivity rests on the performance of such variables, together with the climate of a region. Areas with degraded soils with low relative pH, nutrient, soil organic matter (SOM), cation exchange capacity (CEC), water retention etc. levels tend to experience the best of yield responses (Dokoohaki et al., 2019). In 2017, Jeffery et al. conducted a meta-analysis of 109 papers which demonstrated significant yield response variations depending on geographical positioning.

Ruysschaert et al (2016) facilitated a North Sea ring trial between 7 different countries (UK, The Netherlands, Belgium, Germany, Denmark, Sweden and Norway) all with varying soil types and soil parameters. It was found that BC applications increased the soil organic content (SOC) at all field sites which had low initial SOC (<3%). BC applications were found to increase pH across all field sites but only the Norway site, which had an initially low pH was found to be significant. There were significant grain yield increases in the Sweden and UK sites, while the Norway site also experienced a significant straw yield increase.

A quantitative review by Biederman and Harpole (2013) performed a different meta-analysis from 114 sources containing 371 studies, providing the first review of BC effects on multiple ecosystem functions and responses. On average, the incorporation of BC to soils transpired to increase crop yields (above and belowground), productivity above ground and plant potassium tissue concentration (Biederman and Harpole, 2013). Within this meta-analysis was a study from Asai et al. (2009) where both an improved rice cultivar (*Oryza sativa cv.* Apo) and a traditional rice variety (*Oryza sativa var. Vieng*) on average experienced increased leaf chlorophyll content when measured using soil plant analysis development (SPAD) and grain yields compared to the controls (SPAD 38.9 to 38.7 and yield 4.7 to 4.5, respectively).

Another study by Hossain et al. (2010) demonstrated that cherry tomato (*Lycopersicon esculentum*) yields increased by 64% when BC was applied to chromosol soil compared to the control. Overall, soil productivity increased, compared to the controls in rhizobia nodulation, soil microbial biomass, and soil nutrients (K, P, N & C) as well as elevations in soil pH levels, making soils less acidic (Biederman and Harpole, 2013). In Colombia, an unproductive savanna oxisol experienced increasing yields (from 28 to 140%) of maize (*Zea* *mays*) in the second, third and fourth years after the addition of a solitary application of 20t ha^{-1} of BC (Major et al., 2010).

However, not all crop responses to BC applications are positive. In a study by Jay et al (2015), short term BC applications failed to increase various growth parameters and yields of strawberry (*Fragaria ananassa*) fruit, spring barley (*Hordeum vulgare*) grain/ear counts and potato (S*olanum turberosum*) tubers, even with the presence of fertilizers. In 2020, Melaku et al. showed that similar short-term BC applications required increased fertilizer inputs to achieve the same crop yield potential as the control.

As a soil amendment tool, BC can directly benefit arable soils most in need of amelioration through improving soil density, texture, structure, porosity and particle size distribution (Fox et al., 2014). These improvements can lead to an increased cation exchange capacity (CEC) and together can affect a soil's ability to retain essential plant nutrients for assimilation and water retention within the soil thus influencing various plant growth effects and health (Hagner et al., 2016; Hazelton and Murphy., 2016; McKenzie et al., 2004). In Germany, Haider et al. (2020) demonstrated improved maize crop (*Zea mays*) growth in unproductive sandy soils through BC application, leading to better water holding capacity and nutrient retention as a result.

As outlined earlier, plant growth improvements through BC application are obtained via the amelioration of soil health, as well as soil productivity and function. These advantages consist of increased crop yield, productivity, priming of plant defences against biotic and abiotic stresses and more (Wang et al., 2019; Elad et al., 2011). In 2015, Jeffrey et al. demonstrated how BC applications increased crops yields of soybean (*Glycine max L. Merr.*), maize (*Zea mays L.*), wheat (*Triticum aestivum L.*), and rice (*Oryza sativa L.*) by 22, 19, 17 and 16 % respectively.

3.4 The Microscopic Structure and Functionality of BC

The microscopic structure of a BC and its characteristic, beneficial carbonous pores and their frequency are influenced by the types of feedstock used, as well duration and temperature at which they undergo pyrolysis (Żukiewicz-Sobczak et al., 2020; Nartey and Zhao, 2014 ;Hilscher and Knicker, 2011). It is the surface area and charge of these pores that are the physical and chemical influencers in a BC's adsorption functionality (Nartey and Zhao, 2014). Adsorption functionality affects the water/nutrient retention abilities of a BC, while creating a prosperous niche for soil microbial communities (SMCs) to inhabitat (Azeem et al., 2020). The links between various BC structures, characteristics and functionality have been examined and compared using physicochemical analyses (structural and elemental) by scanning electron microscope (SEM) to gain greater insights into the mechanics of a BC's functionality (Yang et al., 2021; Batista et al., 2018; Dong et al., 2017).

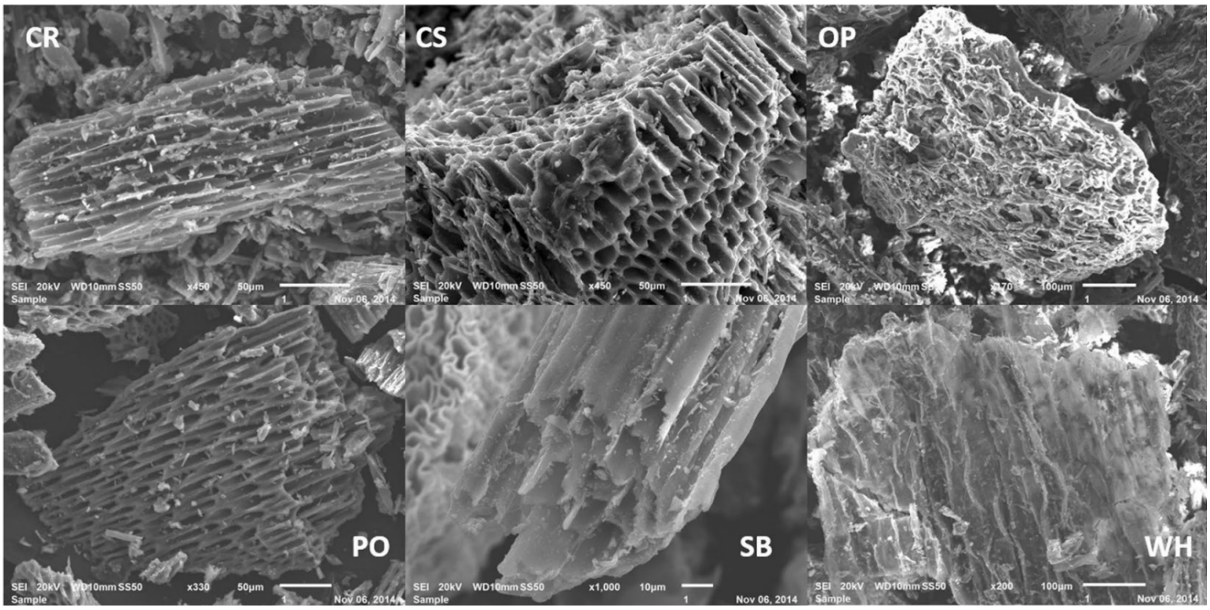

Figure 2: A collection of scanning electron micrographs of various BCs made from 6 different feedstocks. Exhibiting their variations in structural differences. CR = charcoal fines, CS = coconut shell, OP = orange peel, PO = Palm oil bunch, SB = sugarcane bagasse, WH = water hyacinth. Source: Batista et al., 2018.

3.5 <u>The Environmental Need to Replace Peat in Horticulture</u>

Ireland's raised bogs are some of the oldest ecosystems on the planet (Renou-Wilson, 2018). However, habitat destruction under unsustainable peat mining for horticultural co-composting and electricity generation in the 20th century (Mackin et al., 2017) has removed 84% of these active raised bogs (ARBs) (Renou-Wilson et al., 2019). These habitat losses prompted the EU to safeguard active raised bogs under the EU Habitats Directive, requiring Ireland as an EU member state to implement conservation measures (Mackin et al., 2017). This was achieved through ARBs being designated and regularly monitored as special areas of conservation (SACs) (Schouten, 2002).

The need to protect environmental regulatory capabilities of ARBs (Evans et al., 2014), their benefits towards biodiversity and carbon sequestration (Wilson et al., 2013) have contributed to the phasing out of peat in the EU by 2024 (Renou-Wilson et al., 2019). Peat harvesting in Ireland has ceased since 2019, following a high court ruling However, with a horticulture industry worth €477m and no economically viable peat alternative yet available, the peat-reliant sectors of mushroom, vegetable and ornamental cultivation and some 17,000 related jobs are at risk unless alternatives can be found (Hilliard, 2021; McCormack, 2021).

BC has been investigated as a potential alternative for peat within peat-based compost used in horticulture (Margenot et al., 2018). Like peat, BC is the carbonized biomass of plants (Steiner and Harttung., 2014) however, the main difference between them is the pyrolysis of biomass. Research shows the benefit of adding BC with peated composts, yet studies are lacking into using BC as the replacement for peat in horticultural soil free mixes (Wang et al., 2019; Margenot et al., 2018; Steiner and Harttung., 2014).

3.6 Abiotic and Biotic Stress Suppression

BC applications suppress biotic stresses i.e. disease-causing plant pathogens in a number of ways. Firstly, by (i) promoting beneficial organisms which antagonise pathogens through nutrient competition or antibiotic production (Bonanomi et al., 2015). Beneficial organisms are attracted to the nutrients provided by the pore volume, surface area and negative surface charge of a BC through the mechanism of sorption (Chen et al., 2012).

By (ii) inhibiting soilborne plant pathogens via the presence of volatile organic compounds (VOCs) e.g. furan (C_4H_4O) in a BC which incite direct toxicity to the pathogens (Graber et al., 2010). Persistent free radicals (PFRs) e.g. semiquinones, phenoxyls, cyclopentadienyls and more, are also inhibitory to microbial pathogens by inducing oxidative stress (Zhu et al., 2017).

By (iii) inducing plant pathogenic resistance against such foliar disease-causing fungal pathogens as *Leveillula taurica* and *Botrytis cinerea* (Elad et al., 2010). This defence mechanism is regulated by the interactions in the rhizosphere between plant growth promoting rhizobacteria/fungi (PGPR/PGPF) and plant roots (Harel et al., 2012). Arbuscular mycorrhizal fungi (AMF) interactions in the rhizosphere also induce systemic resistance against pathogens like *Fusarium oxysporum* by upregulating plant antioxidant production of L-Ascorbic acid, reducing cellular glutathione through increased redox reactions (Begum et al., 2020; Akhter et al., 2015; Winkler et al., 1994).

By (iv) enhancing disease resistance due to improved nutrient assimilation e.g. increased defence against root rot (*Phytophthora sojae*) and others (Jaiswal et al., 2020). Increased nitrogen adsorption and assimilation increases plant nitrogen utilization efficiency, helping to promote root morphology, thus increasing plant resistance to soilborne pathogens (Feng et al., 2021). Nitrogen utilization is increased through upregulation of genes related to glutamate dehydrogenase expression (Zhang et al., 2020), improving root growth and strengthening plant resistance against pathogens in the rhizosphere such as *P. sojae* (Jaiswal et al., 2020).

By (v) disruption of intra and inter-specific communication molecules within microbial communities (Zhu et al., 2017). BC can disrupt molecules essential for quorum sensing within microbial communities (Gao et al., 2016) e.g. a signalling molecule of the acyl-homoserine lactone (AHL) group, N-3-oxo-dodecanoyl-L-homoserine lactone crucial for the regulation of gene expression in gram negative soil borne microbes, like *Pseudomonas aeruginosa* (Masiello et al., 2013). BC induced signal inhibition occurs via the sorption capacity of BC (Gao et al., 2016). However, a BC's feedstock and pyrolysis conditions greatly affect its sorption capacity - BC made from honey mesquite (*Prosopis glandulosa*) pyrolyzed at 700 °C exhibited ten times the AHL cell to cell signal inhibition than that of the same BC but pyrolyzed at 300 °C (Masiello et al., 2013).

By (vi) disrupting pathogenic mobility within the soil (Bonanomi et al., 2015; Lehmann et al., 2011). Through adsorption, BC can impede and deactivate the mobilization of toxic metabolites i.e. mycotoxins and cell wall degrading enzymes i.e. polygalacturonase from pathogens such as *Fusarium oxysporum* (Jaiswal et al., 2018). All of these mechanisms linked to BC induced pathogen resistance contribute to greater plant growth promotion (Wang et al., 2019; Lehmann et al., 2011; Elad et al., 2011).

Abiotic stresses are also suppressed by BC (Bonanomi et al., 2015). In a study by Meng et al (2019) BC was found to decrease fomesafen toxicity and fomesafen assimilation by wheat plants while concurrently increasing wheat plant performance, soil mycobiome and microbiome diversity and the promotion of beneficial plant-microbe interactions in the rhizosphere.

Zhang et al (2020) demonstrated that soybean crops experiencing the negative effects of salinity and drought stress could be alleviated by the application of BC. In a high temperature stress experiment using BC and P applications on two separate rice (*Oryza sativa*) cultivars, both were found to be more resistant to the heat stress.

Contamination of crops by heavy metals present in agricultural soils can be detrimental to plant and consumer health, due to the production of harmful reactive oxygen species like hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (Engwa et al., 2019; Mani and Sankaranarayanan., 2018). The sorption capacity of BC can bioremediate soils of various heavy metals and rates of pollution (Senthilkumar and Prasad, 2020). In an experiment by Shakya and Agarwal (2019), BC made from discarded pineapple peel and pyrolyzed at 350 °C yielded the greatest adsorptive result of a group of BCs, by bioremediating hexavalent chromium (Cr (VI)) from an aqueous solution at 41.7 mg/g. The polarity of the BC's surface area was found to be the influencing factor on the adsorption of Cr (VI).

Heavy metal contamination of agricultural land causes abiotic stress to the crop plant as well as has a drastic effect on humans. Increased metal concentration in plants leads to the production of reactive oxygen species which results in cell death and thus affects the crop production in plants.

In this present study, two BCs, one from oak (*Quercus sp.*) and other from rush (*Juncus effusus*) were mixed separately with a peat-free (PF) compost. These treatments were then individually tested on perennial ryegrass (*Lolium perenne*) and oilseed rape (*Brasicca napus*) in various growth experiments on plant heigh and yield.

4. MATERIALS AND METHODS

4.1 Overall Experimental Aims

The aims of this project were to:

- Evaluate the effects oftwobiochar (BC) and peat-free (PF) compost treatments on the plant growth of perennial ryegrass (PRG) (*Lolium perenne*) and oilseed rape (OSR) (*Brassica napus*).
- To ‘charge’ and improve treatments via 6-month outdoor incubation while also abiotically stressing both plants so as to observe plant performance under such conditions.
- Observe and gain quantitative physical structure results and qualitative elemental results of the microscopic structures for both biochars via scanning electron microscope (SEM).
- Gain further insight into the relationship between structure and elemental analyses of both biochars.
- Map the porous surface area of the biochars.
- Embed biochar in epoxy resins so as to obtain greater structural mapping analyses under SEM.
- Obtain SPAD measurements from stressed OSR plants at the end of growth trials in glasshouse experiments.

4.1.1 Data Observations and Analyses

Data observations (histograms and line graphs) were generated by Google Sheets. Images and spectra were generated by a JEOL JSM-IT200 InTouchScope™ SEM and subsequently mapped and analysed using ImageJ. Statistical analyses (Welch two-sided t-tests, 2-way ANOVA tests and tukey post hoc tests) were generated by Rstudio. SPAD measurements were obtained using the Photosynq Multispeq™ phytometer.

4.2 Biochar Source, Pyrolysis and PFC

The two BCs used for these experiments were produced via pyrolysis of feedstocks of (a) oak (*Quercus spp.)* and (b) rush (*Juncus effusus*) in a mobile pyrolysis unit (fig.3). They were kindly provided to the School of BEES by Bernard Carey of Biomass to Biochar Ltd (BTBL) for research purposes.

The OBC pyrolysis regime had a temperature of between 450-600 °C and a duration of roughly three hours. The regime for the Rush Biochar had a temperature of between 400-500 °C and a duration of aroundtwohours.

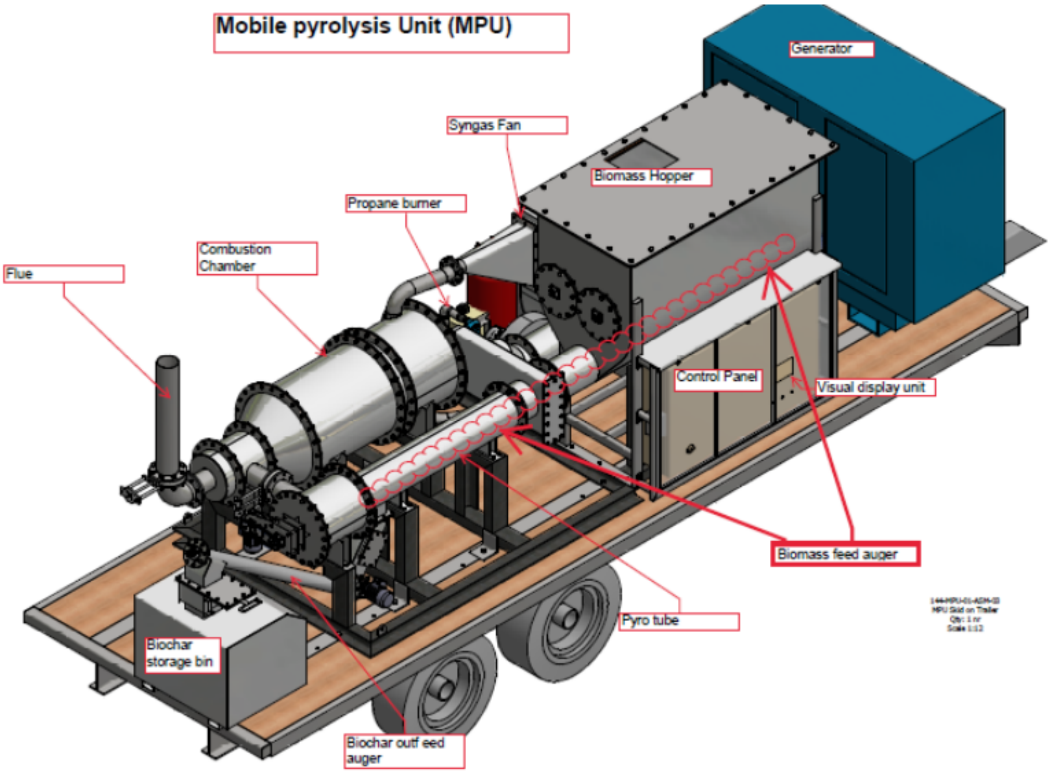

Figure 3: A diagram showing an example of a MPU and its various components used in the production of biochar, syngas and bio-oils. Source: https://www.biomasstobiochar.ie/mpu.html

The PFC used for these experiments was purchased from Fruit Hill Farm, Colomane, Bantry, Co. Cork, Ireland. The PFC had the following feedstock ratios:

- 55% coconut pith/coir, 20% green waste compost and 25% wood fibre compost.
- Predative mites (*Stratiolaelaps scimitus*) were added as a specific biological control agent of sciarid fly (*Sciaridae spp*).

4.3 <u>Treatments</u>

- Treatment 1 = Oak biochar with peat free compost
- Treatment 2 = Rush biochar with peat free compost
- Treatment 3 = Charged oak biochar with peat free compost (6-month incubation to ‘charge’)
- Treatment 4 = Charged rush biochar with peat free compost (6-month incubation to ‘charge’)
- Control = Peat free compost

4.4 Glasshouse Experiment Watering and Harvesting

4.4.1 Watering

All treatments throughout the glasshouse experiments in this thesis were kept sufficiently hydrated by having all replicate plant pots placed in self containing trays. These trays were then filled with enough water to keep treatments moist to the touch. Trays were inspected weekly and water poured into trays accordingly, if needed.

4.4.2 Plant Fresh Weights and Dry Weights

At the end of the 6-week growth trials, plants were removed from their pots and had any excess soil removed. Using a precision balance, the plant fresh weights were taken and put into brown paper bags. Each replicate number and treatment type were then inscribed onto each bag and placed in a tray. Once all fresh weights were taken the tray was then placed into a drying oven (60°C) for a period of at least 48 hours (fig.4). Afterwards the trays were removed, brown bags discarded, and all plant dry weights were recorded using a precision balance.

Figure 4: The plant drying oven in the UCC glasshouse with trays filled with fresh weight plant material, segregated into PRG and OSR groups
Photos: Eric Hynes, 2021.

4.5 Treatment Preparation

Amounts of either oak or rush biochar pieces were pulverized using a pestle and mortar (fig.5). 1.6 g aliquots (Aherne, 2017; Murphy, 2017; O'Sullivan, 2015) of pulverized biochar were then weighed out with weighing scales (fig.6). 426 cm^3 (aka 9 cm) plant pots were filled with PFC. Both biochar and PFC were subsequently mixed heterogeneously in an open container using a laboratory spoon (fig.7). The BC and PFC treatment was then transferred back into 426 cm^2 plant pots. This sequence was completed 20 times so as to have 20 pots of test material (10 pots for PRG and 10 pots for OSR). Following on, 20 pots of PFC were also used as control to the experiment. All test and PFC pots were put intotwotrays (10 test pots and 10 control pots for each tray) and transferred to the UCC glasshouse for use in subsequent experiments.

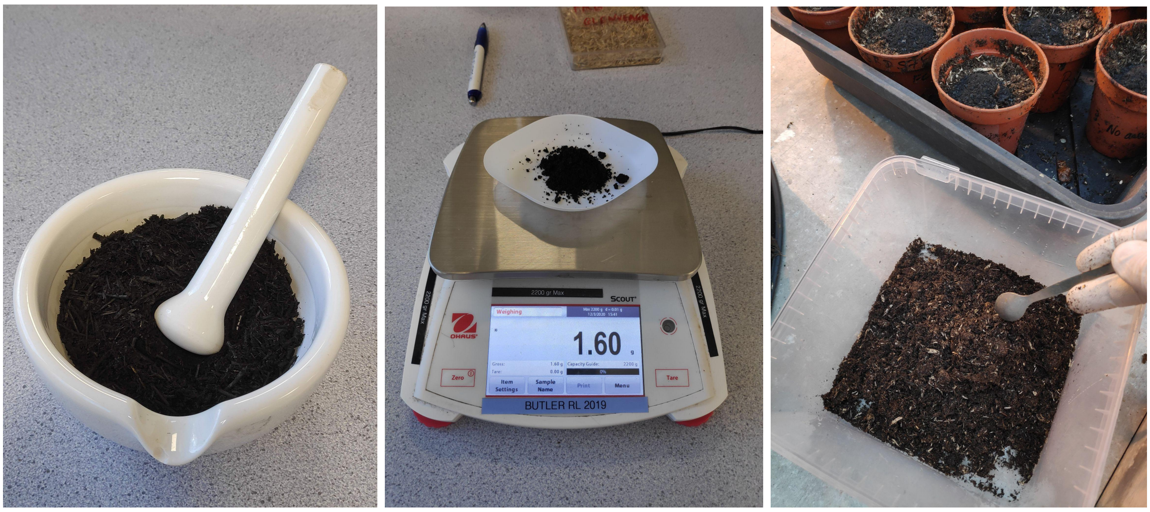

Figure 5-7: Pulverising of biochar using a pestle and mortar, followed by weighing out of 1.6g of pulverized biochar and subsequent mixing with PFC before potting. Photos: Eric Hynes, 2021.

4.6 Glasshouse Experiment #1: Testing Plant Growth Response of Perennial Ryegrass and Oilseed Rape using Oak Biochar with Peat Free Compost

Once the trays were transferred to the glasshouse, one tray was labelled for PRG testing and the other for OSR testing. In the PRG tray, 10 PRG seeds were carefully and evenly sown into each test plant pot. In the OSR tray, 5 OSR seeds were carefully and evenly sown into each test plant pot. Once all the seeds were sown for the experiment, plant pots were arranged as shown in table 1 of the next section.

4.6.1 Hypothesis

- **OBC+PFC affects PRG/OSR growth.**

4.6.2 Experimental Design

The experimental design for glasshouse experiment #1 can be found below in Table 1. This approach was used to ensure randomisation within the experiment's design.

Table 1: Shows the complete randomized design (CRD) of plant pots within each tray. A = test plant pot, B = control plant pot.

A	B	A	B	A
B	A	B	A	B
A	B	A	B	A
B	A	B	A	B

4.7 Glasshouse Experiment #2: Testing Growth Response of Perennial Ryegrass and Oilseed Rape to Rush Biochar with Peat Free Compost

The same methods were used here as in sections 4.4 & 4.5 but with a Rush Biochar instead of an OBC.

4.7.1 Hypothesis:

- **RBC+PFC affect plant growth.**

4.7.2 Experimental Design

The experimental design here remained the same as in section 4.5.2.

4.8 Glasshouse Experiment #3: Testing Plant 'Growth Response' of Perennial Ryegrass to Charged Oak Biochar with Peat Free Compost and Charged Rush Biochar with Peat Free Compost with Abiotic Stress Exposure

Following on from the two previous experiments which had mostly neutral to negative results, newer experiments were designed in a bid to induce positive plant growth and yield responses in the potted glasshouse experiments.

4.8.1 Preparing Charged BC

Large amounts of COBC+PFC and CRBC+PFC (same ratio as OBC+PFC and RBC+PFC but x10) were left in their own individual large containers outside of the UCC glasshouses. They remained there for 6 months, exposed to the atmosphere, in order to charge and induce positive plant growth responses. They would then be subsequently used in later plant growth experiments.

The 4 large containers (2 x COBC+PFC and 2 x CRBC+PFC) used for the long-term experiment had 5 drainage holes drilled into each container. These holes were evenly distributed around the circumference of the base of the large containers. After this, small rocks were placed above each hole, in an effort to improve drainage and for the more efficient flow of rain through the treatments (fig.16). This was followed by the loading of 4.26 L of PFC into the container and the successive adding of 16 g of pulverized BC (fig.17). This was then carefully and thoroughly mixed together using a garden trowel.

Figure 8 & 9: An empty, large container with rocks added above modified drainage hole areas, followed by the large amounts of BC and PFC treatment, ready to be mixed. Photos: Eric Hynes, 2021.

At the end of the 6-month period, these treatments were transferred into the same size pots used in experiments 1 & 2. The next phase of the experiment was to test PRG plant growth in the presence of COBC+PFC and CRBC+PFC as well as abiotic stressors (salt stress and drought stress) and to observe the interactions that may occur.

The control (PFC) for these experiments were not incubated over a 6 month period. PFC was potted directly from compost bags, appropriately.

4.8.2 Hypotheses

- **COBC+PFC affects PRG growth and abiotic stress resilience.**
- **CRBC+PFC affects PRG growth and abiotic stress resilience.**

4.8.3 Experimental Design

The experiment has 90 x 426 cm^3 pots and 10 PRG seeds were then carefully sown into them. Experiments using COBC+PFC were designated with 30 replicates. Pots of 10 were separated in a tray and used for the salt stress test. Another 10 were separated in another tray and used for the drought stress test and the remaining 10 were isolated as a control. This same method was repeated for the CRBC+PFC experiments (30 replicates) and control experiments (30 replicates).

Table 2: Shows the experimental design of Glasshouse Experiment #3 demonstrating the positioning of each 10 replicates within each self-contained tray. A = COBC+PFC, B = CRBC+PFC, C = PFC, D = drought stress, S = salt stress, NS = no stress.

	D	S	NS
A	10	10	10
B	10	10	10
C	10	10	10

4.8.4 Inducing Plant Stress via Abiotic Stress Exposure

All seeds were allowed a 2-week period to germinate and grow before any abiotic stressors were applied to the respective replicate plant pots.

4.8.4.1 Salt Stress

Using a technique designed by He et al. (2018) plants undergoing salt stress testing were given 20 ml of 17.532 g/L NaCl solution every second day into the plant pots via syringe for greater accuracy. Firstly, a high precision analytical weighing scale, a weighing boat and a small laboratory spoon were used to weigh out 17.532 g of NaCl. Measures of tap water were taken from the UCC glasshouse for consistency with the same water used for watering of plants in previous experiments. Tap water (1 L) was measured using a large graduated cylinder. The NaCl and tap water were subsequently mixed as a solution in a glass media bottle and transported back to the glasshouse for the experiments. These steps were repeated to keep a ready supply of NaCl solution for plant salt stress testing throughout the experiment.

4.8.4.2 Drought Stress

Plants to be drought stress tested were put aside in drought stressing trays. Each plant pot replicate was given 20ml of tap water per week and monitored regularly so plants did not die of dehydration. If plants appeared observably near death as such, then a booster hydration shot of 10ml tap water was given to any replicate pots in need of the boost.

4.8.4.3 No Stress

The same number of replicates underwent a no-stress regime, acting as the control in the presence of either treatment and no BC control. The replicates had water applied the same way as in section 4.3.

4.9 Glasshouse Experiment #4: Testing Plant Growth Response of Oilseed Rape to Charged Oak Biochar with Peat Free Compost and Charged Rush Biochar with Peat Free Compost with Abiotic Stressor Exposure

The methods of preparation for replicates, trays and experimental design remained the same here as in section 4.8. Except for the sowing of 5xOSR seeds per replicate pot instead of PRG seeds.

4.9.1 Hypotheses

- **COBC+PFC affect OSR growth while under abiotic stress.**
- **CRBC+PFC affect OSR growth while under abiotic stress.**

4.10 Glasshouse Experiment #5: Testing Plant Health/Stress Response of Oilseed Rape to Charged Oak Biochar with Peat Free Compost and Charged Rush Biochar with Peat Free Compost with Abiotic Stressor Exposure

4.10.1 Methodology of Acquiring Data on Plant Stress

At the end of the glasshouse experiment #4, SPAD measurements were taken from each OSR plant within each replicate. The Photosynq Multispeq™ and its partner software Multispeq v2.40 was downloaded from Google Play and installed onto a smartphone to allow the pairing of smartphone and the handheld photosynq device. After pressing the button at the back of the device, a leaf from an OSR plant was carefully placed where the LED sensor for spectroscopy is located on the underside of the device.

Using a trigger in the smartphone software, SPAD measurements were taken from the leaf and subsequently transmitted to the smartphone and noted (fig.24). Pressing the button on the back of the device once more to carefully release the leaf, further measurements of the other leaves of all other OSR plants were taken and noted. SPAD measurements demonstrate leaf reflective chlorophyll content and chlorophyll fluorescence. A higher SPAD value of a leaf is usually an indicator of good plant health.

Figure 10: A demonstration of the Photosynq Multispeq™ taking SPAD measurements from a sample leaf, via the paired device and smartphone. Managed through the v2.40 software. Photo: Eric Hynes, 2021.

4.10.2 <u>Hypotheses</u>

- **COBC+PFC affects OSR health and the health of OSR plants under abiotic stress.**
- **CRBC+PFC affects OSR health and the health of OSR plants under abiotic stress.**

4.11 <u>SEM Microscopy Experiment #1: The Embedding of Rush Biochar and Oak Biochar Samples in Resin for Structural and Mapping Analyse</u>

To gain insights into the microstructures of both biochars, it was decided that SEM analyses would provide the best data of such aspects of the material. The structural parameters of pore frequencies, biochar pore surface area and biochar sample surface area were mapped so as the data acquired from both biochars could be compared. Thus, helping to contrast the differences between the two types.

For the structural analyses of porous microstructures such as biochar the fixing of samples for SEM analyses in an epoxy resin is the preferred embedding media option (Besserer et al., 2016). Good adhesion, minimal sample shrinkage, vapor pressure and low viscosity are some of reasons for its choice (Besserer et al., 2016).

4.11.1 Methodology of Preparation of BC Samples for Analysis

10 pieces of each BC type were separated from larger pieces of each BC type. They were carefully cut on top of aluminium foil using a scalpel (fig.26).

Figure 11: Shows pieces of BC being cut into smaller pieces using a scalpel, on top of a small piece of tinfoil. Photo: Eric Hynes, 2021.

4.11.2 Preparing BC Samples and 'O-Rings'

Twenty pieces of 27 mm diameter grey pipe were cut roughly 15 mm in length by a UCC technical officer. These 'o-rings' had one side sanded down using two different grades of sandpaper (240 grit, 600 grit) on sanding plates. Pieces of brown sticky tape of similar square shape and size were cut from the sticky tape roll, and placed on the workbench, sticky side up. Each of the o-rings were then placed, sanded side down onto the sticky side of each piece of tape. Using a forceps, a BC sample was then placed into the centre of each o-ring, firmly sticking the sample to the sticky tape (fig.12).

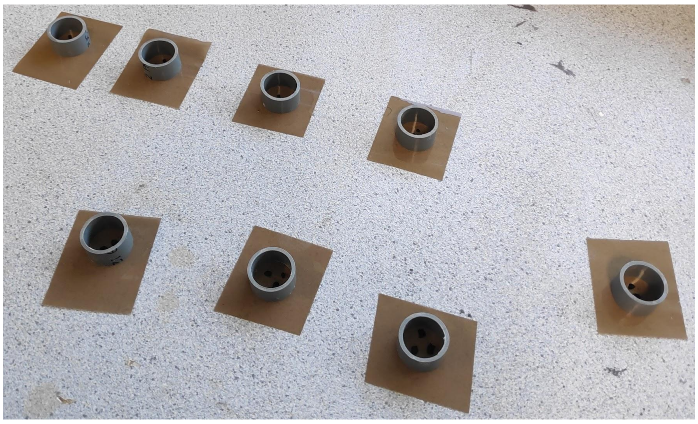

Figure 12: Shows brown sticky tape left on the workbench, sticky side up. O-rings are stuck to the sticky tape with samples of biochar on the inside, securely stuck to the tape. Photo: Eric Hynes, 2021.

4.11.3 Methodology of the Preparation of Epoxy Resin and O-Rings

Before starting to use the resin materials, laboratory goggles and facemask were ensured to be worn before using the highly toxic and carcinogenic resins. Using a ratio 2:1 of EpoThin™ Epoxy Resin to EpoThin™ Epoxy Hardener, 80ml resin and 40ml hardener measured in a beaker and poured into a disposable coffee mug for mixing. A glass rod was used to slowly and carefully stir the mixture for two minutes taking extra care not to allow bubble formation (fig.13). Once the mixture was ready, it was subsequently poured carefully into the o-rings, on top of the biochar samples attached to sticky tape. Then, the resin and samples were left to harden overnight for a 24-hour period (fig.14).

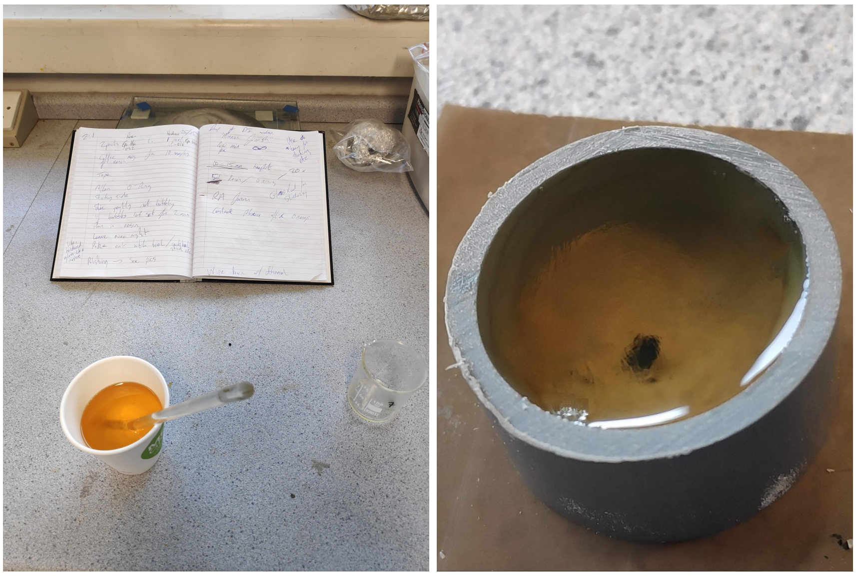

Figure 13 & 14: Shows the preparation of epoxy resin and careful following of the noted protocol from a lab-book. Figure… shows an o-ring with the resin set after 24 hours and a piece of RBC visibly fixed at the opposite side of the resin/o-ring. Photo: Eric Hynes, 2021.

Once hardened, each resin filled o-ring was sanded down using a 'figure of 8' sanding technique, to mitigate any lob-siding of the o-ring samples. Resin samples for SEM analyses are expected to have a 'mirror finish' appearance at the end of the sanding down process. To achieve this, each o-ring had around 20 minutes of sanding on each grade of grit sandpaper (coarse 240 grit to ultra-fine 2500 grit - fig.30) and finally on to the Kemet Polishing Pad with a 6 µm cloth to be used in conjunction with a Kemet Diamond Suspension 3µm solution (fig.16) to put the final mirror-finish glaze on to the sample. After this, samples were ready to be inputted into the SEM chamber for analysis and photography (post SEM image analysis via ImageJ).

Figure 15 & 16: Shows the sanding plates of varying grades of sanding grit. As well as the Kemet polishing pad, cloth and diamond solution. Photo: Eric Hynes, 2021.

4.11.4 Hypothesis

- **OBC displays distinct structural differences to that of a RBC.**

4.12 SEM Microscopy Experiment #2: The Preparing of OBC and RBC Samples for Qualitative Energy-Dispersive X-ray SpectroscopyElemental Analyses

Furthering on from section 4.10, an experiment on the elemental analyses of BC was the next step in utilising the efficiency of SEM to compare and contrast one biochar against the other. Energy-dispersive X-Ray spectroscopy (EDS) was performed on 10 samples of each BC to gain further insight into the elemental constituents on the materials.

4.12.1 Hypothesis

- **OBC displays the presence of different elemental constituents to that of a RBC.**

4.12.2 Methodology of Preparing BC Samples on SEM Stubs

The same methods used in section 4.10.1 were repeated here.

Once the 10 small pieces of each BC were ready, 4 x 12.55 mm diameter, high purity aluminium SEM pin stubs were taken from a pack of 100 (2 x stubs per biochar type). 4 x 12 mm double sided, electrically conductive, carbon adhesive discs (aka Leit tabs) were taken from a pack of 100 and stuck carefully to the top of the stubs. Both the stubs and Leit tabs were manufactured and ordered from Agar Scientific Ltd.

Taking a piece of bluetack, a SEM stub was carefully pushed into the adhesive bluetack and placed in the centre of a light microscope stage. Here, using the light microscope to carefully observe and guide, small fragments of BC samples were lifted by forceps and placed onto the Leit tab and the orientation fixed slightly, if needed (fig.41). After all specimens were satisfactorily placed onto the stubs, the bluetack and SEM stubs with samples stuck to the Leit tab were carefully lifted and placed into the SEM stub specimen mount storage box. These were then transported to the SEM and subsequently loaded into the SEM for EDS analyses.

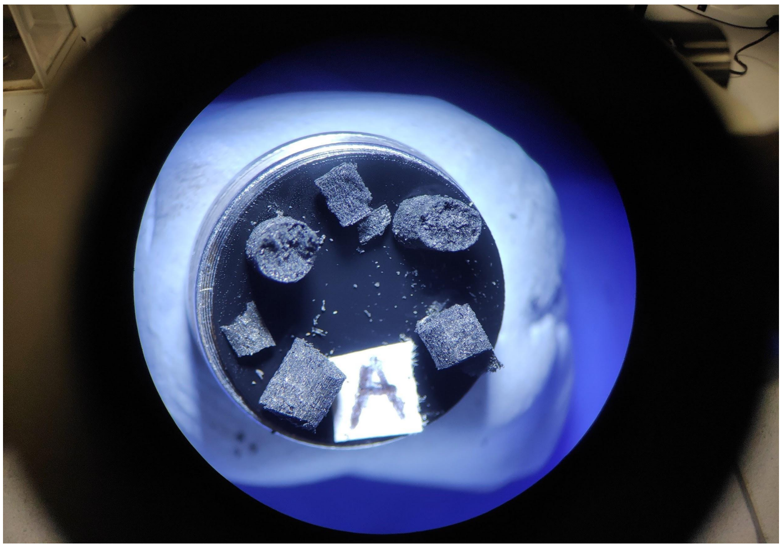

Figure 17: Shows pieces of fragmented RBC, carefully placed on top of a cohesive Leit tab resting on top of the SEM pin stub, through the lens of a light microscope. Photo: Eric Hynes, 2021.

5. RESULTS

5.1 Glasshouse Experiment #1 Results

5.1.1 Oilseed Rape Plant Heights

A two-sample t-test was performed on the week 6 data in fig.18 and it was found that plant height was not affected by OBC+PFC. The PFC had a slightly higher influence on plant growth.

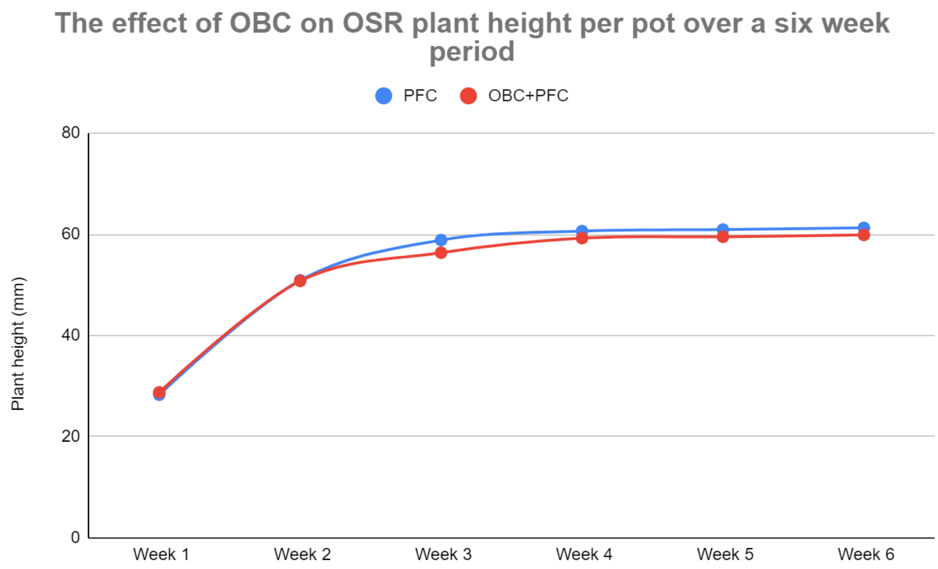

Figure 18: Shows the difference in plant heights taken weekly over a 6-week period between OSR plants grown in PFC and OSR plants grown in the OBC+PFC. 10 replicates per treatment.

5.1.2 Oilseed Rape Fresh Weights and Dry Weights

A two-sample t-test was performed on the week 6 data in fig.19 and it was found that plant fresh weights were not affected by OBC+PFC, with a p-value of 0.5465. The same test was carried out on plant dry weights and were found to not be affected by OBC+PFC, with a p-value of 0.519. The PFC had a slightly higher influence on plant growth.

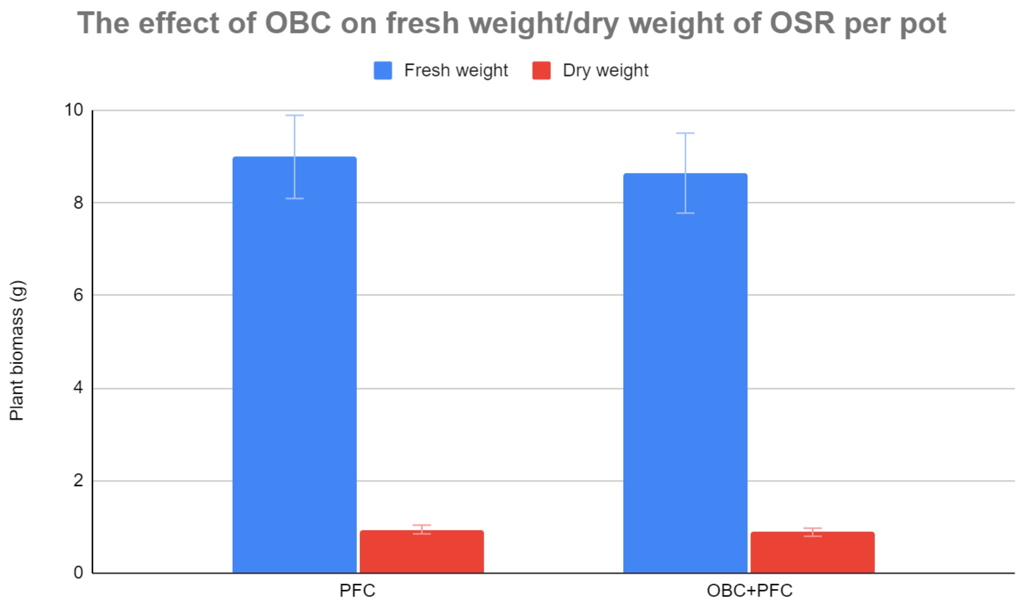

Figure 19: Shows the difference in plant fresh weights and dry weights taken after 6 weeks of growth between OSR plants grown in PFC and OSR plants grown in the OBC+PFC. 10 replicates per treatment. Error bars represent standard error.

5.1.3 Perennial Ryegrass Plant Heights

A two-sample t-test was performed on the week 6 data from fig.20. It was found that plant heights were not affected by OBC+PFC, with a p-value of 0.6404. On week 6 the OBC+PFC had a slightly higher influence on plant growth.

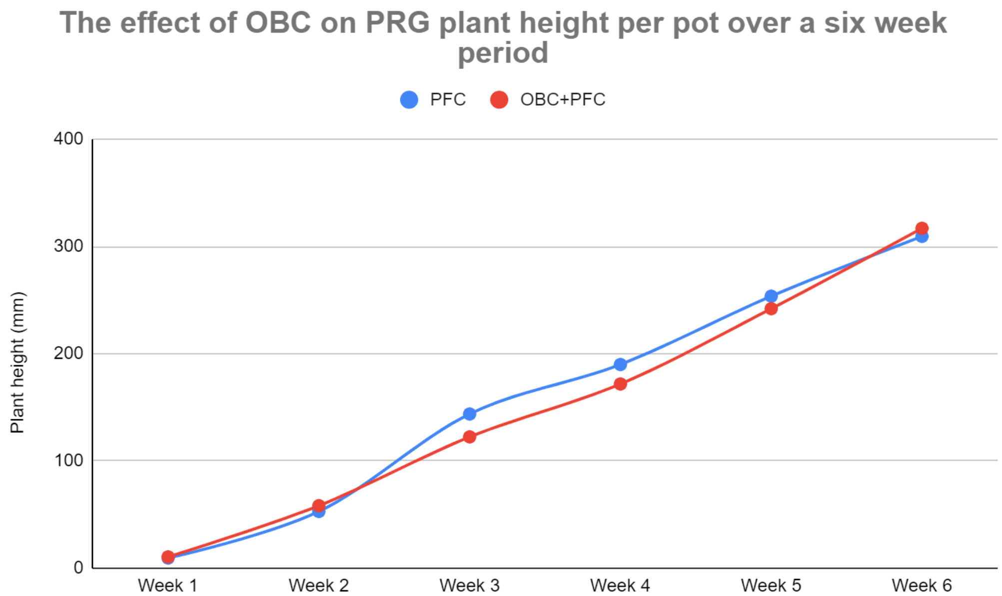

Figure 20: Shows the difference in plant heights taken weekly over a 6-week period between PRG plants grown in PFC and PRG plants grown in the OBC+PFC. 10 replicates per treatment.

5.1.4 Perennial Ryegrass Fresh Weights and Dry Weights

A two-sample t-test was performed on the data from fig.21. It was found that plant fresh weights were not affected by OBC+PFC, with a p-value of 0.2201. The same test was carried out on plant dry weights, which were found to not be affected by OBC+PFC (p-value = 0.333). On week 6 the PFC had a slightly higher influence on plant yield.

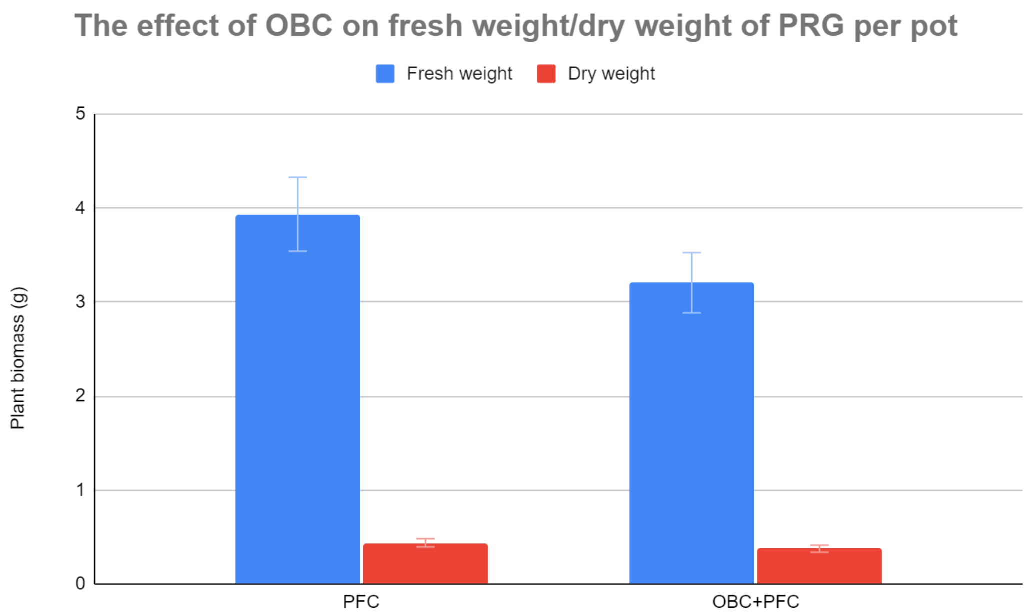

Figure 21: Shows the difference in plant fresh weights and dry weights taken after 6 weeks of growth between PRG plants grown in PFC and PRG plants grown in the OBC+PFC. 10 replicates per treatment. Error bars represent standard error.

5.2 Glasshouse Experiment #2 Results

5.2.1 Oilseed Rape Plant Heights

A two-sample t-test was performed on the week 6 data of fig.22. It was found that plant heights were not affected by RBC+PFC (p-value = 0.4803). Week 3 & 4 were omitted from the data due to UCC and the glasshouses being inaccessible during the holiday season. The PFC had a higher influence on plant height.

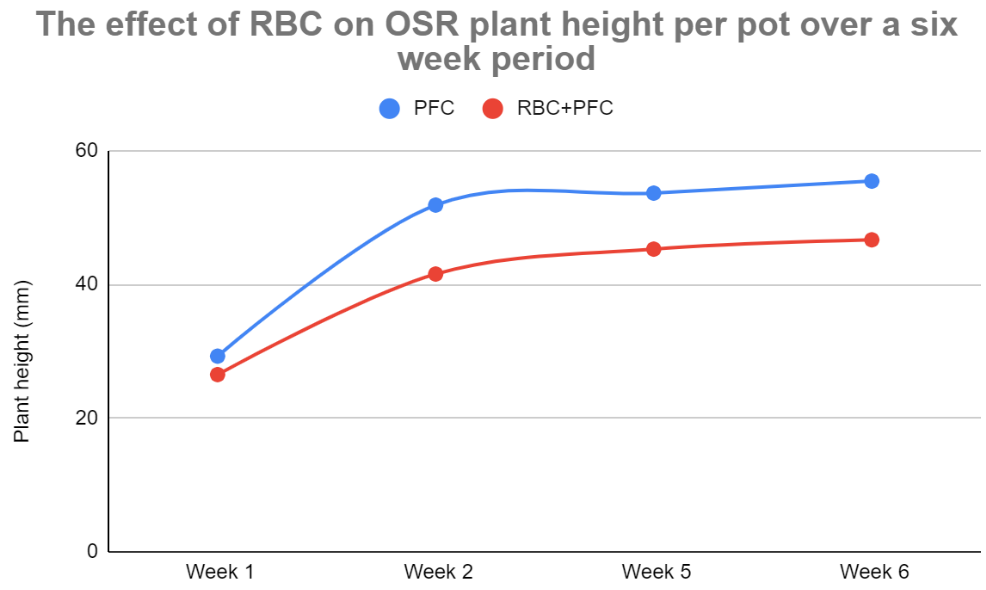

Figure 22: Shows the difference in plant heights taken weekly over a 6-week period between OSR plants grown in PFC and OSR plants grown in the RBC+PFC. 10 replicates per treatment.

5.2.2 Oilseed Rape Fresh Weights and Dry Weights

A two-sample t-test was performed on the week 6 data of fig.23. It was found that plant fresh weights were not affected by RBC+PFC, with a p-value of 0.3115. The same test was carried out on plant dry weights and OSR plants were not affected by RBC+PFC (p-value = 0.424). The PFC had a higher influence on plant growth.

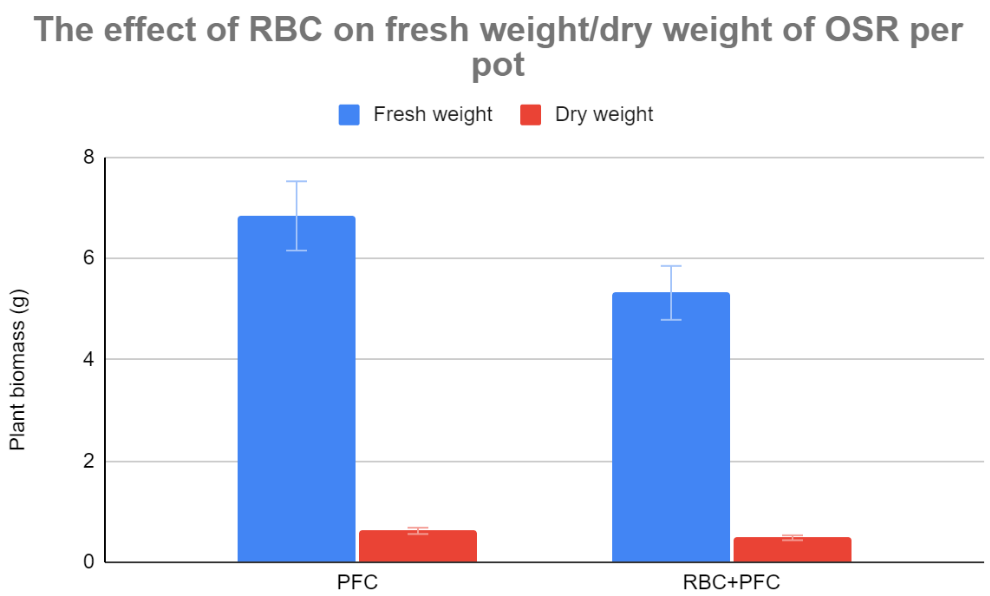

Figure 23: Shows the difference in plant fresh weights and dry weights taken after 6 weeks of growth between OSR plants grown in PFC and OSR plants grown in the RBC+PFC. 10 replicates per treatment. Error bars represent standard error.

5.2.3 Perennial Ryegrass Plant Heights

A two-sample t-test was performed on the data of fig.24. It was found that plant heights were significantly affected by RBC+PFC, with a p-value of 0.0268.

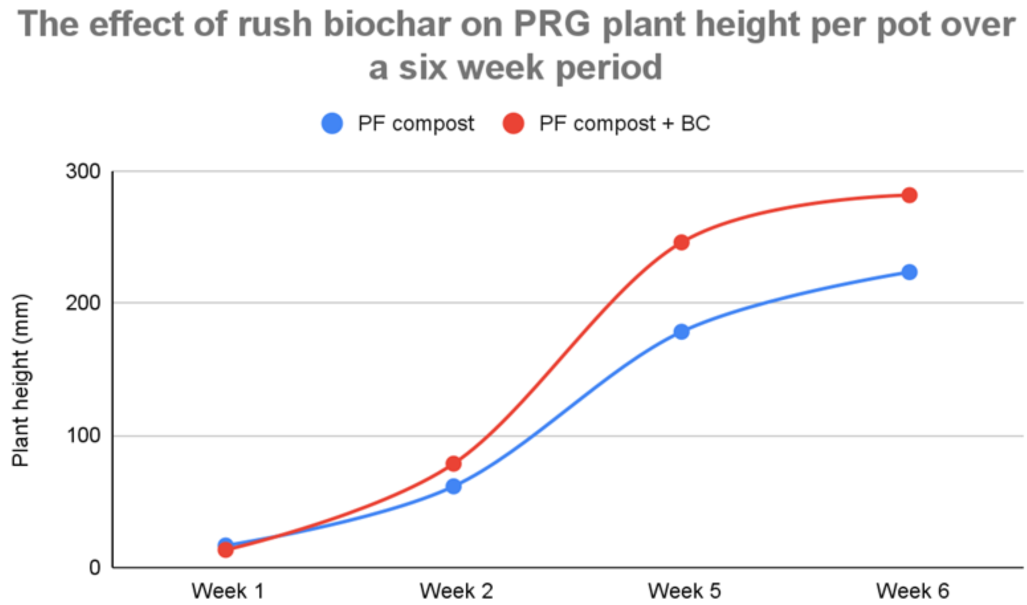

Figure 24: Shows the difference in plant heights taken weekly over a 6-week period between PRG plants grown in PFC and PRG plants grown in the RBC+PFC. 10 replicates per treatment.

5.2.4 Perennial Ryegrass Fresh Weights and Dry Weights

A two-sample t-test was performed on the data from fig.25. It was found that plant fresh weights were increased by RBC+PFC (p-value = <0.001). The same test was carried out on plant dry data, showing plant dry weights had been significantly increased by RBC+PFC (p-value = 0.012). The RBC+PFC has a significant effect on increasing plant yields.

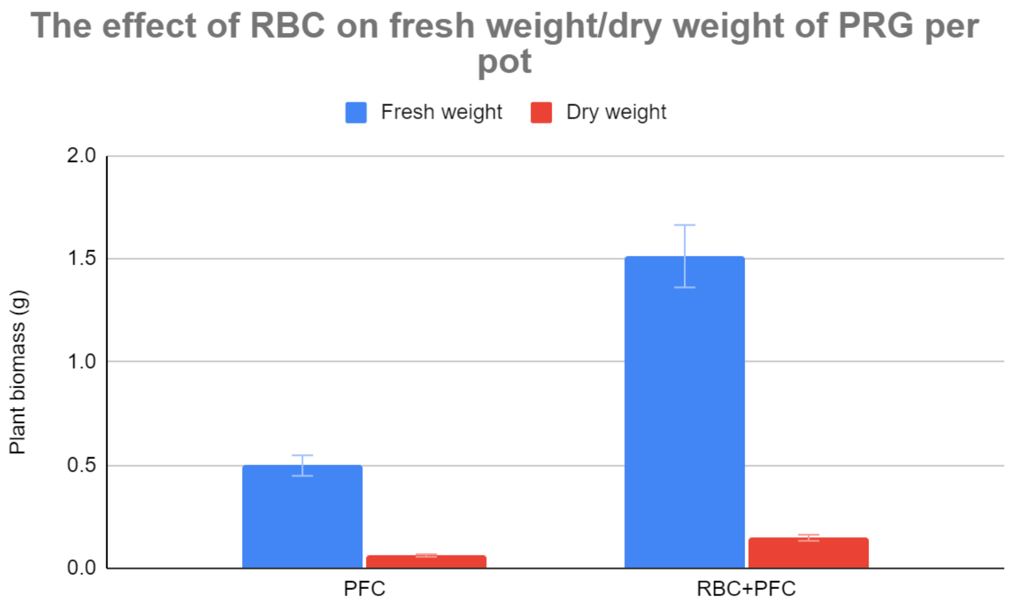

Figure 25: Shows the difference in plant fresh weights and dry weights taken after 6 weeks of growth between PRG plants grown in PFC and PRG plants grown in the RBC+PFC. 10 replicates per treatment. Error bars represent standard error.

5.3 Glasshouse Experiment #3 Results

5.3.1 Perennial Ryegrass Plant Heights

A two-way ANOVA was carried out on the plant height data from fig.26. A p-value of <0.001 was generated for the three categories of the effect of treatment, stressor and treatment:stressor interaction on plant height. This shows the data analyses to be highly significant and that the presence of both COBC+PFC, CRBC+PFC and PFC did influence the growth of PRG.

The PFC group saw the greatest increases in plant height, with PFC + drought stress and PFC + no stress having best effects within the group. PFC + salt stress significantly decreased plant height.

Within the CRBC+PFC group CRBC+PFC + drought stressor had the greatest effect on plant height, followed by CRBC+PFC + no stressor. Again, the addition of a salt stressor decreased plant height in comparison to others within the group.

The COBC+PFC group had the greatest decrease in plant height. The COBC+PFC + salt stressor and COBC+PFC + no stressor had similar effects on plant height with COBC+PFC + drought stressor having the overall greatest decrease in plant height out of all treatments and stressors combinations.

A Tukey post hoc test was subsequently carried out on the same data. All three treatments were found to be different from each other (p-value = <0.001) in terms of effect on PRG height. The PFC and drought stressor combination had the most statistically unique effect to all other combinations, followed by the PFC and no stressor.

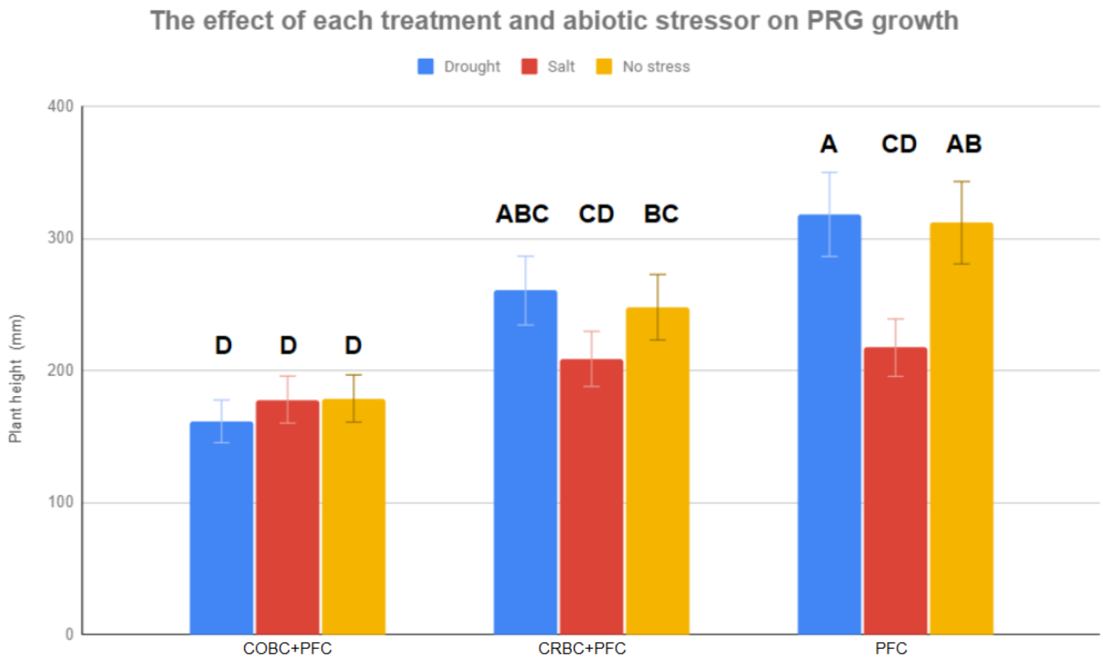

Figure 26: Shows the effect of each treatment:stressor on the growth of PRG plants and the interaction between them. Any samples sharing a common letter are not significantly different. 10 replicates per combined treatment and stressor. Error bars represent standard error.

5.3.2 Perennial Ryegrass Fresh Weights

A two-way ANOVA was performed on the plant fresh weight data belonging to the below figure (fig.19). A p-value of <0.001 was generated for the two categories of treatment and treatment:stressor interaction. COBC+PFC and CRBC+PFC did have an effect on plant fresh weights.

The PFC group saw the greatest increases in plant fresh weight, with PFC + no stress and PFC + drought stress having marginally better effects within the group, respectively. PFC + salt stress significantly decreased plant fresh weight.

Within the CRBC+PFC group CRBC+PFC + drought stressor had the greatest effect on increasing plant fresh weight. The CRBC+PFC + salt stressor and CRBC+PFC + no stressor had a similar effect.

The COBC+PFC group had the greatest decrease in plant fresh weight. The greatest increases within the group on plant fresh weight were COBC+PFC + no stressor and COBC+PFC + salt stressor, respectively.

A Tukey post hoc test was then carried out on data from fig.27. COBC+PFC and CRBC+PFC were both significantly different from the PFC group (p-value = <0.001). Furthermore, COBC+PFC and CRBC+PFC were found to be significantly different in effect from each other (p-value = <0.01).

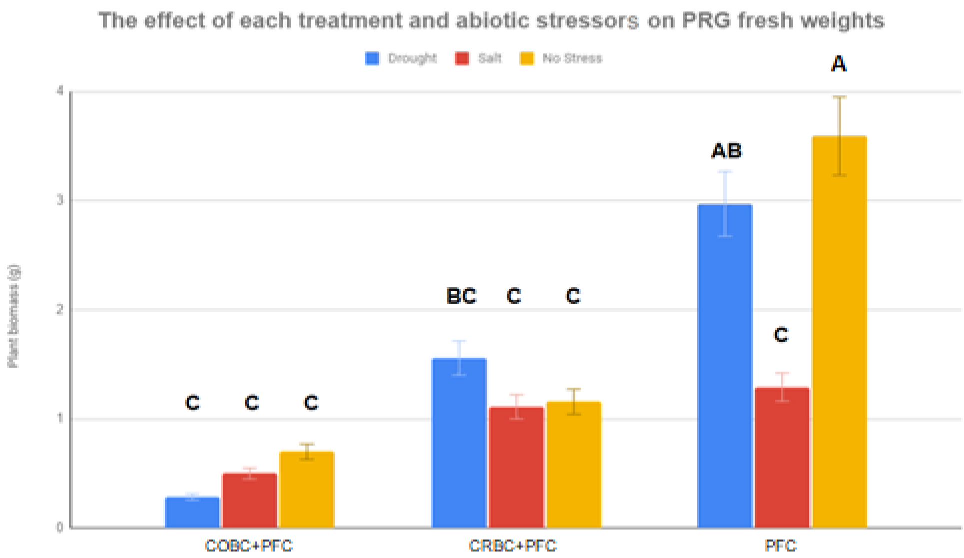

Figure 27: Shows the effect of each treatment:stressor on the growth of PRG plants and the interaction between them. Any samples sharing a common letter are not significantly different. 10 replicates per combined treatment and stressor. Error bars represent standard error.

5.3.3 Perennial Ryegrass Dry Weights

A two-way ANOVA was carried out on the data from fig.28. This generated a p-value of <0.001 for both COBC+PFC and CRBC+PFC showing they both had an effect on PRG plant weight, while under salt and drought stress.

The PFC group saw the greatest increases in plant dry weight, with PFC + no stress and PFC + drought stress having the marginally best effects within the group. PFC + salt stress significantly decreased plant dry weight.

Within the CRBC+PFC group, CRBC+PFC + drought stressor had the greatest effect on increasing plant dry weight. The CRBC+PFC + salt stressor and CRBC+PFC + no stressor had a similar effect.

The COBC+PFC group had the greatest decrease in plant dry weight. The greatest increases within the group on plant dry weight were COBC+PFC + no stressor and COBC+PFC + salt stressor, respectively. COBC+PFC + drought stressor had the overall least effect on increasing plant growth out of all treatments and stressor combinations.

A Tukey post hoc test showed a p-value of <0.001 for all three treatments showing they were significantly different from each other. The PFC and no stressor combination had the greatest effect on PRG weight, followed by the control and drought combination.

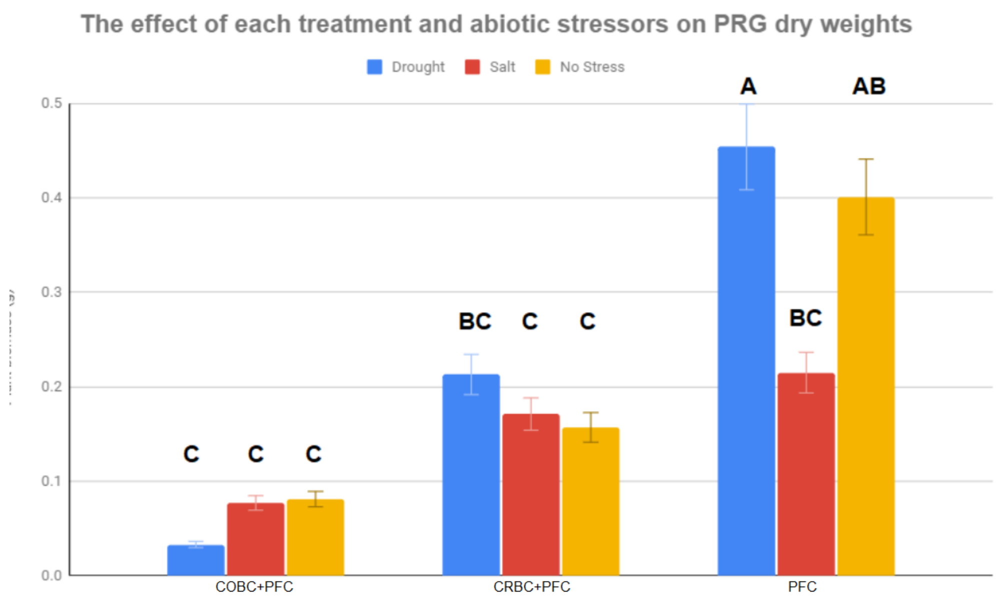

Figure 28: Shows the effect of each treatment:stressor on the growth of PRG plants and the interaction between them. Any samples sharing a common letter are not significantly different. 10 replicates per combined treatment and stressor. Error bars represent standard error.

5.4 Glasshouse Experiment #4 Results

5.4.1 Oilseed Rape Plant Heights

A two-way ANOVA was performed on the OSR height data from fig.29. All three groups had a p-value of <0.001 and significantly affected growth of OSR plants.

The PFC group saw the greatest increases in plant height, with PFC + no stress and PFC + salt stress having the best effects within the group, respectively. PFC + drought stress decreased plant height in comparison.

Within the CRBC+PFC group, the CRBC+PFC + salt stressor had the greatest effect on plant height. CRBC+PFC + drought stressor and CRBC+PFC + no stressor had similar effects

The COBC+PFC group had a similar decrease in plant height to that of the CRBC+PFC group.

A Tukey post hoc test showed that the COBC+PFC and CRBC+PFC groups were significantly different from the PFC treatment (p-value = <0.001). Both COBC+PFC and CRBC+PFC groups were significantly different from each other (p-value = 0.01). The greatest effect on OSR plant height was the PFC and no stressor combination followed by the PFC and salt stressor.

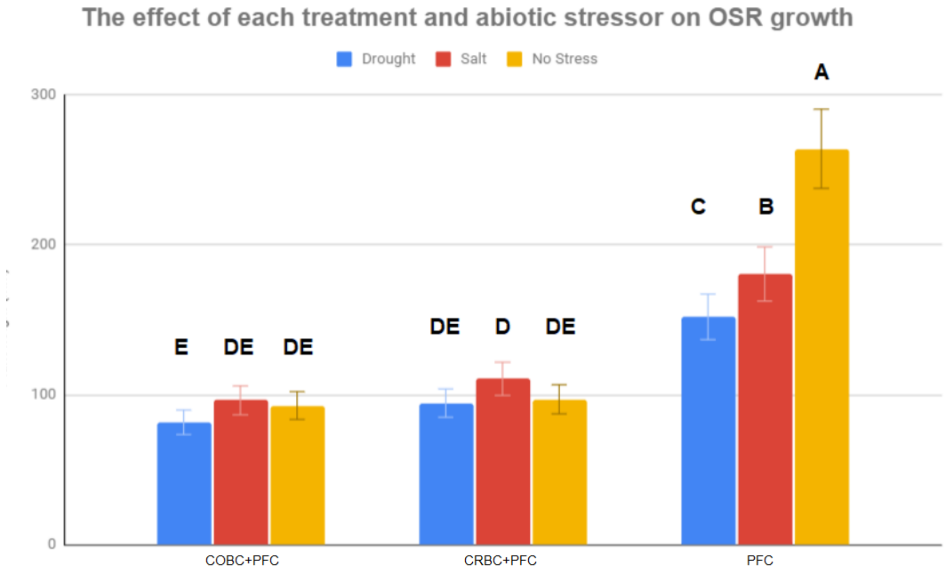

Figure 29: Shows the effect of each treatment:stressor on the growth of OSR plants and the interaction between them. Any samples sharing a common letter are not significantly different. 10 replicates per combined treatment and stressor. Error bars represent standard error.

5.4.2 Oilseed Rape Fresh Weights

A two-way ANOVA was performed on the OSR height data from fig.30. The COBC+PFC and CRBC+PFC groups had a p-value of <0.001 and significantly affected growth of OSR plants.

The PFC group had the greatest increase in plant fresh weight. PFC + no stress saw an extreme increase in plant fresh weight compared to all other treatment and stressor combinations. This was followed by PFC + salt stress.

All other treatments and stressor combinations had similar negative effects on plant fresh weights.

A Tukey post hoc test showed that the COBC+PFC and CRBC+PFC groups were significantly different from the PFC treatment (p-value = <0.001). The PFC and no stressor combination had the greatest impact on plant weight.

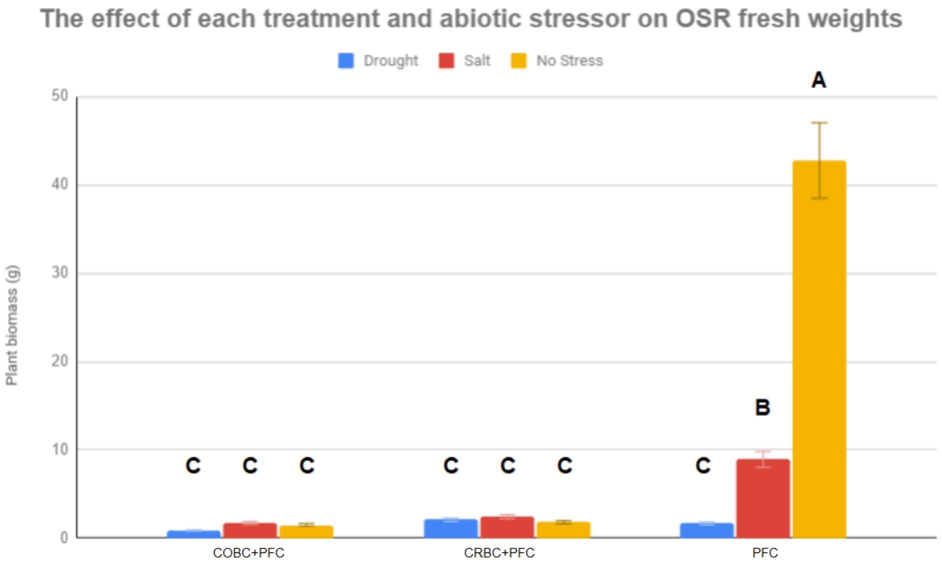

Figure 30: Shows the effect of each treatment:stressor on the fresh weights of OSR plants and the interaction between them. Any samples sharing a common letter are not significantly different. 10 replicates per combined treatment and stressor. Error bars represent standard error.

5.4.3 <u>Oilseed Rape Dry Weights</u>

A two-way ANOVA was performed on the OSR height data from fig.31. The OBC+PFC and CRBC+PFC groups had a p-value of <0.001 and significantly affected growth of OSR plants.

The PFC group had the greatest increase in plant dry weight. PFC + no stress saw an extreme increase in plant dry weight compared to all other treatment and stressor combinations. This was followed by PFC + salt stress and PFC + drought stress, respectively.

The COBC+PFC and CRBC+PFC groups both had similar negative effects on plant dry weights.

A Tukey post hoc test showed that the COBC+PFC and CRBC+PFC groups were significantly different from the PFC treatment (p-value = <0.001). The PFC and no stressor combination had the greatest impact on plant weight.

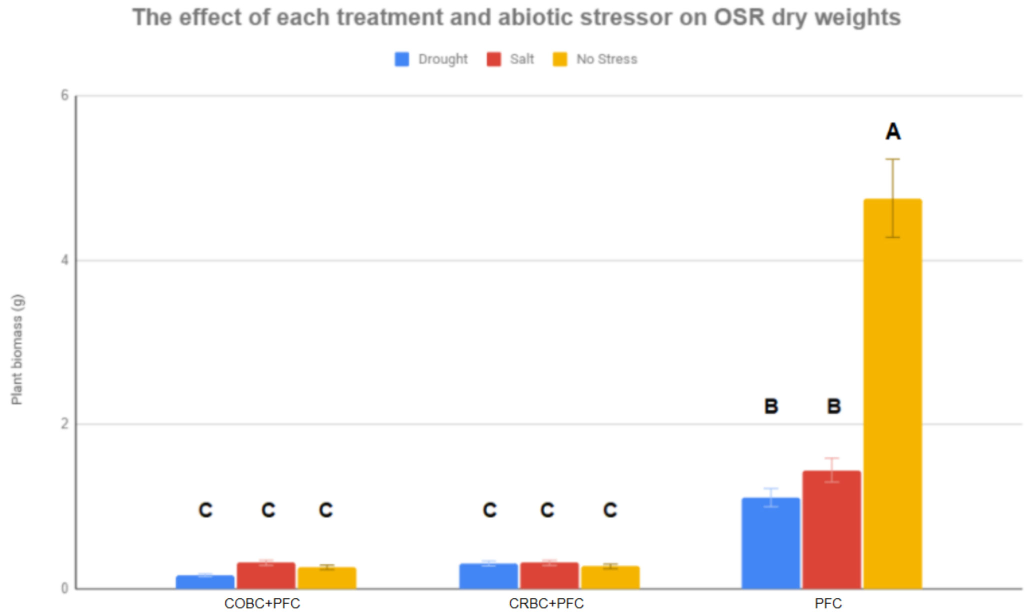

Figure 31: Shows the effect of each treatment:stressor on the growth of OSR plants and the interaction between them. Any samples sharing a common letter are not significantly different. 10 replicates per combined treatment and stressor. Error bars represent standard error.

5.5 Glasshouse Experiment #5 Results

A two-way ANOVA was performed on data from fig.32. All three groups had a p-value of <0.001 and significantly affected SPAD measurements.

The PFC group saw the highest SPAD values, with PFC + drought stress and PFC + salt stress having the best effects within the group, respectively.

Within the CRBC+PFC group, CRBC+PFC + salt stressor had the highest SPAD values, followed by CRBC+PFC + drought stressor and then CRBC+PFC + no stressor.

For the COBC+PFC group, COBC+PFC + salt stressor had the highest SPAD values, followed by COBC+PFC + drought stressor and then COBC+PFC + no stressor.

A Tukey post hoc test showed that the PFC group saw the greatest effects on SPAD measurements, with the drought stressor within the same group exhibiting the greatest effect.

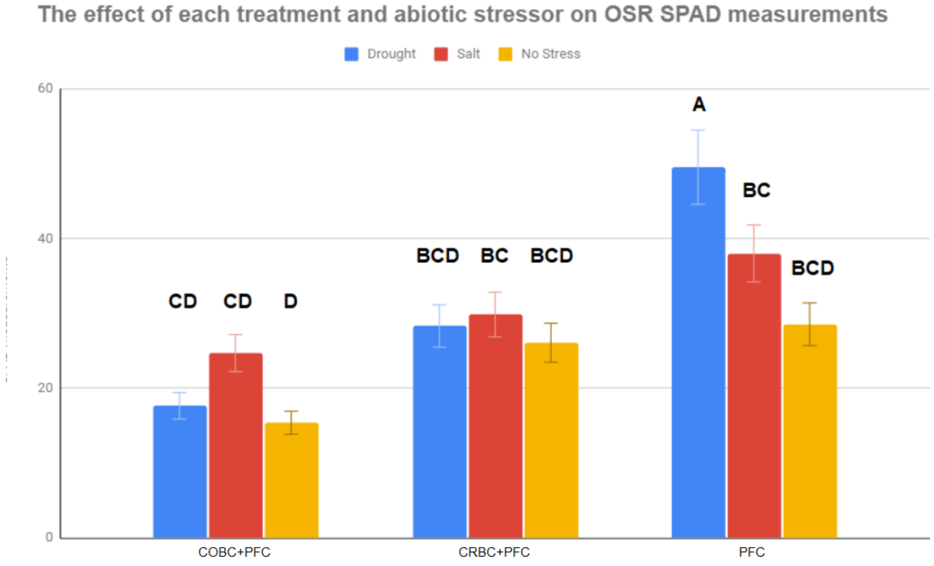

Figure 32: Shows the effect of each treatment:stressor on the plant health/stress of OSR plants and the interaction between them. Any samples sharing a common letter are not significantly different. 10 replicates per combined treatment and stressor. Error bars represent standard error.

5.6 <u>Microscopy Experiment #1 Results: Determining the Mean Pore Surface Area of Each Biochar</u>

A two-sample t-test was performed on the data from fig.33 and the mean pore surface area was not significantly different between both types of biochars (p-value = 0.078).

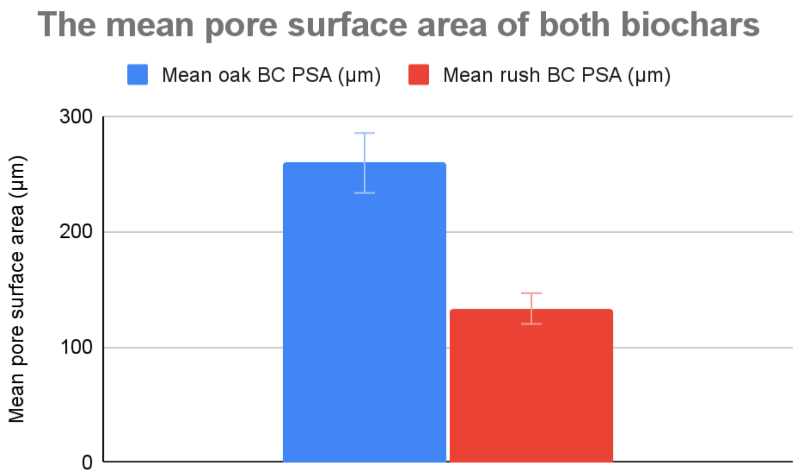

Figure 33: Shows the mean pore surface area of each BC type. 10 replicates per biochar. Error bars represent standard error.

5.7 <u>Microscopy Experiment #2 Results: Determining the Mean Pore Frequency per Biochar Surface Area</u>

A two-sample t-test was performed on the data from fig.34 and mean pore surface area was not significantly different between both types of biochars (p-value = 0.1083).

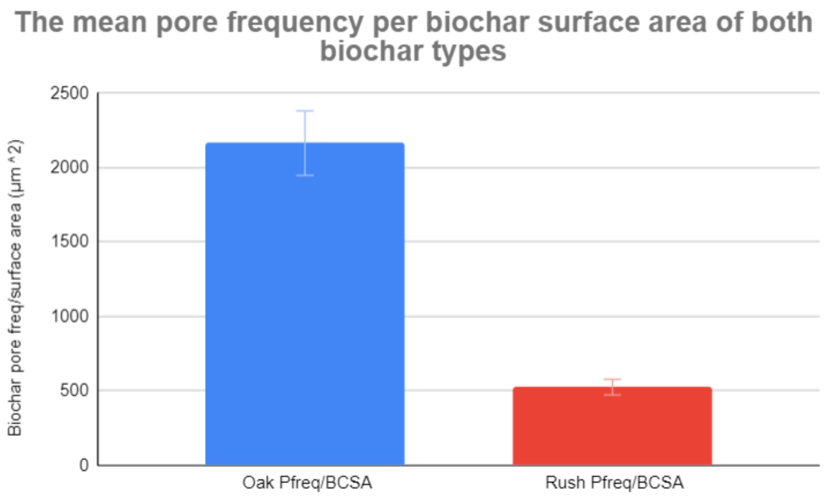

Figure 34: Shows the mean pore frequency per biochar surface area of both BC types. 10 replicates per biochar. Error bars represent standard error.

5.8 <u>SEM Images of Oak Biochar</u>

The SEM images below (fig.35-38) exhibit the vastly porous nature of the structure of OBC. The BC pieces were photographed by high def SEM at various magnifications and subsequently mapped using ImageJ.

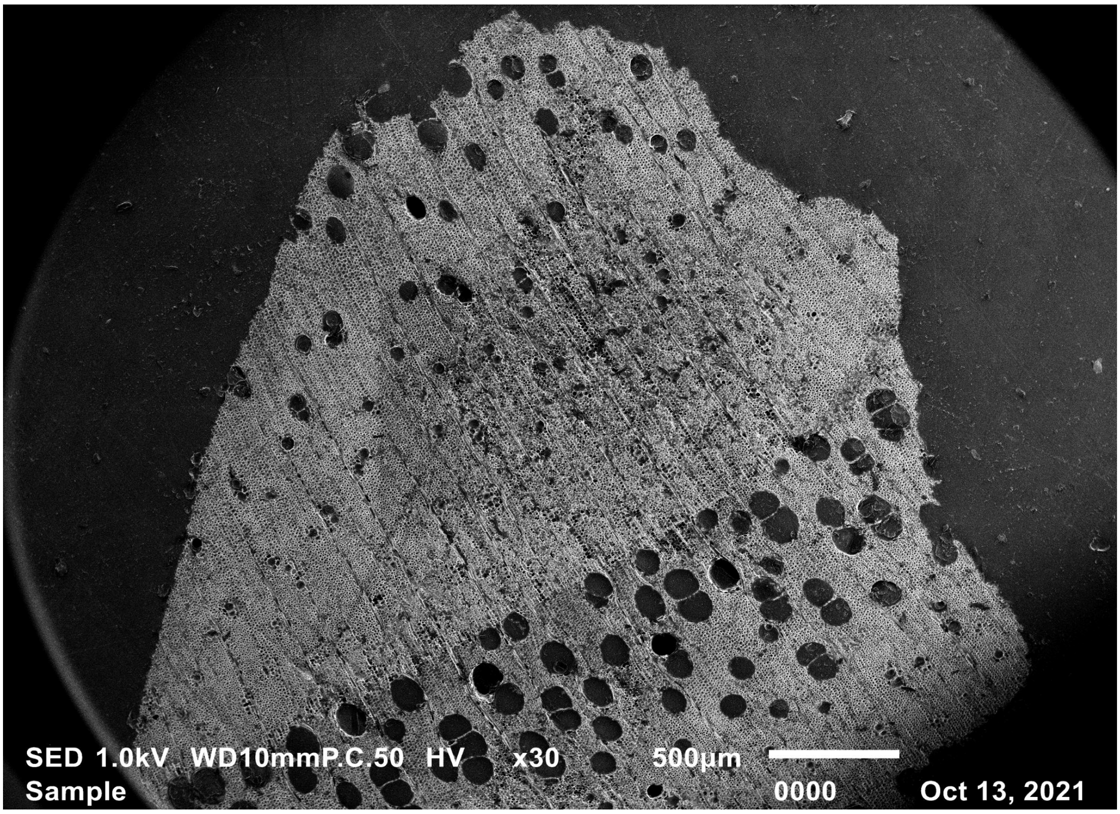

Figure 35: Shows a sample of OBC embedded in epoxy resin at minimum magnification (x30). Photo: Eric Hynes, 2021.

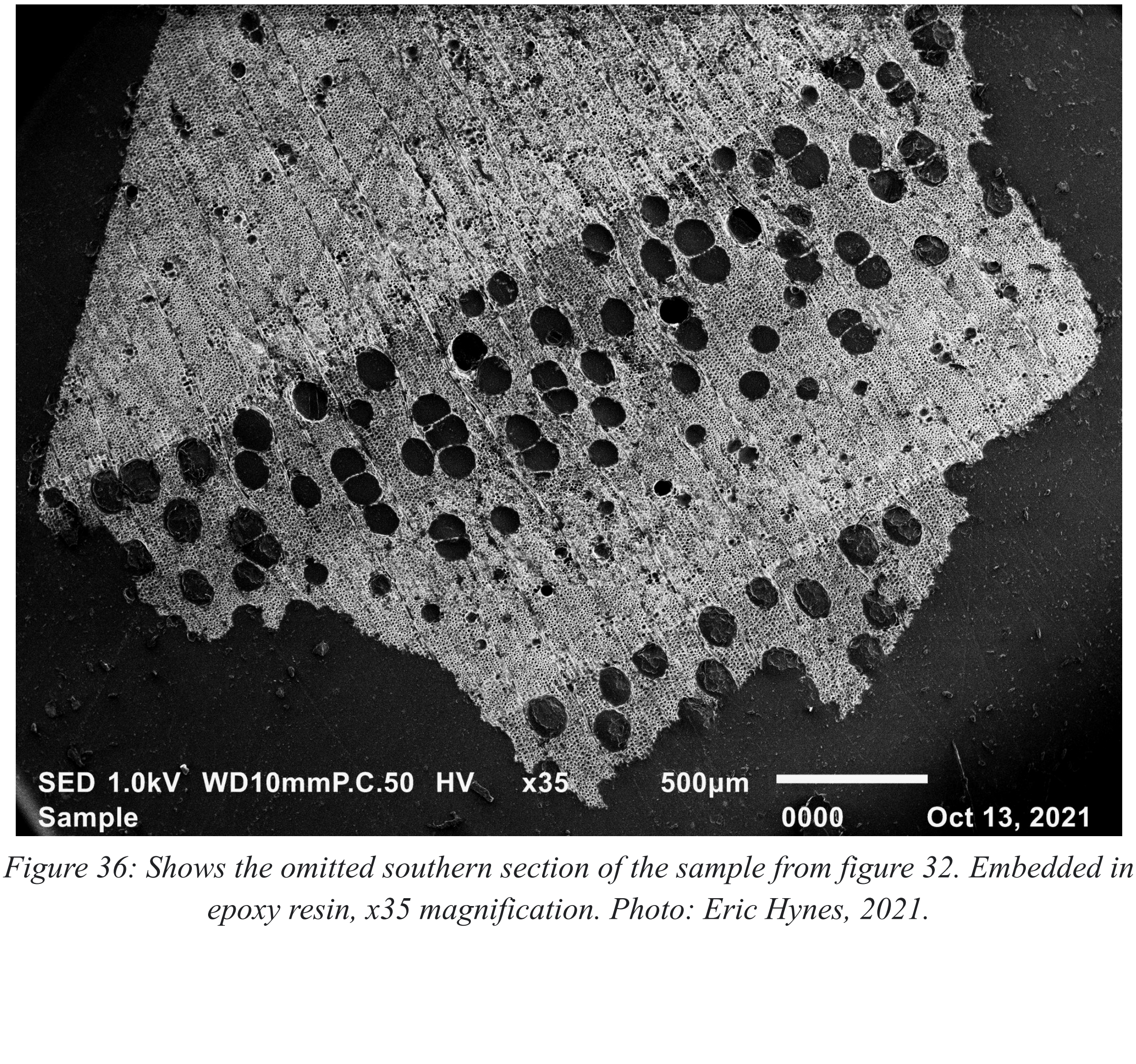

Figure 36: Shows the omitted southern section of the sample from figure 32. Embedded in epoxy resin, x35 magnification. Photo: Eric Hynes, 2021.

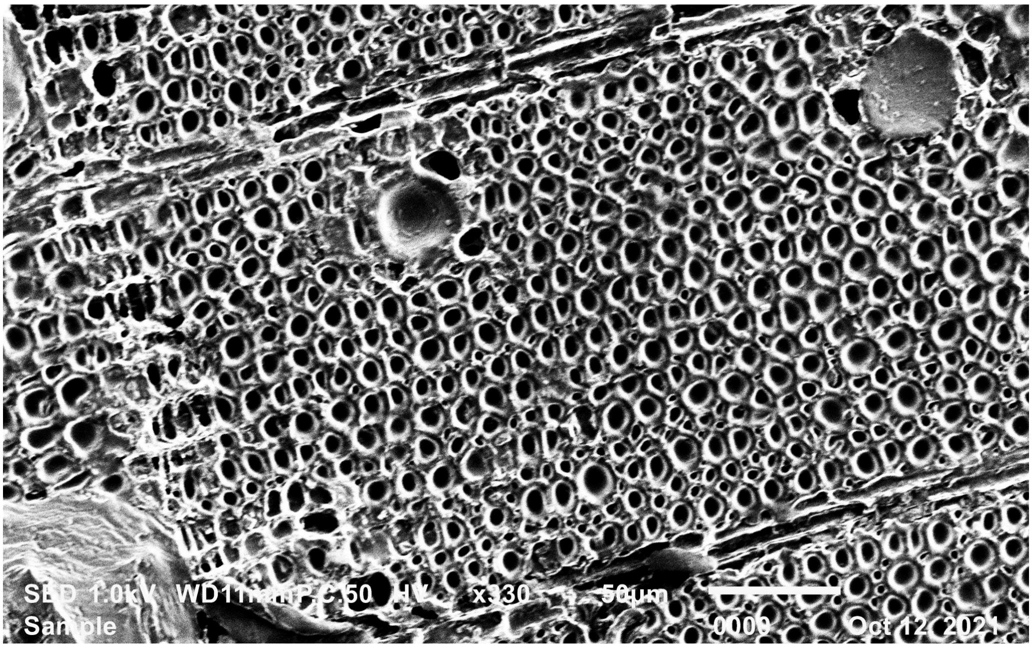

Figure 37: An up-close perspective of the strong and numerous presences of deep pores of OBC. Embedded in epoxy resin, x330 magnification. Photo: Eric Hynes, 2021.

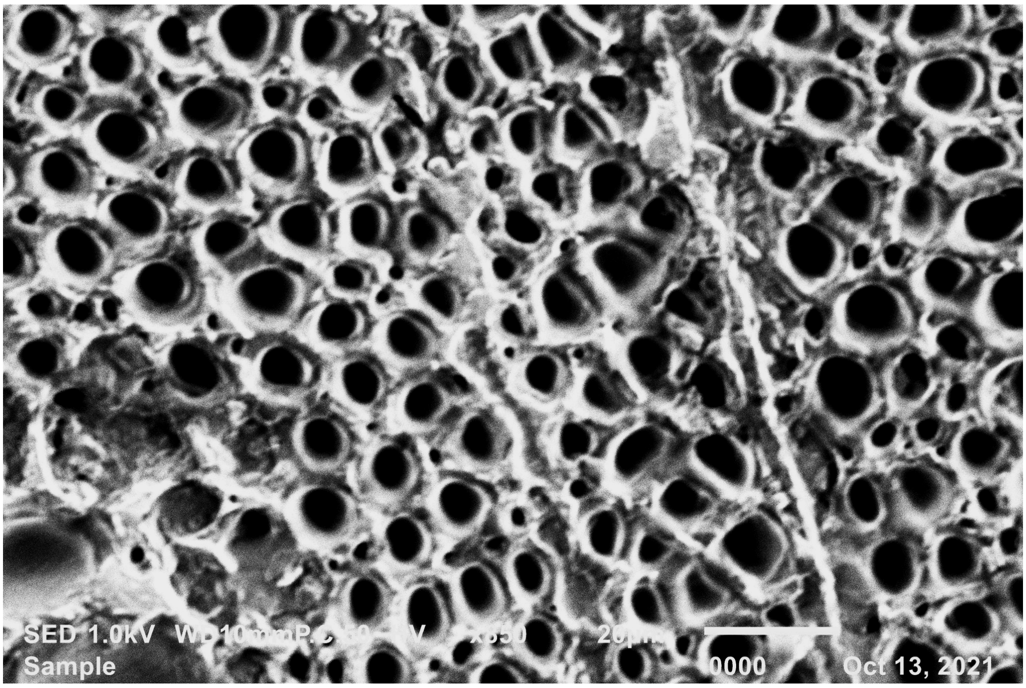

Figure 38: An up-close perspective of the strong and numerous presences of deep pores of OBC. Embedded in epoxy resin, x850 magnification. Photo: Eric Hynes, 2021.

5.9 SEM Images of Rush Biochar

The SEM images below (fig.39-41) exhibit the sparse nature of RBC pores. The epidermal tissues of the plant (vascular bundle etc) is where these pores reside after pyrolysis occurs. While the larger central area of the culm pith see no formation of pore structures. The BC pieces were photographed by high def SEM at various magnifications and subsequently mapped using ImageJ.

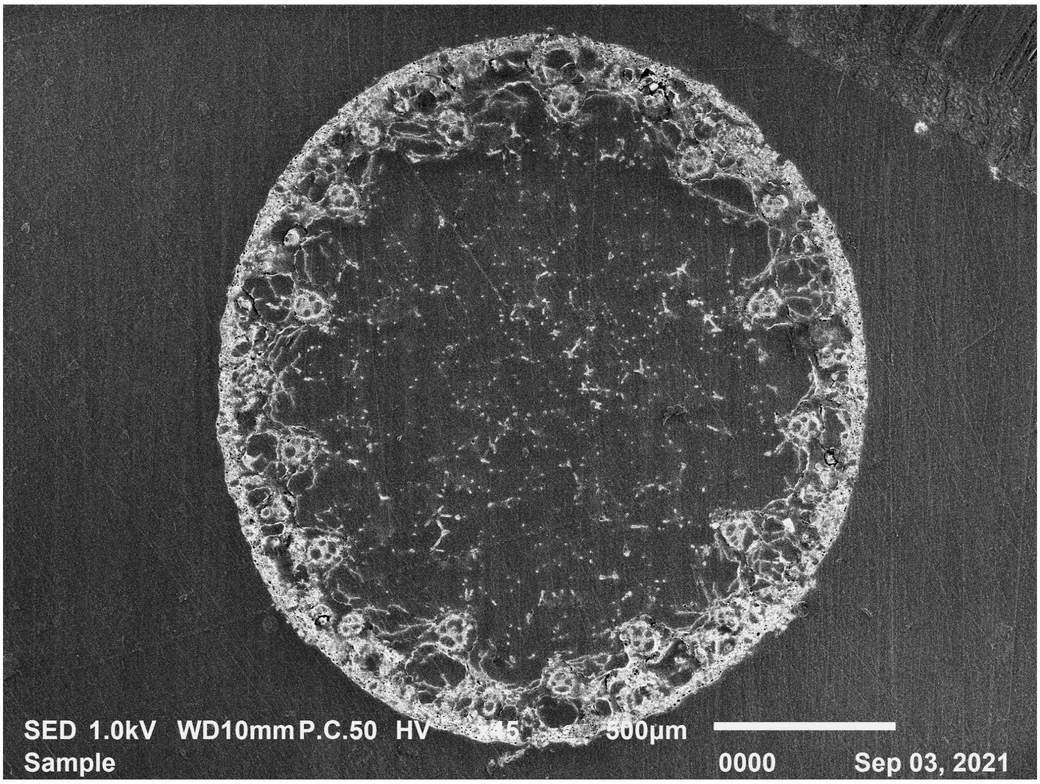

Figure 39: Shows a whole cross section of a RBC embedded in epoxy resin at magnification (x45). Photo: Eric Hynes, 2021.

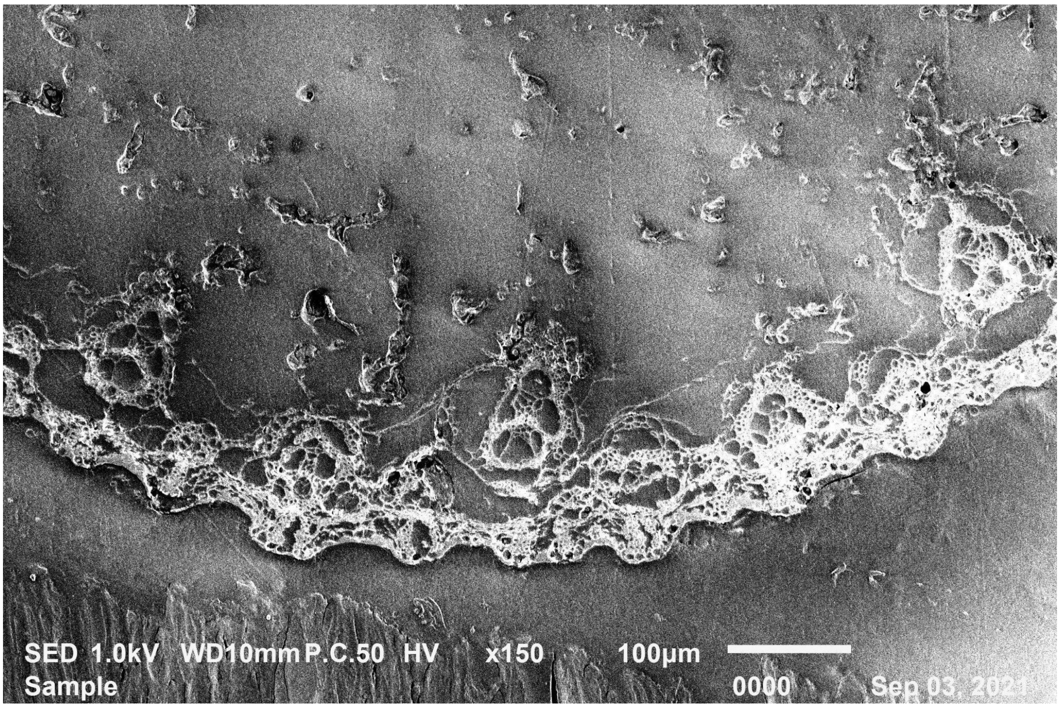

Figure 40: Shows a section of RBC embedded in epoxy resin (x150 magnification). Photo: Eric Hynes, 2021.

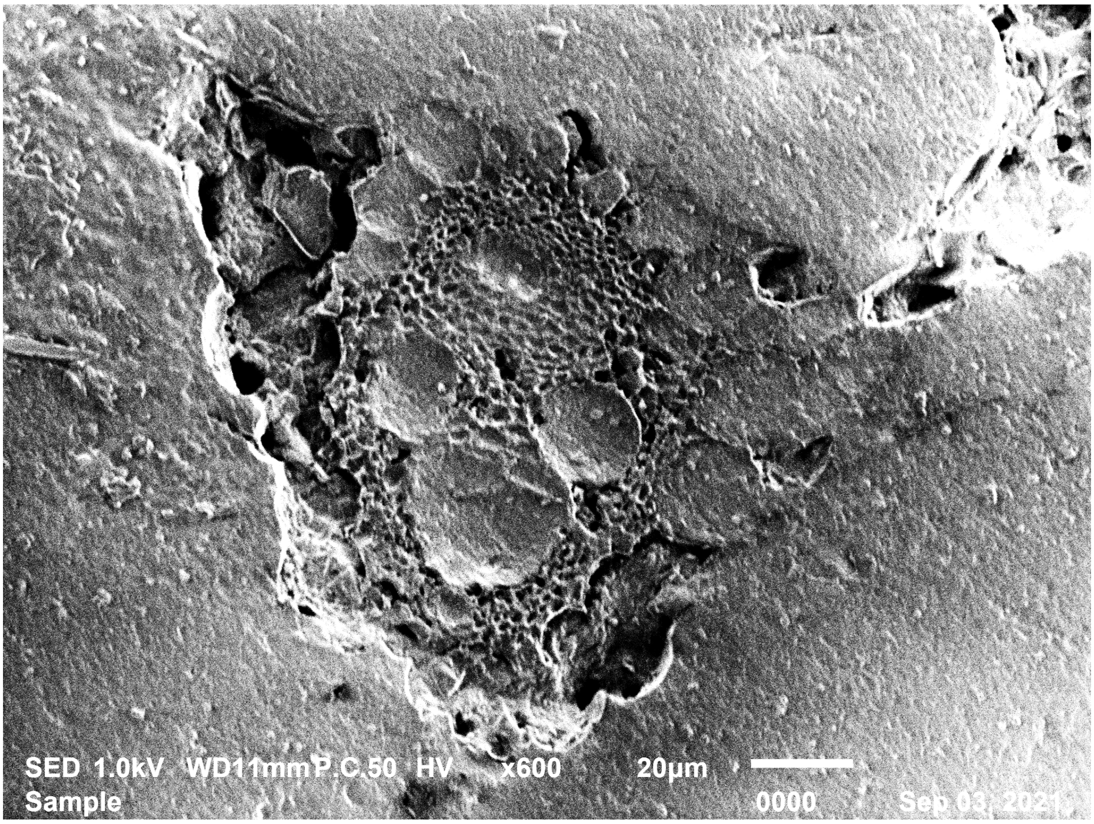

Figure 41: Shows a close up of a xylem and phloem plant anatomy of a RBC embedded in epoxy resin at magnification (x600). Photo: Eric Hynes, 2021.

5.10 <u>Microscopy Experiment #2.1 Results: Qualitative Energy-Dispersive X-ray Spectroscopy Elemental Analyses of Oak Biochar</u>

Observations of the qualitative data produced by the 10 EDS spectra below confirm the presence primarily of carbon, and secondarily oxygen in all 10 samples of OBC tested by EDS in the SEM. The spectra for replicates #1-8 (fig. 42-49) confirmed the presence of just carbon while the remaining 2 replicates (fig.50-51) confirmed the presence of both carbon and oxygen, where spikes of carbon were up to 8 times greater than the spikes of oxygen.

It should be noted that even though there are observable spikes of data in the region of the x-axis where oxygen's presence should be confirmed, it can be seen the 'O' has not been assigned on the EDS spectra (except fig.50-51). If the duration of EDS performed on the samples was perhaps extended or shortened the presence of carbon may well have been detected.

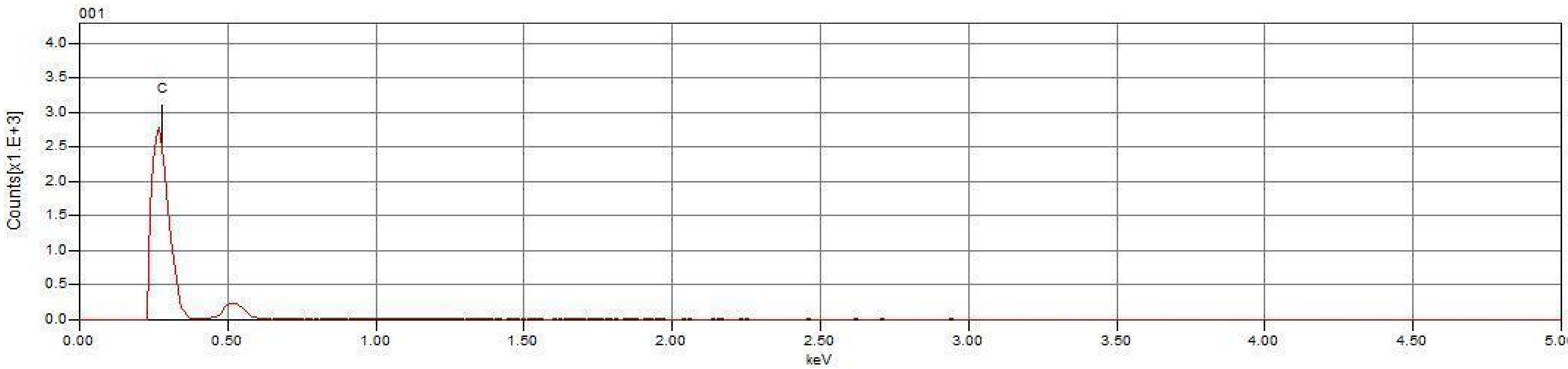

Figure 42: An EDS spectrum of OBC (replicate #1) showing the presence of carbon.

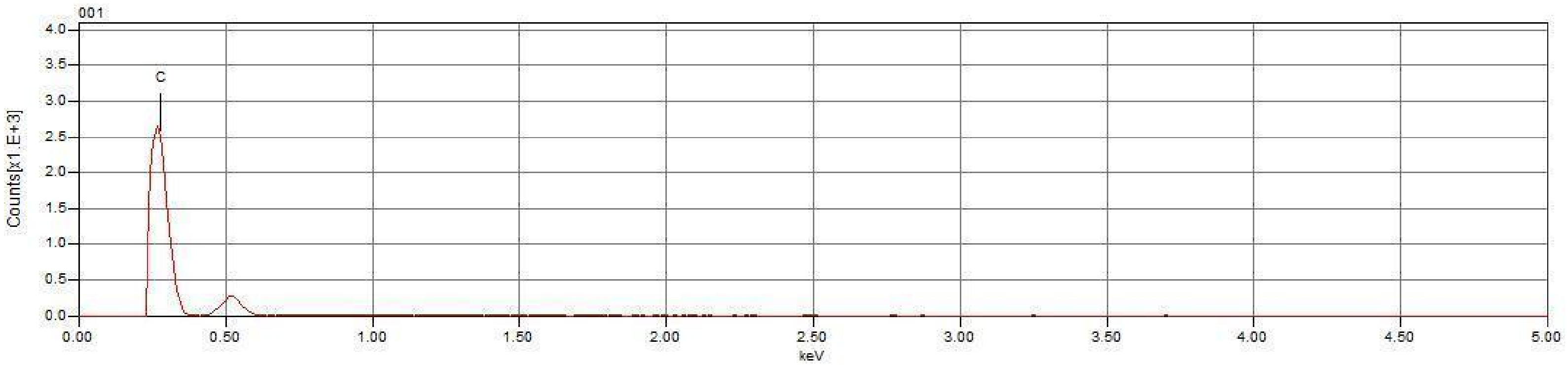

Figure 43: An EDS spectrum of OBC (replicate #2) showing the presence of carbon.

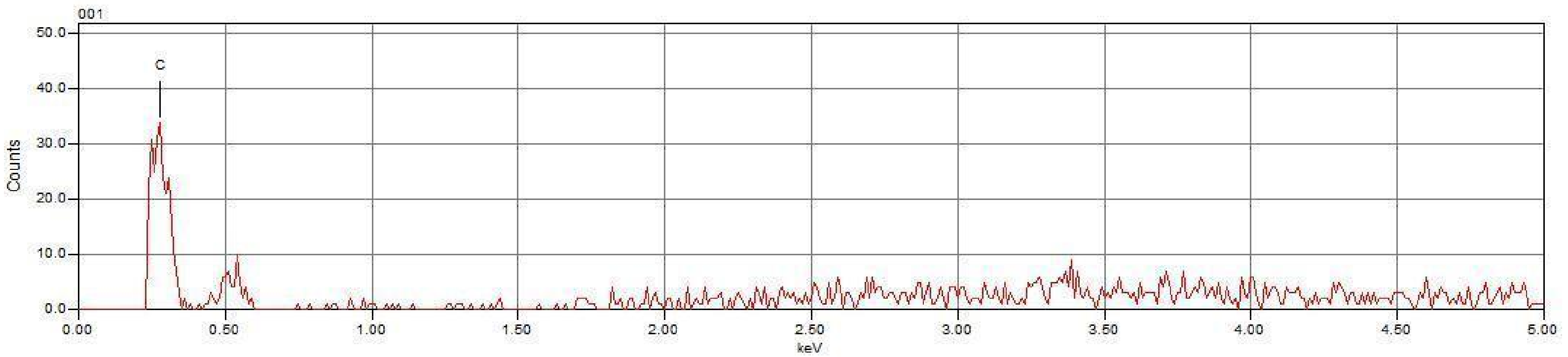

Figure 44: An EDS spectrum of OBC (replicate #3) showing the presence of carbon.

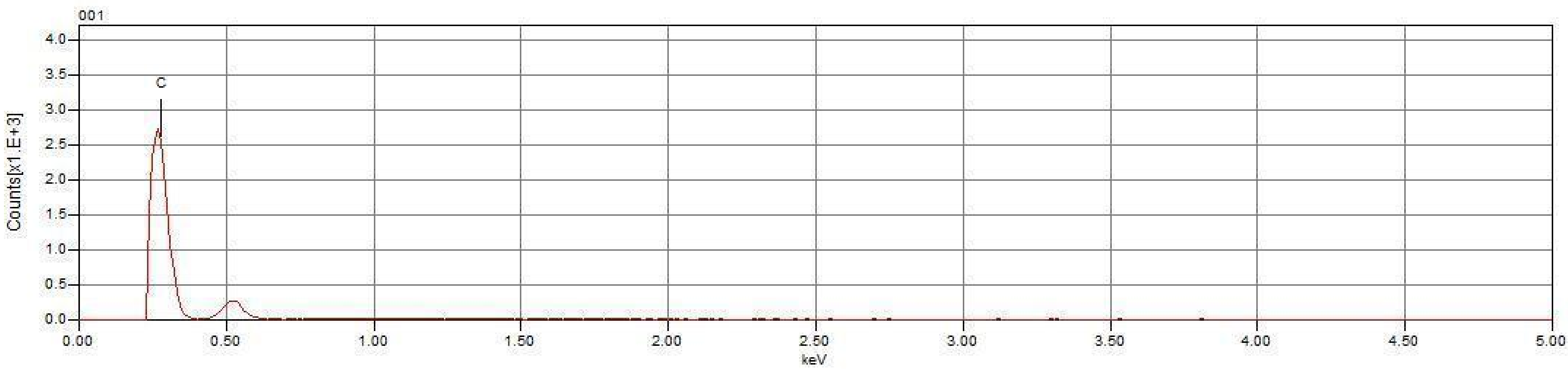

Figure 45: An EDS spectrum of OBC (replicate #4) showing the presence of carbon.

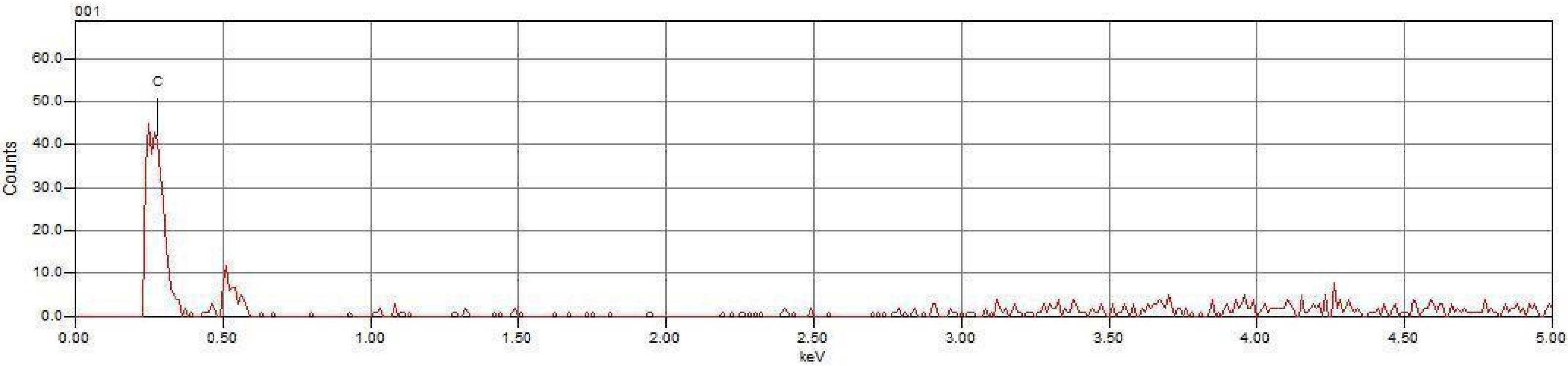

Figure 46: An EDS spectrum of OBC (replicate #5) showing the presence of carbon.

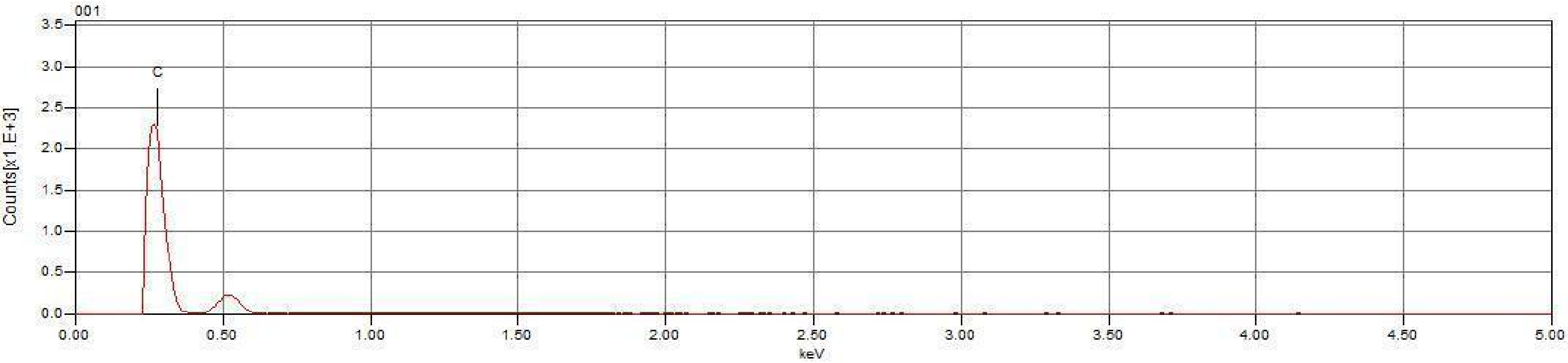

Figure 47: An EDS spectrum of OBC (replicate #6) showing the presence of carbon.

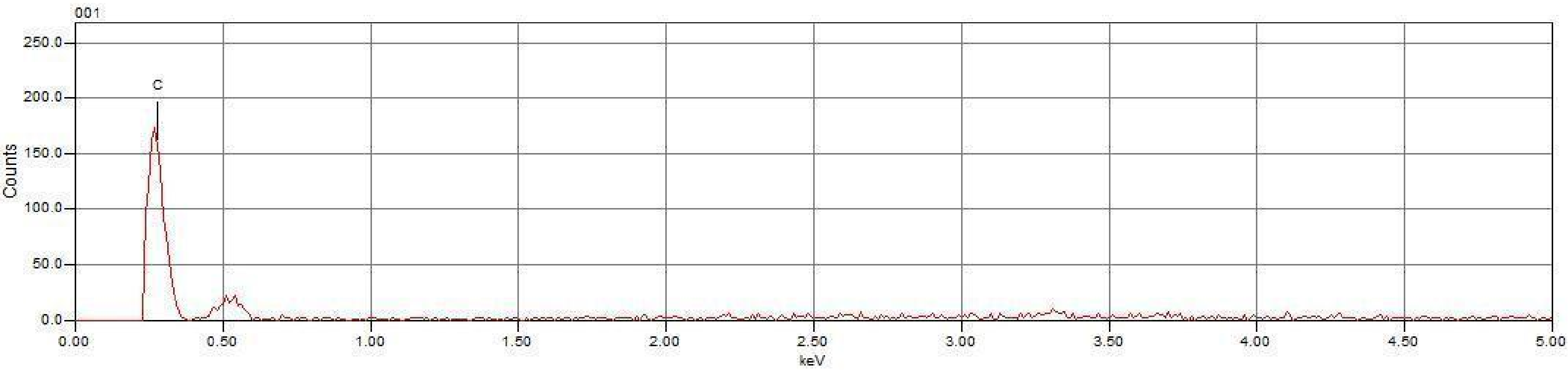

Figure 48: An EDS spectrum of OBC (replicate #7) showing the presence of carbon.

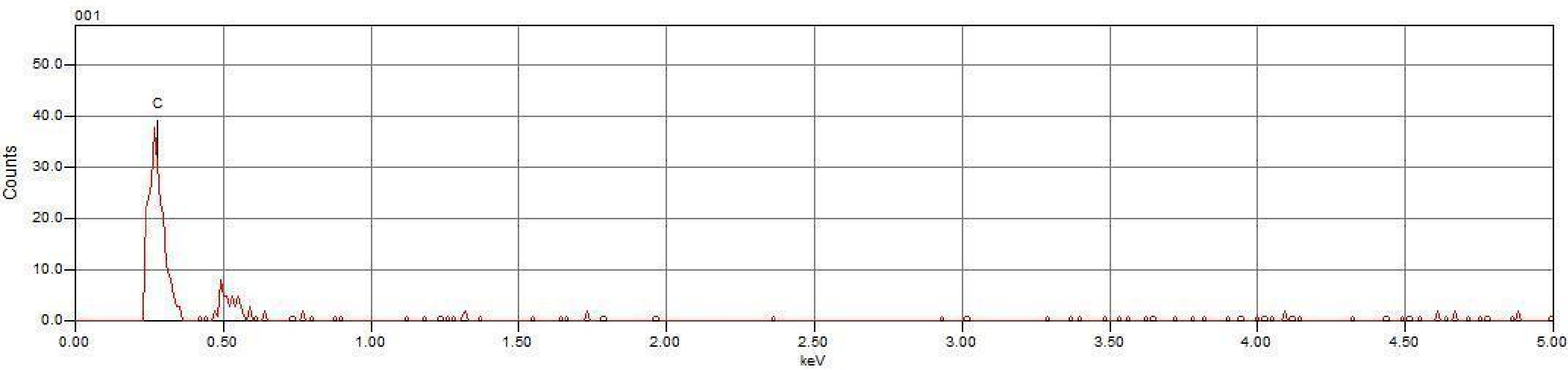

Figure 49: An EDS spectrum of OBC (replicate #8) showing the presence of carbon.

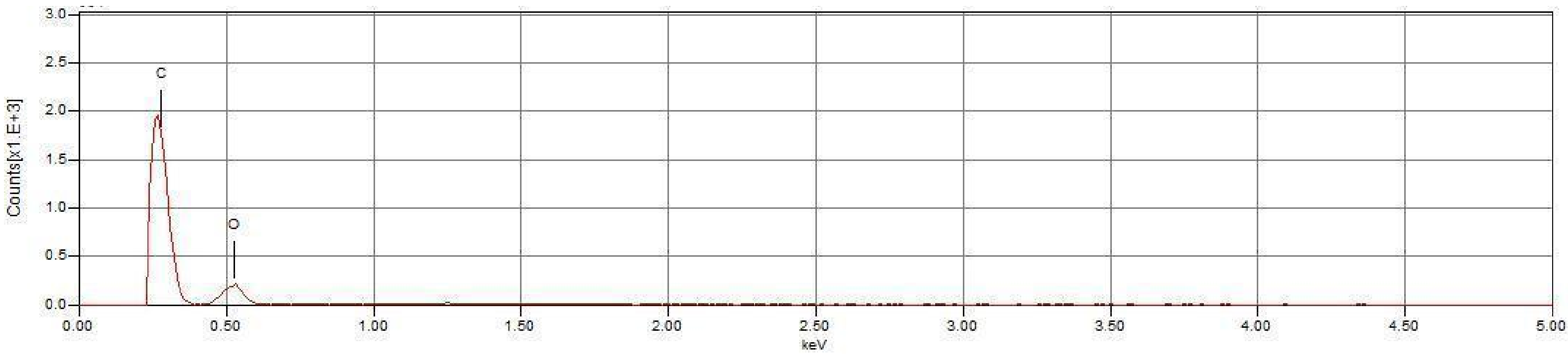

Figure 50: An EDS spectrum of OBC (replicate #9) showing the presence of carbon and oxygen.

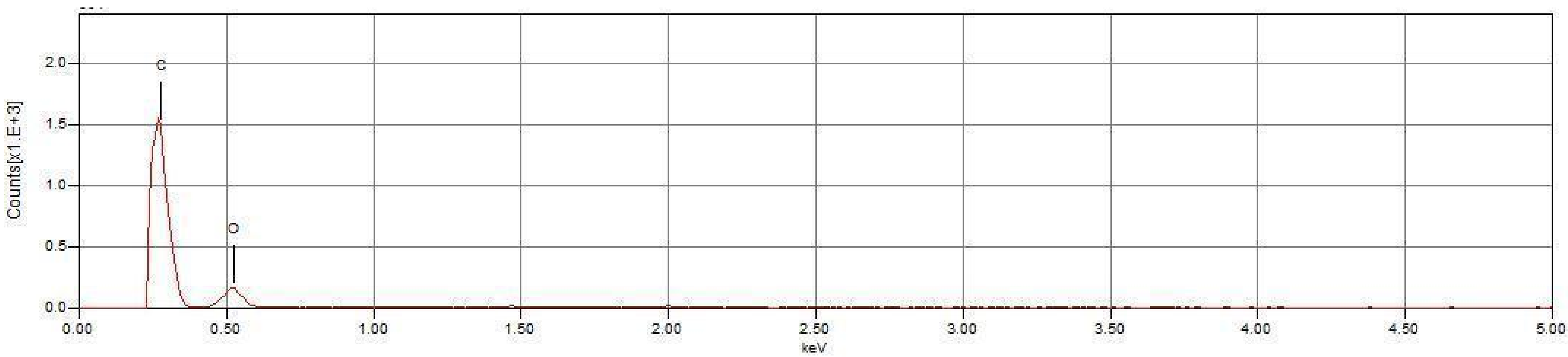

Figure 51: An EDS spectrum of OBC (replicate #10) showing the presence of carbon and oxygen.

5.11 <u>Microscopy Experiment #2.2 Results: Qualitative Energy-Dispersive X-ray Spectroscopy Elemental Analyses of Rush Biochar</u>

Observations of the qualitative data produced by the 10 EDS spectra above confirm the presence of carbon, oxygen, chlorine, potassium, sodium, magnesium, calcium and silicon in all 10 samples of RBC tested by EDS in the SEM. The elements confirmed for each replicate (fig. 53-62) was as follows:

- Replicate #1: oxygen, sodium, phosphorous, chlorine, potassium and calcium
- Replicate #2: oxygen, sodium, magnesium, silicon, chlorine and potassium
- Replicate #3: chlorine and potassium
- Replicate #4: phosphorous chlorine potassium
- Replicate #5: carbon, sodium, phosphorous, chlorine and potassium
- Replicate #6: sodium, chlorine and potassium
- Replicate #7: chlorine and potassium
- Replicate #8: sodium phosphorous chlorine potassium
- Replicate #9: chlorine and potassium
- Replicate #10: phosphorous chlorine potassium

It should be noted that even though there are observable spikes of data in the region where carbon's presence should be confirmed, it can be seen that 'C' has not been assigned on the

EDS spectra (except fig.57). If the duration of EDS performed on the samples was perhaps extended or shortened the presence of carbon may well have been detected

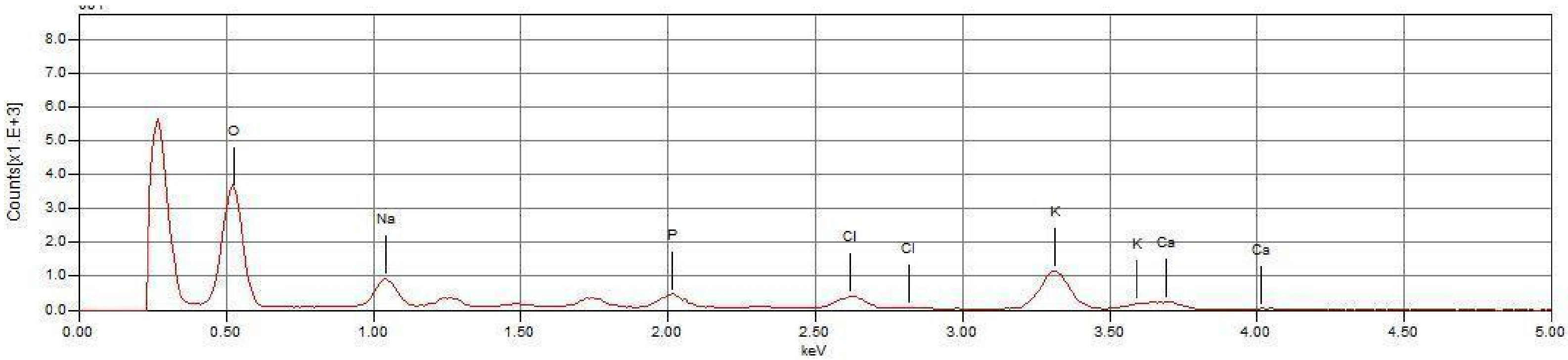

Figure 53: An EDS spectrum of OBC (replicate #1) showing the presence of elements.

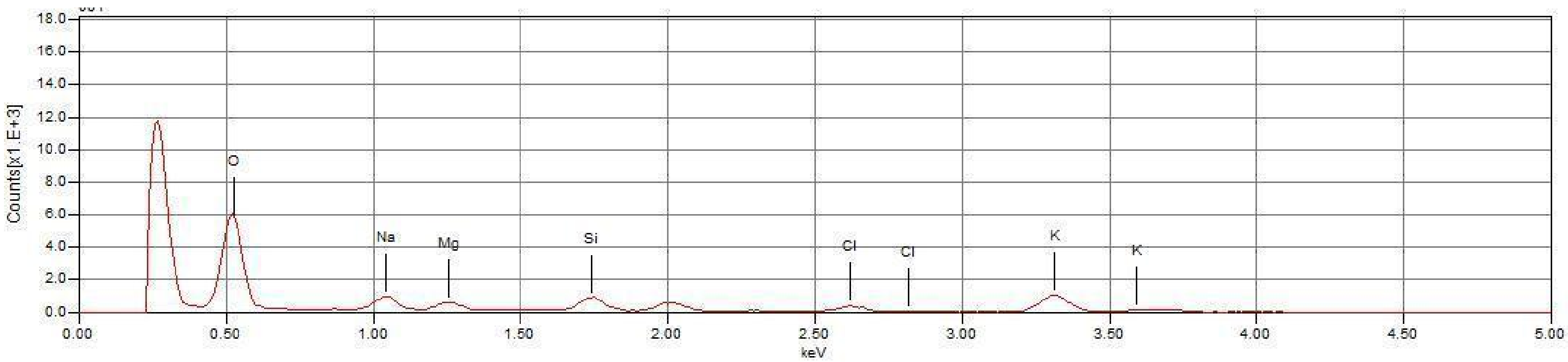

Figure 54: An EDS spectrum of RBC (replicate #2) showing the presence of elements.

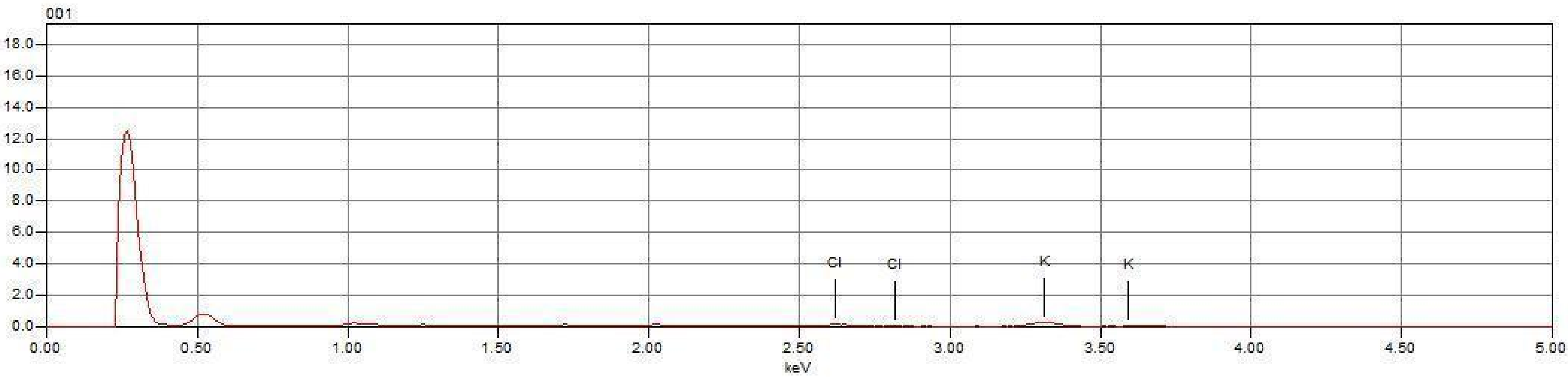

Figure 55: An EDS spectrum of RBC (replicate #3) showing the presence of elements.

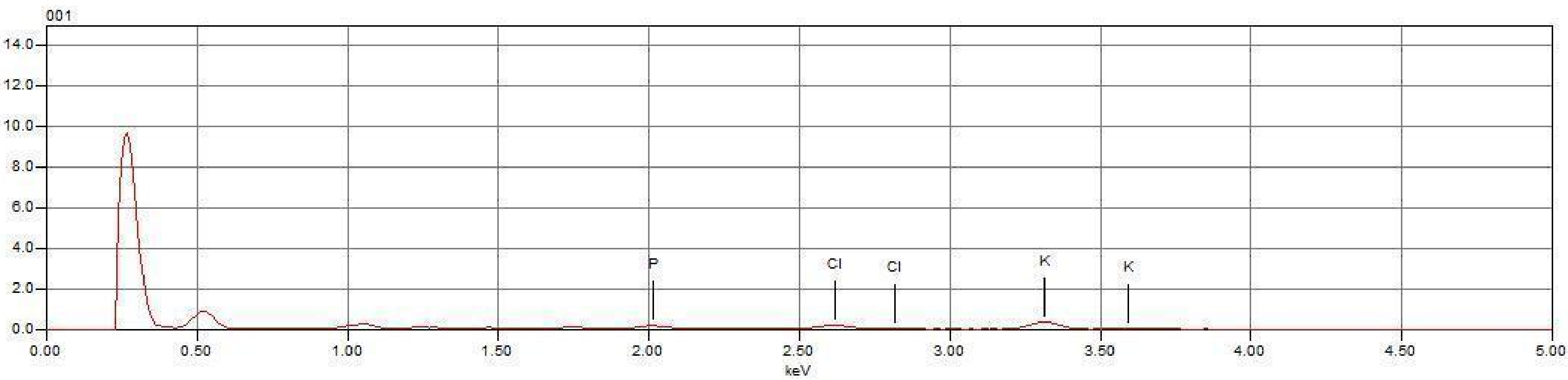

Figure 56: An EDS spectrum of RBC (replicate #4) showing the presence of elements.

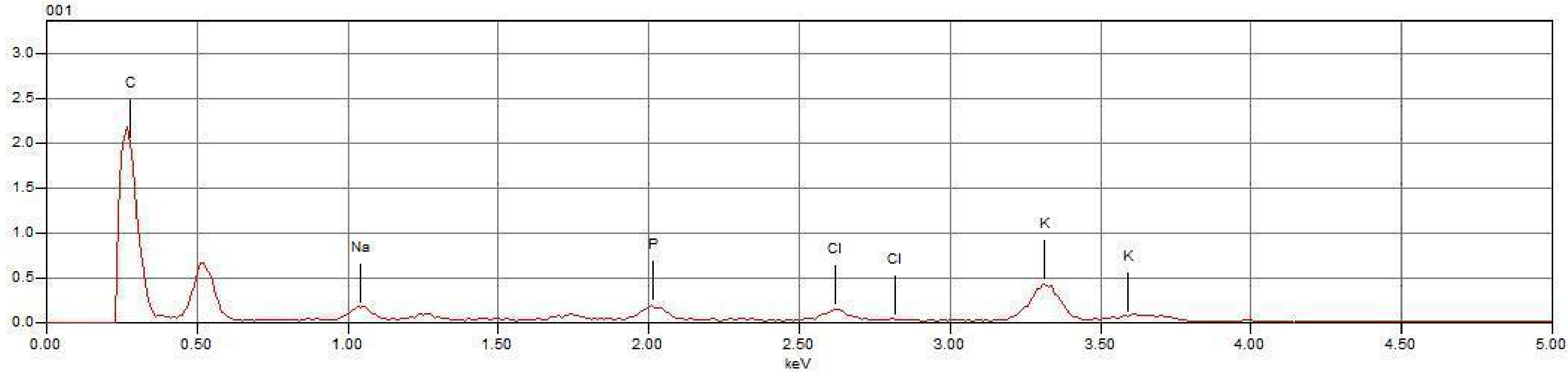

Figure 57: An EDS spectrum of RBC (replicate #5) showing the presence of elements.

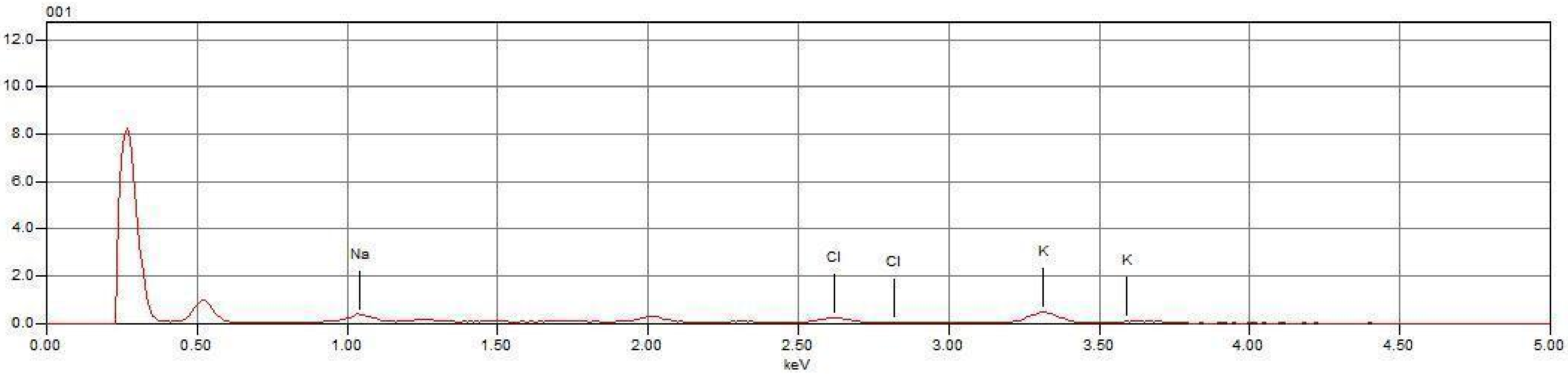

Figure 58: An EDS spectrum of RBC (replicate #6) showing the presence of elements.

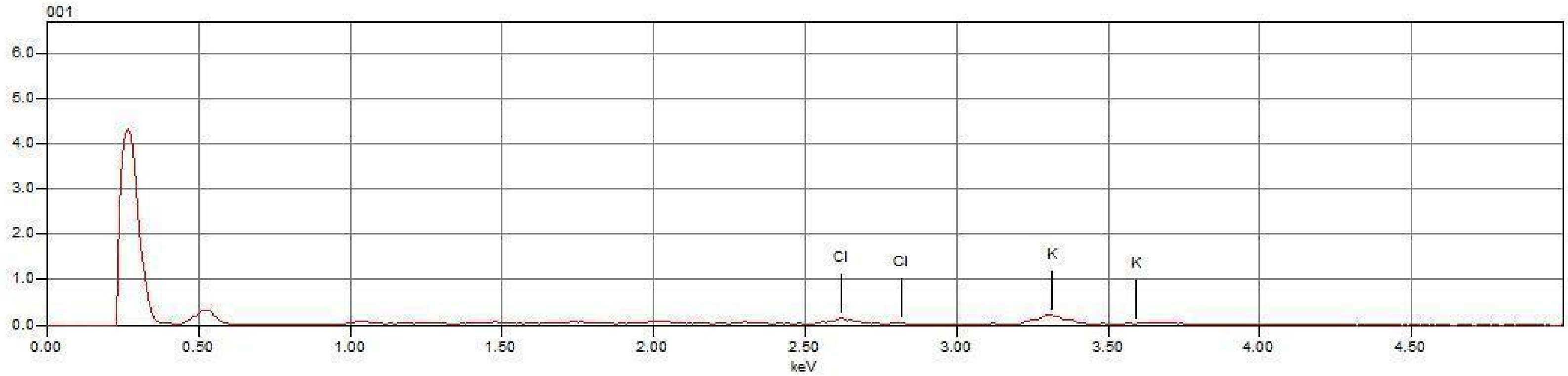

Figure 59: An EDS spectrum of RBC (replicate #7) showing the presence of elements.

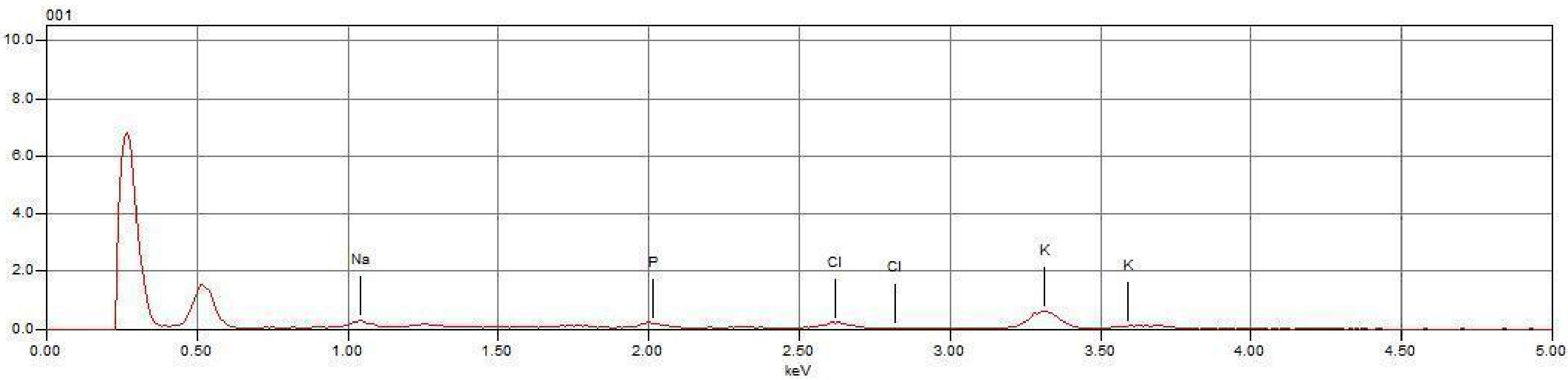

Figure 60: An EDS spectrum of RBC (replicate #8) showing the presence of elements.

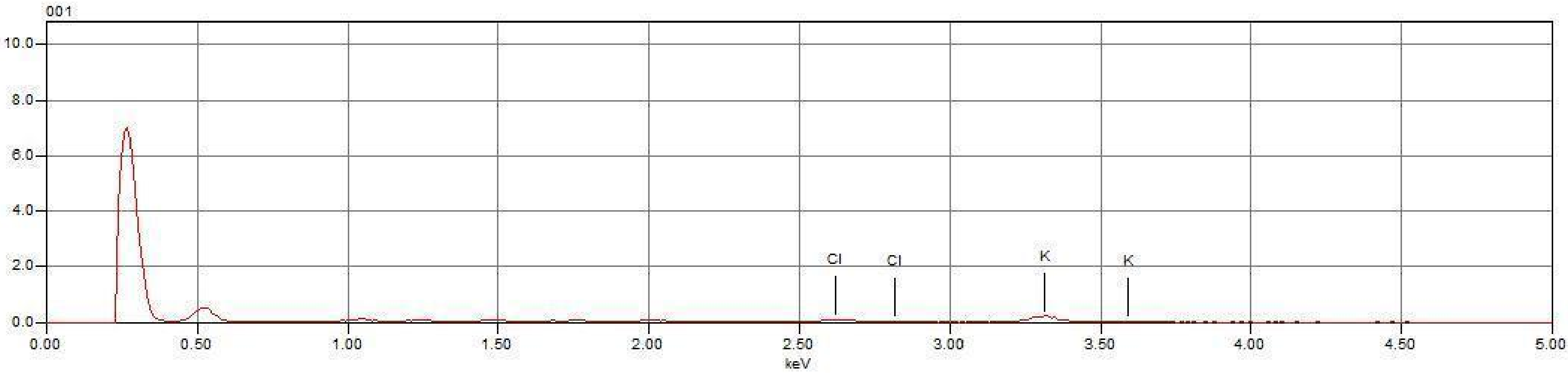

Figure 61: An EDS spectrum of RBC (replicate #9) showing the presence of elements.

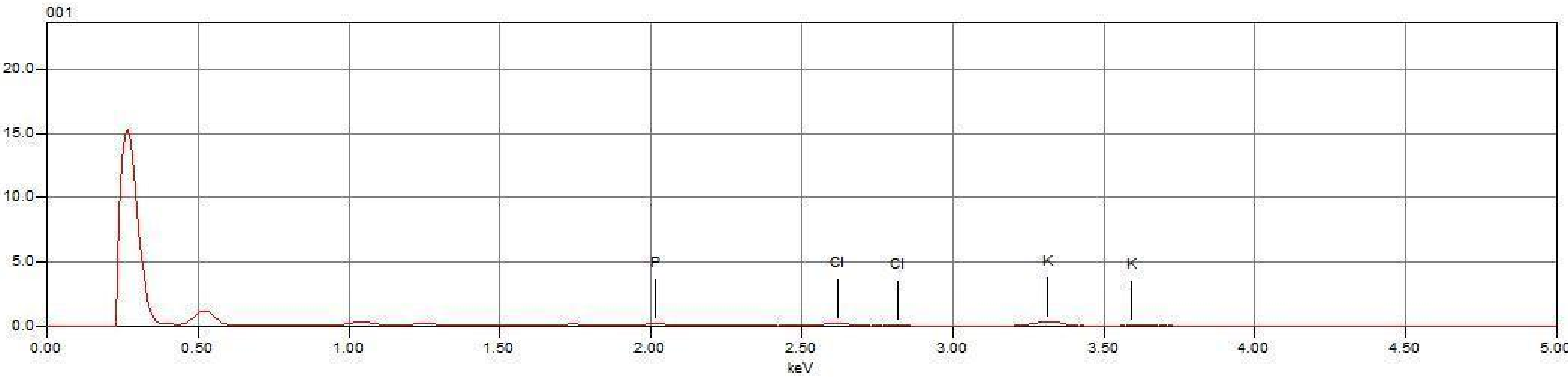

Figure 62: An EDS spectrum of RBC (replicate #10) showing the presence of elements.

6. DISCUSSION

6.1 Oilseed Rape Plant Growth Response to Oak Biochar with Peat Free Compost and Rush Biochar with Peat Free Compost

6.1.1 Response to Oak Biochar with Peat Free Compost

A hypothesis was to test that OBC+PFC could have an effect on OSR growth and yield. Looking at the data presented in section 5.1.1 (fig. 18), this mixture does not appear to affect the growth of OSR. Growth of OSR over the 6-week growth period was uniform across both treatments until after week 2 where growth remained similar until the end of the trial. Therefore, results were found to be statistically insignificant. The plant fresh weights and dry weight data were also found to not be significant.

Statistical significance aside, an observation of a recurring theme was noted. OSR plants grown without OBC+PFC had higher yields than those grown with the treatment.. All OSR plant measurements with OBC+PFC were negatively affected compared to the control. There have been many studies on short term applications of BC or COMBI (co-composted biochar) such as in this thesis, which have shown positive initial effects on soil N_2O emissions but have failed to have a positive effect on plant growth and yield (Melaku et al., 2020; Bonanomi etal., 2017; Darby et al., 2016; Jay et al., 2015).

In 2015, Jay et al demonstrated that short term applications of hardwood BC from *Castanea sativa* into a sandy loam soil in the UK saw no statistically significant increases in plant growth. Specifically, they found no increases in potato (*Solanum tuberosum L.*) shoot growth, tuber quality or yield; strawberry (*Fragaria x ananassa*) leaf growth, yield or fruit quality; and spring wheat (*Hordeum vulgare L.*) plant growth or yield and grain quality. As mentioned before, those soils in greatest need of amelioration tend to have the most positive responses to BC treatments.

Naveed et al (2021) demonstrated that COMBI used in chromium contaminated soils, growing OSR had positive results. They found that the plant growth of OSR (height, shoot dry weight, root dry weight, root length) and phytostabilization were significantly increased while also significantly reducing enzymatic antioxidant activities against chromium related stress.

A putative reason for the absence of an increase in crop growth of OSR with a short-term oak biochar treatment is the lack of additional P being made available to the plant (Jay et al., 2015). Initial biochar applications without the application of extra P can lead to direct decline in available P (Jay et al., 2015; Nelson et al., 2011).

6.1.2 Response to Rush Biochar with Peat Free Compost

To test whether RBC+PFC had an effect on OSR growth and yield was another hypothesis of this thesis. In glasshouse experiment #2, the control and test replicates demonstrated an observable but insignificant difference ($p = 0.4803$) in plant height over the 6-week growth period. Plants treated with RBC+PFC appeared to have reduced growth compared to that of the control (fig.22). Again, there was an observable but insignificant difference between the means for OSR plant fresh and dry weights (fig.23) with a p-value of 0.424, results were not significant.

In section 5.2 OSR plants were negatively affected by RBC+PFC compared to the control. A study by Thers et al (2020) showed that after an initial growing season of OSR in a sandy loam soil with a wheat (*Triticum aestivum L.*) straw BC (a grass BC more similar to RBC), there was no significant increase in plant growth or yield. Wang et al (2019) showed in a meta-analysis that grain yield was only significantly increased (up to 39.7%) in cereal crops ($p < 0.001$) like maize (*Zea mays*), barley (*Hordeum vulgare*), quinoa (*Chenopodium quinoa*), wheat (*Triticum aestivum*) and oat (*Avena sativa*) compared to other plants like bok choy (*Brassica rapa*) and OSR.

Another study demonstrated how OSR grown in a sandy loam soil was not affected after each growing season by the presence of a wheat (*T. aestivum L.*) BC, even after three consecutive years of application (Hansen et al., 2017). A meta-analysis by Wang et al (2019) of crops grown with a COMBI treatment showed that only a statistically significant increase in yield was experienced by cereal crops. That other plants such as OSR did not show a significant positive difference in yield, however the control (compost) did show a significant increase.

However, another study showed that wheat (*T. aestivum L.*) straw biochar inoculated with inorganic phosphate solubilizing bacteria (iPSB), saw positive OSR growth due to the biochar sorption abilities, making available phosphate made possible by the iPSB, available to the plant (Zheng et al 2019).

6.2 Perennial Ryegrass Plant Growth Response to Oak Biochar with Peat Free Compost and Rush Biochar with Peat Free Compost

6.2.1 Response to Oak Biochar with Peat Free Compost

A hypothesis was to test that OBC+PFC could have an effect on PRG growth and yield. Looking at the data from the tables presented in section 5.1.3 there appeared to be little difference between plant height ($p = 0.7683$), fresh weight ($p = 0.5465$), dry weight ($p = 0.519$) and dry weight values. All three parameters were found to be insignificant. Mean plant heights varied at the end of week 6, OSR plants were found to be positively affected by the test treatment than the control, but only slightly.

Plant yields have been known to be neutrally or negatively affected by the over-liming effect of a BC (Vijay et al., 2021). Woody BC (such as oak) can have an inherently high pH and when in the presence of soil which is acidic can have a negative or neutral impact on plant growth and yield (Shetty and Prakash., 2020). On average composts are between 6 - 8 pH (Taylor et al., 2016), however some composts can be as low as 4.5 (Jamal et al., 2018).

6.2.2 Response to Rush Biochar with Peat Free Compost

The effect of RBC+PFC (fig.24) on plant height appears to have had a positive effect on PRG growth here compared to the PFC treatment and all previous test treatment trials in experiment #1 and #2. Looking at the plant fresh and dry weights the same can be observed, both weights for the test treatment have been more positively and significantly affected than the control treatment. This would suggest that RBC+PFC had a greater effect on PRG as a target crop.

Głąb et al (2020) demonstrated in a sandy loam soil potted, glasshouse experiment that 2 grass biochars Miscanthus (*Miscanthus x giganteus*) and wheat (*Triticum aestivum L.*) both had positive effects on PRG growth and productivity. Their results saw highly significant effects of almost double the biomass yields and root dry matter density than that of the control. Głąb et al also found that root length density, root volume density and root surface area were all significantly greater than that of the control.

In 2016, Vandecasteele et al saw a significant increase in PRG of 34.7% when using a holm oak (*Quercus ilex*) COMBI treatment in field trials.

6.3 Oilseed Rape Plant Growth Response to Charged Oak Biochar with Peat Free Compost and Charged Rush Biochar with Peat Free Compost with Abiotic Stress Exposure

To obtain positive results for OSR growth and yield in the presence of OBC+PFC and RBC+PFC, large amounts of the treatments (the same ratios and components used in glasshouse experiments #1 and #2, uncharged) were left outside to ‘charge’. This was an attempt to incubate the BC and PFC and determine its plant growth promoting effect. Allowing the BC more exposure for nutrient absorption in the presence of the PFC and atmosphere.

After subsequently repotting for the following revised experiments, the same plant species were tested against both charged treatments concurrently with an abiotic stressor.

6.3.1 Oilseed Rape Response to Charged Oak Biochar with Peat Free Compost and Charged Rush Biochar with Peat Free Compost with Abiotic Stress Exposure

COBC+PFC and CRBC+PFC did have an effect on OSR plants. However, these were both negative effects compared to the control.

In this experiment an attempt was made to establish if a BC based, growth promoting treatment media could potentially fill the gap left by the phasing out of peat used for peat-based composts. Since many before have used BC in combination with composts as COMBI to induce positive plant growth (Wang et al., 2019; Bonanomi etal., 2017; Darby et al., 2016; Jay et al., 2015) either with or without added fertilisers, it was hypothesised that leaving mixtures outside for long periods could significantly charge the treatments for subsequent repotting. Schmidt (2008) had said that a minimum of two weeks was required to charge a BC without any extra chemical/fertiliser components needing to be added.

However, when comparing the observational and statistically significant differences between experiments #1 and #2 to these charged treatment experiments, the latter appears to have had a much greater (albeit negative) effect on plant growth.

It is likely that this decreased growth effect on OSR is a matter of nitrogen immobilisation caused by the overly high ratio of nitrogen to carbon within the charged treatment (Vijay et al., 2021). When microbes typical of the soil or in the mixture are experiencing growth and proliferation, they require a balanced N:C ratio of 1 mol of N for every 10 mol of C as food (Bonanomi et al 2017). If there is not a sufficient amount of nitrogen in the mix when an extra carbon source is added, this leads to the scavenging by microbes of available N in the soil/mixture. Thus, leaving too little N for the plant to assimilate and greatly affecting the growth of the plant (Bonanomi et al 2017). Another cause of nitrogen immobilisation is the insufficient pyrolysis of a feedstock into biochar, which can leave a lot of unnecessary carbon available to soil microbes (Brewer and Brown., 2012). Thus, negatively affecting plant or crop growth.

Despite all the above, many COMBI experiments have improved crop yields when applied either in the first year of application or in the subsequent years following an initial COMBI application.

Willow wood co composted with bagasse, green waste, poultry fertiliser increased grain yields of maize by 10% to 13% compared to the control (Agegnehu et al., 2016). The same COMBI experiment saw increases of peanut pod yields by 17 % to 24% compared to the control.

A COMBI made with pyrolyzed wood chippings, compost and horse, poultry and cow manure was left to charge for 60 days (Kammann et al., 2015). This COMBI mixture was grown with quinoa and in the first growing season obtained a very large yield of 305% whilst the mixture of uncharged biochar showed a 60% decrease in yield compared to the control. The composting process used was proven by scanning electron microscopy to have a direct effect on the BCs organic content (Kammann et al., 2015).

6.4 Perennial Ryegrass Plant Growth Response to Charged Oak Biochar with Peat Free Compost and Charged Rush Biochar with Peat Free Compost with Abiotic Stress Exposure

Both treatments were demonstrated to have a statistically significant effect on the growth on the PRG plants. However, these effects were shown to be negative compared to the control, rather than the desired effect of positive growth promotion.

6.4.1 Perennial Ryegrass Response to Charged Oak Biochar with Peat Free Compost and Charged Rush Biochar with Peat Free Compost with Abiotic Stress Exposure

Much like the response of OSR in section 6.3.1, the PRG response to the control:abiotic stressor combination out-performed both charged treatments. However, the charged rush:abiotic stressor treatments was the most statistically significant and similar to the control:abiotic stressor treatments. Echoing the performance of RBC+PFC with PRG plants (sections 5.1.3 and 5.1.4) the CRBC+PFC was statistically the better treatment out of the two charged treatments. Between the three combination treatments the salt stressed plants remained statistically similar.

As with section 6.3.1, nitrogen immobilisation is a likely mechanism within the charged treatments leading to decreased yields of the tested plants. The over-liming effect could also be a potential factor playing a role.

Another mechanism that may have influenced further decreases in plant growth and yields would be the exudation of allelopathic compounds by weeds which have colonised the large tubs of COBC+PFC and CRBC+PFC as a substrate over the 6-months. As much as was possible (every few weeks) every effort was made to remove the haulm-based portion of the juvenile weeds present (*fig.63 & 64*). Common Irish weeds such as Dandelion (*Taraxacum officinale*) Cat's Ear – (*Hypochaeris radicata*) Nipplewort – (*Lapsana communis*) Ragwort – (*Senecio jacobaea*) have been known to exudate allopathic compounds aqs biotic stressors to crops currently or subsequently growing in the substrate they are present (Możdżeń et al., 2021; Jankowska et al., 2014; Jacobs and Sing., 2009; Kim et al., 2005).

Figure 63 & 64: Firstly shows juvenile weeds growing on the charged biochar treatment substrate and the latter shows most of the weeds removed. Photos: Eric Hynes, 2021.

Remaining plant litter such as extensive roots systems (fig.65) left behind by germinated weeds (once again, every effort was made to remove as much as possible) can directly affect crop root growth and thus overall crop growth and yields or indirectly by mediating microbial activity within the pots (Bonanomi et al 2017). All of which could be working synergistically with the N-immobilization and liming mechanisms, thus affecting subsequent crop growth further.

Figure 65: Extensive roots left behind by the growth of weeds in the large tubs of biochar treatments being charged outside of the glasshouses. Photos: Eric Hynes, 2021.

Regularly overturning a compost (every three to seven days) or composting feedstock is needed to provide oxygen for microbial respiration, aerobic digestion, kills weed seeds and antagonises germinated weeds and provides greater moisture distribution (Dahlquist et al., 2007).

Lashari et al (2013) demonstrated that a COMBI mixture including wheat biochar, pyroligneous solution (aka wood vinegar - a pyrolysis by-product) and poultry fertiliser gave a 36.5% increase to wheat yields which were salt stressed in an antisol soil, the second year of crop growth. This result was in comparison to the control without the pyroligneous solution and also after neutral results obtained in the first growing season.

Schmidt et al (2017) demonstrated that a COMBI co composted with NPK fertilizer and resulted in yields around 20% with a standard deviation of 5.1% over 4 experiments testing different cash crops. However, another COMBI, this time co composted with cow urine produced yield of up to 123% with a standard deviation of 76.7% over 13 experiments testing different cash crops. However, these sorts of powerful N fertilising approaches could have potential detrimental effects to the environment via soil nitrate leaching (Haider et al., 2016).

6.5 Oilseed Rape Stress Response to Charged Oak Biochar with Peat Free Compost and Charged Rush Biochar with Peat Free Compost with Abiotic Stress Exposure

SPAD measurements taken by the Photosynq Multispeq™ showed that the three groups of treatment and stressor combinations all had significantly different effects from each other on the plant health of OSR. Unfortunately, the surface area of PRG was too small for the Photosynq Multispeq™ to be able to take a measurement. Therefore, it was omitted from this experiment.

SPAD values are a measurement of either reflecting chlorophyll content and chlorophyll fluorescence. A higher SPAD value is a measurement of higher concentrations of chlorophyll in a leaf. A greener, more chlorophyll dense leaf is a healthier leaf. Thus, a higher SPAD value means a healthier leaf and plant (Chunjiang et al., 2007).

In section 5.5 (fig.32) the PFC group had the overall greatest effect on SPAD values and plant health. Whereas, both the CRBC+PFC and COBC+PFC groups negatively affected plants growing under stress.

Figure 66: The poor appearance of OSR plants growing in the PFC + drought stress plant pots (bottom right) with contrasting CRBC+PFC + drought stress plant pots (top left). Photo: Eric Hynes, 2021.

So far, there is a gap in the literature of evidence for the negative effects of plants grown in the presence of a BC and stressor. This research has shown in this case, that BC exacerbated the effects of drought and salt stressors on plant growth. This is confirmed by the results of the PFC group through section 5.5.

6.6 Quantitative Analyses of Both Biochars Using Scanning Electron Microscope

The results from section 5.6 show that the mean pore surface area of an OBC is almost double (259.45 µm) that of a RBC (133.24 µm) (fig.33). However, the two sample t-test which was run for these findings was not significant and this is most likely due to the very high variance sizes of the pore surface areas of both BC types.

The results from section 5.7 demonstrated that the mean pore frequency per BC surface area for OBC was over 4 times (2164.79μm^2) that of the RBC (526.01μm^2) (fig.34). However, as above, the two-sample t-test which was run for these findings was not significant and this is most likely due to very high variance found between the pore frequencies of the BCs (especially OBC) and varying surface area sizes of each biochar sample.

The sheer magnitude of structural differences exerted between the two types of biochars can be explained by the percentage of lignin, cellulose and hemicellulose content constituting the biomass of a given species of feedstock (Rangabhashiyam and Balasubramanian, 2019).

Trees (such as oak) and woody plants are known to have high levels of lignin to cellulose and hemicellulose content (Novaes et al., 2010). Pore composition (size and frequency) of a biochar is influenced by the percentage of lignin content of a feedstock and the pyrolysis temperature (Břendová et al., 2017). The temperature needed for polymer decomposition to form a BC from a high lignin feedstock is between 250-500°C (Kong et al., 2014). The frequency of micropores can be increased by elevating the pyrolysis temperature and the amount of lignin available to the pyrolysis regime (Břendová et al., 2017).

Grasses (such as rush) and herbaceous plants have inherently low amounts of lignin compared to a high cellulose and hemicellulose content and are said to have a lignocellulosic biomass (Waliszewska et al., 2021). The temperature needed for polymer decomposition to form a BC from a lignocellulosic feedstock is between 200-350°C (hemicellulose) and 305-375°C (Kong et al., 2014). Thus the lack of lignin content and lower temperature needed for polymer decomposition results in far fewer pores. This explains the large differences in pore frequencies between thetwotypes of BC.

6.7 Qualitative Analyses of Both Biochars Using Scanning Electron Microscope

The qualitative results generated by energy dispersive X-Ray spectroscopy in sections 5.10 and 5.11 show us that C and O were the elements present in both BC types. However, with the OBC it can be seen that these elements were the only one's present. Whereas with the RBC there was also Cl, K, P, Na, Mg, Ca and Si present in the samples.

The presence of C and O in biochar samples are due to a couple of reasons. The condensed aromatic structures of the feedstock biomass have C as the principal element, controlling the organic phase of the BC (Uchimiya et al., 2011). Furthermore, O is a central elemental to the organic, polar functional groups (Uchimiya et al., 2011). These both influence the reactivity of a BC within a soil ecosystem (Bakshi et al., 2020).

The presence of the Cl, K, P, Na, Mg, Ca and Si elements found in the RBC are based on whether a low or high heating rate was used and the overall pyrolytic temperature. For lignocellulosic biomasses a low rate of 2 - 5°C/min should be utilized (Mohanty et al., 2013). The agricultural and geographical location of where a plant has grown can also influence what microelements which are present in a lignocellulosic biomass (Bakshi et al., 2020).

7. CONCLUSIONS AND RECOMMENDATIONS

Overall, the addition of an OBC and RBC to a peat-free compost as separate growth media treatments had mostly neutral to negative effects on plant-growth promotion. However, the positive effect of RBC on PRG in RBC+PFC and 4 would suggest that PRG may be an ideal target plant for RBC or as part of a BC and peated/PFC treatment. Furthermore, PRG has known green manure capabilities which directly affect soil health (Vystavna etal., 2020) e.g. the plants extensive root system mitigates nitrogen loss via leaching and soil physical structure via amelioration of soil compaction or drought affected soils (Mauro et al., 2015). If PRG was used synergistically with a RBC or COMBI treatment could further benefit a particular soil experiencing low soil health/productivity.

The effort to develop a potentially new COMBI method/alternative growth media to induce positive plant growth was largely unsuccessful. As detailed in this project, the COMBI approach to BC application has seen many benefits in crop production/soil health amelioration. However, these COMBI studies share a common theme - BC was added at the start of composting (a COMBI standard), therefore aerobic digestion of plant material and reactions with BC is key to charging a BC. Antonangeloe et al (2021) showed that mixing a BC with a mature compost yielded neutral to negative results in plant growth, compared to the standard COMBI method.

The Irish horticultural industry is in desperate need of a replacement to peat which is still being imported into the country in large quantities. They are calling on the Irish government to allow peat harvesting from Irish bogs while increasing research funding for suitable alternatives i.e. BC (O'Sullivan., 2021). The banning of peat for environmental reasons makes little sense when such quantities are being imported for horticultural and domestic uses.

Given the lack of positive plant growth increases in temperate soils, further BC potted trials should also include the target soil type with its COMBI mixture i.e. podzol with a standard COMBI mixture. Directing further BC research to find the right target crops/soil types and pyrolysis regime of a feedstock to optimize COMBI production is essential for creating stable alternative growth media. Furthermore, increasing our understanding of the pH and electrical conductivity of a BC and target soil type will allow us to optimise amendment capabilities for greater sustainable agriculture.

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