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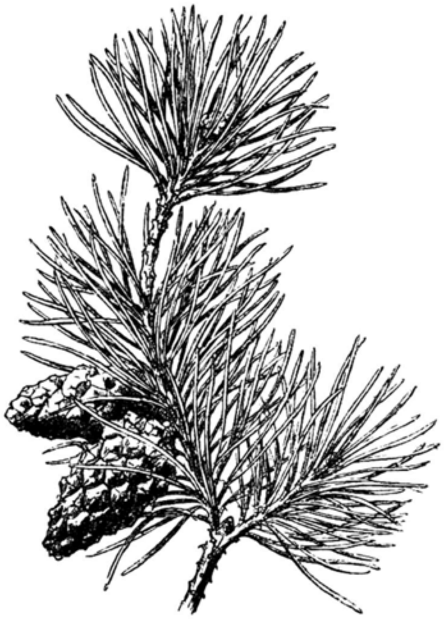
Biochar in forest establishment: synergies between nutrient delivery, growth and carbon storage

Hamish Alexander Creber

Submitted for the degree of Doctor of Philosophy
The University of Edinburgh
School of GeoSciences
2022

Dedicated to Sephera, who supported me and made this work possible.



Scotch Pine.
Pinus sylvestris.
— CONIFERÆ. —

Declaration

The work presented in this thesis is original and was composed by the author, unless indicated otherwise as part of a joint publication (part of chapter 6 and Appendix 2 only). The candidate confirms that appropriate credit has been given where reference has been made to the work of others and contributions are detailed at the end of each Chapter. No part of the thesis has been submitted for any other award or professional qualification. Where work has been published the contribution of co-authors was provided.

Part of Chapter 6 was produced by for Scottish Forestry under the title; Disturbance is key: time to delivery of net climate mitigation from woodland creation. Insights from two empirical soil carbon models. Hamish Creber[1], Mike Perks[2], Saran Sohi[1], Robert Matthews[2] & Elena Vanguelova[2] ([1] University of Edinburgh [2] Forest Research). The contributions of each author are detailed in Section 6.6.

Appendix 2 was a supplementary project to this thesis, which was conceived and designed by the author and published; K. Lei, H. Creber, R. Bol, A. Tietema and S. P. Sohi (2022). "Preferences of Pinus sylvestris seedling roots for different phosphorus sources under phosphorus-deficient conditions." Plant and Soil. (DOI: https://doi.org/10.1007/s11104-022-05682-0).

Hamish Alexander Creber

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Lay Summary

Forests are vital for sustainable timber production, carbon storage and ecosystem services. Historic planting in the U.K. has created a legacy of forests located on soils which have low productivity and are considered as nutrient poor. Forest harvesting practices are degrading some forest soils, with an incremental decline in soil nutrients. Climate change is also impacting forest establishment. More frequent drought events can cause seedling death or reduced growth, with young seedlings more susceptible to environmental stresses. Forestry must seek innovative solutions, such as biochar, as the industry adapts to future pressures.

Biochar is a charcoal-like product that is produced by heating biomass, such as wood, at high temperatures and low oxygen, in a process called pyrolysis. The process stabilises the carbon, that is stored in the biomass, preventing it from decomposing when it is added to the soil. Biochar has been found to improve soil productivity by improving a wide range of interlinked soil properties. The use of biochar to enhance seedling growth in forest planting on sites where the soil has low fertility, which prevents rapid tree growth, has not been widely studied.

Biochar was added to a range of common tree species and compared to conventional fertiliser. Biochar was found to *i*) increase seedling growth and improve seedling nutrition. *ii*) prevent plant stress, and associated growth decline, due to drought by locally improving the ability of soil to retain water around the seedling roots. *iii*) enhance the root system of treatments and symbiotic fungi (which acquire water and nutrients for the seedling) which improves the ability of the seedling to access water and nutrients held within biochar and soil. *iv*) aid the carbon storage of woodland planting by increasing growth and compensating for soil carbon loss in some soil types, which makes woodland creation more effective at delivering carbon storage.

The adoption of biochar by the forestry industry has the potential to limit some of the challenges facing forest productivity due to soil nutrient decline and climate change. Producing biochar from selected sawmill co-products has the potential to create biochar with ideal properties for use in forest planting. Nutrients can be cycled back into forest soils, which can contribute to enhanced circularity and a sustainable biochar. The use of biochar which has been produced to address critical soil limitations should be considered an appropriate method of localised soil improvement, benefiting forest growth and carbon storage.

Abstract

The increasing demand for sustainably produced timber and the expansion of land-based greenhouse gas removal technologies is driving the growth of the forestry sector in the U.K. This is creating increased competition for the finite land resource. Historic planting on nutrient limited upland soils, coupled with open nutrient cycles due to high intensity silvicultural systems, is creating a productivity crisis across upland plantation forestry. Climate change is expected to further impact the productivity of U.K forests and managing soil health is vital in ensuring crop resilience to our changing climate. Biochar is an established greenhouse gas removal technology (GGR) that can increase the carbon storage capacity of land and enhance the efficacy of other land based GGR technologies, such as woodland creation. The agronomic benefit of biochar is well understood, however, there has been minimal research thus far on the potential application of biochar in productive silvicultural systems as a novel method of forest fertilisation.

This thesis explores the potential utilisation of targeted biochar application to improve seedling growth on nutrient poor soils during the establishment phase of forest cycles. A selection of distinct biochar types, ranging from high to low available phosphorus concentration, were tested in a controlled environment growth experiment on Scots pine (*Pinus sylvestris* L.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) along with conventional phosphorus fertilisation intervention. A specified biochar was also developed to address the primary soil limitations on seedling establishment in phosphorus limited soils. The vascular cambial zone of roundwood is relatively high in phosphorus and is a readily available sawmill co-product produced during ring debarking. This feedstock was used to create VCZ biochar. The physical and chemical properties of this specified biochar address the limitations of low phosphorus upland forest soils. Biochar amendment was further tested in a large-scale field experiment on a typical phosphorus limited restock establishment site. The effects of biochar on seedling growth and morphology were variable, according to biochar properties, dose and seedling species. The moderate native biochar phosphorus content of VCZ biochar, coupled with high porosity and internal surface area, improved above ground biomass growth, stimulated root proliferation and enhanced mycorrhizal abundance. Optical and SEM microscopy was used to investigate the importance of root and hyphal interaction with biochar structure and the accessibility of available nutrients within biochar particles in relation to seedling nutrient uptake strategies. High phosphorus fertilisers and high phosphorus biochar were found to limit the development of below ground biomass and suppress mycorrhizal fungi, in turn impacting above ground biomass development. The mechanisms which contribute to the effect of specified biochar on seedlings was explored. Biochar is a direct source of micro and macro-nutrients and can also improve localised soil nutrient availability and accessibility through sorption, pH and biofertilisation. Foliar analysis showed that biochar can be used to alleviate seedling phosphorus deficiency. The effect of biochar on soil water holding capacity (WHC) was also found to be a critical factor in improving establishment and increasing drought resilience.

This thesis details the impact of biochar on forest carbon. The implication of forest establishment practices, including biochar amendment, on carbon balance and year of net carbon storage was modelled to assess the optimal scenarios for carbon storage on nutrient poor soils. Early growth improvements from low dose targeted biochar amendment have the potential to reduce the time to net carbon storage by three to six years on low yield organo-mineral planting sites. It can be concluded that the targeted application of specified biochar can improve early-stage seedling growth and enhance the development of the rhizosphere, providing greater silvicultural benefit compared to conventional forest fertilisation. This in turn can create longer term benefits to forest health, crop stability and future rotation yields. Enhancing the localised soil conditions through specified biochar amendment should be considered as a viable management intervention to increase forest resilience, species suitability, carbon sequestration and close nutrient cycles.

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In no particular order I would like to thank a number of people for supporting me in completing this PhD thesis and all of the tribulations along the way. Firstly, I would like to thank my wife Sephera for all the sacrifices she has had to make in supporting me over the past years and picking up the slack at home whenever I have been struggling. Well done to our lovely children Ruaridh and Finnian for putting up, some of the time, with the long evenings and weekends when I have had to sequester myself in the home office. If you are still reading, I will confess there is a slight order to this.

I am forever grateful for the invaluable assistance of my principal supervisor and friend, Dr Saran Sohi. His mentorship and unwavering support have helped me immeasurably. Our regular meandering conversations were a definite highlight. My supervisor at Forest Research, Dr Mike Perks has also been a tenant of support. He has provided excellent advice throughout the project and kept the project from veering off-course. Without his help the extensive seedling growth trials and experimental work would simply not have been possible. I would also like to thank Prof. Ondrej Masek, my co-supervisor, and Prof. Kate Heal for gladly providing help and advice whenever needed.

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As part of my time at the University of Edinburgh I was lucky enough to work on an important biochar project with Alex Clarke, which has led to our founding of Black Bull Biochar. His support and understanding in the final year of my PhD has kept me motivated and driven me to completion. This thesis is a testament to his unwavering faith and a step on our journey to commercialise biochar, realising the environmental benefits.

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Chapter 1

Introduction and Background

Sustainable plantation forestry performs vital roles in addressing climate change and meeting NetZero commitments (Morison et al., 2012; Nasi, 2022). Plantation forests provide a source of timber, provide ecosystem services and store carbon, allowing the protection of native semi-natural woodlands when sustainably managed (Sing et al., 2017). In the U.K., plantation woodlands are managed according to high industry standards in order to maintain productivity, mitigate negative environmental impacts (particularly to protect water), enhance ecosystem services and improve forest resilience (Forestry Commission, 2017). The history of afforestation in the U.K. has created a legacy of forests established on nutrient poor soils, where establishment relied on widespread chemical fertiliser application (Taylor, 1991). Cumulative net soil nutrient loss, particularly phosphorus (P), as a result of harvesting practices is causing a decline in forest ecosystem P pools which can limit growth in the establishment phase and increase time to canopy closure (Hume et al., 2018). Without novel soil management strategies, forest productivity and resilience to abiotic stresses will become a critical issue in U.K. forestry, exacerbated by climate change (Atkinson et al., 2022). The viability of productive forests will decline in certain areas without intervention, along with the range of suitable species, limiting timber production and carbon storage, and will have wider ecosystem implications.

Biochar is an established technology which has been proven to improve soil productivity in agriculture and increase soil carbon stock (Lehmann et al., 2021; Schmidt et al., 2021). The effect of biochar on the soil environment and the rhizosphere is highly dependent on soil type, application rate and biochar properties (Yang et al., 2019). Biochar can improve soil nutrient cycling, rhizosphere development, soil water properties and directly deliver nutrients to the soil (Edeh et al., 2020; Zhang et al., 2019). Biochar has been shown to increase root growth and enhance root structure, as roots forage for biochar and proliferate in biochar proximity (Gujre et al., 2021; Xiang et al., 2017). Mycorrhizal fungi have also been found to access nutrients held with biochar (Hammer et al., 2014; Jaafar et al., 2014). The application of biochar to the soil can increase the available nutrient pool and improve the ability of plants to acquire available nutrients (Hossain et al., 2020). The function of biochar in soil can be altered through feedstock, pyrolysis and application parameters to target soil properties which limit crop growth. By altering these parameters, a specified biochar and biochar use system can be developed to optimise performance, creating a “specified biochar” (or “tailored biochar” in some studies) (Joseph et al., 2021). Operational and environmental constraints limit blanket application in forest systems, however low dose targeted application has the potential to provide considerable localised soil improvement in the proximity of the rhizosphere of seedlings during establishment (Lei et al., 2022).

Biochar has the potential to partially mitigate some of the soil related factors which limit forest growth in the establishment phase and survival, reducing the time to canopy closure (Wilson et al., 2001). Improved growth rate and establishment success could have profound impacts on forest carbon dynamics and offset soil carbon loss during establishment (Friggens et al., 2020). By creating a specified biochar from sawmill co-products, nutrients can be cycled into nutrient poor forest sites to partially close nutrient loops. To understand the potential of biochar to improve establishment success in nutrient limited planting sites it is vital to investigate the interaction between biochar, soil and seedling. This will provide mechanistic insight into the effects of specified biochar on seedling morphology and soil properties, allowing appraisal of the technology and a pathway for industry adoption. It is also important to assess the effects of biochar on the soil environment, seedling morphology and seedling nutrition compared to conventional mineral nutrient additions in order to compare the impact of management interventions.

1.1 Thesis Aim and Objectives

The overall aim of this thesis is to provide new insight into the potential of targeted low dose application of specified biochar as an alternative to conventional fertilisation in upland forestry and how biochar can mitigate soil nutrient decline from clearfell silvicultural systems. This will help appraise the role of biochar in helping mitigate crop resilience to climate change (through nutrient delivery and drought tolerance) on low yield upland sites and assess the potential to broaden the establishment suitability of species. Understanding the impact of biochar induced growth responses in commercial conifer species on woodland carbon balance over the establishment phase is critical in assessing the efficacy of low yield woodland creation as a greenhouse gas removal method and subsequent contribution to net zero commitments.

The specific objectives were;

- to specify the properties of biochar which address the principal soil and environmental factors which limit seedling growth on nutrient poor upland forestry in the U.K. Develop a biochar which matches the identified properties from timber co-products which can improve circularity and contribute to close the nutrient loop, whilst within the constraints of existing silvicultural systems and establishment practices.
- to quantify the morphological response of key conifer productive species to selected biochars and fertilisers to provide insight into the relationship between below ground and above ground biomass development at varying nutrient concentrations.
- to assess the principal mechanisms and treatment properties which contribute to seedling response. The sub-objectives were; a) to identify the contribution of nutrient availability and nutrient accessibility to growth responses, b) establish the role of biochar and mineral fertiliser on root development and soil pH, c) assess the efficacy of nutrient application on seedling nutrient deficiency and d) whether biochar can improve seedling survival and drought tolerance.
- to investigate the proliferation and interaction of ectomycorrhizal fungi with the charosphere and discuss the potential impact on nutrient accessibility and seedling nutrient exploitation strategies.
- to model the net carbon balance, including soil carbon loss, establishment carbon costs and carbon accumulation in soil and biomass, over the first rotation. Assess the viability of establishment practices on low-yield organo-mineral soils across a range of scenarios to determine rapidity of net carbon storage. Model the impact of biochar on carbon balance using quantified growth responses.

1.1.1 Thesis Structure

The objectives of this thesis have been addressed over the seven chapters. Chapter 1 provides an introduction to the work completed and provides the context and objectives of the thesis. A comprehensive background is also included, which details the current issues facing woodland establishment on nutrient poor upland soils and the potential of biochar in addressing these issues. Chapter 2 covers a brief overview of the methods used in this thesis, including the development and specification of the distinct biochars used in experimental work. Chapter 3 provides controlled environment experimental data which explore the effects and nutrient delivery of biochar and conventional fertiliser on seedling morphology and health. Chapter 4 investigates the effect of biochar on establishment success and seedling yield in a nutrient limited field environment. Chapter 5 qualifies the interaction between ectomycorrhizal fungi, rhizosphere and charosphere, and discusses the impacts on nutrient accessibility and below ground biomass development. Chapter 6 presents a novel woodland creation soil carbon which is compared to the existing soil carbon model used in U.K. forest policy. The chapter assesses the policy implications of low yield forest establishment on organo-mineral soils from a carbon storage perspective under a range of scenarios, which include biochar application. Modelled carbon storage for biochar treated trees, using data from experiments, assesses the long-term carbon implications of targeted biochar use on nutrient poor planting sites. The themes discussed in chapters 3 to 6 are brought together in chapter 7, which synthesises the elements of this thesis to present a holistic conclusion to the potential of biochar in upland U.K. forest management and silvicultural systems.

1.2 Background of Study

This section will provide a background on U.K. forest establishment and management practices, highlighting the productivity issues facing forests established on nutrient poor sites. Biochar will also be discussed, particularly in relation to soil function. The potential of biochar use in forestry will be highlighted along with a summary of previous research of biochar utilisation in forest context.

1.2.1 Woodland Establishment and Silvicultural Management

Sustainably managed plantation forests can contribute to greenhouse gas removal and provide a source of timber, in turn enhancing preservation of semi-natural native woodlands (Freer-Smith et al., 2019; Sing et al., 2017). Forest management practices and silviculture determine the outcomes of establishment and will influence crop productivity in response to climate change (Cameron, 2015). This section will provide a background context to the U.K. forest stock and discuss the influence of common forest management practices on crop establishment.

Development of U.K. Plantation Forestry

Woodland distribution in the United Kingdom is disproportionally concentrated on nutrient poor upland soils (Forest Research, 2022). This is a legacy of historical conifer plantation planting practices which favoured low yielding upland sites, which had little agricultural value, despite high ecological and carbon storage potential. In order to manage the current challenges facing low-yield upland forestry it is important to understand the historic context of woodland expansion in the U.K and how landscape evolution influences current and future forest policy (Raum, 2020).

Over centuries the role and importance of U.K woodlands has changed according to land use and resource demand. The expansion of human populations led to the continuous decline in U.K. woodland cover as timber resources were exploited and competition for land intensified. By the early 20th century forest cover in the U.K reached an estimated low of 4.7% of land. Before this time forest policy was reactive to changes in timber demand and woodland creation was seen as out-with the state's remit (Mather, 1992). Cheap timber imports were seen as a viable alternative to home grown timber resources, with 90% sourced from overseas. There was a fundamental shift in forest policy following acute timber shortages and vulnerable supply lines during World War One, after which forestry was seen as an industry vital to national security. This prompted the establishment of the Forestry Commission, which was tasked with increasing U.K. timber production through woodland creation in order to meet domestic demand and prevent future timber crises. U.K. forest cover was rapidly expanded in the inter-war period, which was primarily commercial conifer plantations on low value agricultural ground. Extensive pine (*Pinus spp.*) dominated woodlands and were established on low-phosphorus sandy soils at this time, notably in East Anglia and North East Scotland (Gauld, 1981). Woodland creation required extensive blanket fertiliser application and cultivation to aid successful establishment (Miller, 1969). By 1939 the Forestry Commission had acquired 263,000 ha of land and had established 149,700 ha of new woodland. The bulk of new planting was on newly acquired cheap poor-quality agricultural land purchased by the state, initially focussed on lowland heaths, and later favouring upland heathland and peat moorland (Foot, 2010). In addition to the extensive public planting programme generous grant funding stimulated private landowners to expand forest cover, favouring productive woodlands. In the inter-war period a further 50,500 ha were privately planted, assisted through government grants (Aldhous, 1997). This afforestation programme led to the largest decadal change in land use in

Europe, involving a rapid shift from agriculture to forestry, especially in the uplands (Bunce et al., 2014).

Woodland establishment in the inter-war period, whilst extensive, did not mature sufficiently to significantly contribute to the second acute timber crisis of the 20th century, caused by World War Two. Privately owned and crown owned woodlands, typically broadleaf, were decimated by clearfell harvesting to supply timber during the war. These woodland were widely replaced by productive conifer plantations resulting in a dramatic decline in semi-natural and native woodland areas, with many of these woodlands now designated as plantations on ancient woodland (PAWS) (Kirby & Thomas, 2017). Following World War Two, the government passed further legislation to consolidate timber supply and increase production in the face of growing demand and depleted timber stocks. Publicly owned forest cover steadily increased from 1945 to 1980 as new land was acquired and planted, increasing to nearly 300,000 ha (Oosthoek, 2013). This was concentrated in upland areas and resulted in widespread establishment of plantation forests on peatland and low-productivity soils, which were established using high intensity cultivation methods, extensive drainage and high dose application of fertilisation products (Mason, 2007). During this time an extensive area of peatland was afforested, resulting in low yield forestry and significant soil carbon loss (Sloan et al., 2018). Part of the success of this planting programme can be attributed to improved establishment practices from forest research and technology innovation, however much of the success was due to the extensive introduction of exotic conifer species (particularly Sitka spruce (*Picaea sitchensis*)) (Mason, 2007).

Large-scale private afforestation did not occur until the 1960s, at which time extensive woodland creation was initiated by private investment companies who purchased cheap upland land for productive conifer plantations, a trend that continues to some extent. These were woodlands often established on marginal soils and on exposed sites. This limited potential silvicultural systems due to the risk of wind damage, compounded limited root development due to soil (induration and ironpan) or water (high water table). Silviculture prioritised return of investment and timber income, negatively impacting the potential for multi-objective forest management and ecosystem services (Tompkins, 1989). Modern forest policy is slowly remediating poorly designed and low-productivity woodland through restructuring and phased harvesting and woodland creation requires a high standard of environmental planning and consent. New U.K. planting and restock establishment must adhere to the U.K. Forestry Standard (Forestry Commission, 2017), ensuring appropriate species choice and soil management. However, nutrient poor soils from historic planting require restocking after harvesting, resulting in a decline to crop productivity and requiring adaption to forest management practices (Atkinson et al., 2022; Forestry Commission, 2017).

This history of plantation woodland creation has created a legacy of first or second rotation forests established on nutrient poor soils, where historic planting success has been reliant on antiquated ground preparation techniques and extensive forest fertilisation (Taylor, 1991). These establishment practices are now impractical due to operational constraints and the detrimental impact of high intensity cultivation. Upland forests can be broadly categorised according to soil type, with forest establishment on free draining podzolic soils principally limited by different factors to imperfectly drained gleys, which in turn determines species selection and yield (Ashton & Kelty, 2018; Savill, 2019).

Forest Establishment Management

Whilst there is a gradual shift to low impact silvicultural systems (LISS) and continuous cover forestry (CCF), clearfell harvesting remains the principal method of crop harvesting (Mason, 2007). Clearfell harvesting is particularly common where crops are established on nutrient poor soils. These sites are often exposed, with low crop stability due to limited root development, and have delayed or no thinning which increases the risk to windthrow from implementation of LISS or CCF forestry, making forest restructuring to these silvicultural systems impractical (Cameron, 2002). Cultivation is generally required prior to restock or woodland creation planting (Mason, 1999). Historically, upland sites were widely cultivated by deep plough, which rapidly mineralised soil carbon resulting in significant soil carbon loss (Sloan et al., 2018). There is also evidence the high disturbance cultivation methods increased soil erosion and negatively influenced crop stability due to lateral root development along furrows (Stott & Mount, 2004). Restock and woodland creation cultivation is now limited to low or moderated disturbance cultivation methods. Hinge and trench excavator mounding is now favoured on upland forest soils, improving localised establishment conditions (Sinnett et al., 2008). During ground preparation and cultivation brash is consolidated and soil is exposed to create a planting position clear from competing vegetation and generally higher than surrounding bulk soil. Ground preparation is vital to reduce interspecific competition, stimulate mineralisation of soil organic matter, maintain aerobic soil conditions and create an identifiable planting position for management interventions (Mason, 1999).

Modern forest fertilisation has seen little development since principal research was completed in the 1980s (Taylor, 1991). Interventions in restock establishment sites are generally limited to surface application of NPK mineral fertiliser, directly added to planting positions. Best practice stipulates that the use of chemical fertilisers should be used only as a last resort and it is therefore often applied retrospectively, one growth rates are already suppressed (UKWAS, 2022). The prevalence of brash and stumps on restock sites, which should not be harvested on nutrient poor or high carbon soils to minimise soil disturbance and nutrient loss, prevent blanket fertilisation and limits nutrient application to hand spreading (Forest Research, 2009). The traditional large-scale chemical fertiliser inputs on nutrient poor soils in restock establishment (Taylor, 1991) can have a negative environmental impact, through diffuse pollution and altering soil biofertilisation (Nisbet, 2001). Modern best practice (as described in the U.K. Woodland Assurance Standard (UKWAS, 2022)) stipulates reducing chemical inputs, including mineral fertiliser. Early establishment fertilisation can also be achieved through the in-situ fertiliser contained in containerised seedling growing media, although the long-term effects of containerised production on root architecture and nutrient accessibility is unclear. It is therefore vital that closing nutrient cycles is a more viable soil management objective, rather than mitigating soil nutrient loss through increased nutrient input.

1.2.2 Forest Soils and Establishment

Upland forest growth in the establishment phase is generally limited by climate or soil factors. The principal soil factors which influence growth are soil nutrient regime (SNR) or soil moisture regime (SMR).

Soil Nutrient Regime

Soil nutrient regime is one of the primary environmental factors which limit forest productivity and growth in the early establishment phase (reducing the range of species suitability in low nutrient soils) (Wilson et al., 2001). Soil nutrient content, availability and accessibility are vital to the successful establishment of seedlings on upland forest soils. On moorland and heathland soils in Great Britain nitrogen and phosphorus deficiency can severely restrict the growth of certain conifer species, including Sitka spruce, the main commercial species (Binns et al., 1980; Taylor, 1991). Until the 1970s this was partly attributed to direct competition from heather

(*Calluna vulgaris* L.) and was commonly known as "heather check' (Weatherell, 1953). However, increased planting of Sitka spruce on very nutrient-poor soils revealed that, even after removal of heather by herbicide treatment, growth was still limited by low availability of nitrogen and phosphorus. This can be caused by limited soil nutrient pools and a slow rate of mineralisation (Attiwill & Adams, 1993). Application of nitrogen and phosphorus fertiliser can overcome this deficiency although several applications may be required to achieve full canopy closure (Taylor, 1991). The greatest demands for soil nutrients are during establishment, while the green crown is being formed to canopy closure (Miller, 1990). Once this stage is reached demand for nutrients is reduced due to shading of competing vegetation, improved nutrient recycling and capture of atmospheric nutrients and further inputs of nitrogen or phosphorus should not be required (Prescott, 2002).

There are limited management interventions available to improve establishment success or increase species suitability on nutrient poor planting sites due to the potential negative environmental effects of high dose fertiliser application (Mason, 2007). Miller (1990) highlighted the requirement to develop diagnostic and ameliorative measures that can help improve forest nutrition during establishment, whilst ensuring minimum adverse effects on the environment, over three decades ago. Some improved diagnostic measures have been developed, along with improved understanding of the impacts of management practices on forest nutrient pools, however no new soil amelioration interventions have been adopted (Hume et al., 2018; Martin et al., 2008; Elena Vanguelova et al., 2010).

Soil Nutrient Availability and Phosphorus

Phosphorus (P) is the limiting nutrient to growth and establishment on many nutrient poor forest soils in the U.K. (Binns et al., 1980). Phosphorus is vital element for plants as it is present in biological molecules, including nucleic acids, co–enzymes, phosphoproteins, and phospholipids (Schachtman et al., 1998). Vanguelova and Pitman (2019) have shown that the prevalence of forest P limitation is likely to increase some regions of the U.K. due to elevated atmospheric nitrogen deposition. Soil P can be broadly categorised into four pools, three inorganic pools and one organic pools (Lajtha & Jarrell, 1999). Soil P cycles between these pools by processes which are influenced by soil properties and fertiliser application (Dobermann et al., 2002). Soil organic P is P held within soil organic matter (SOM). P can cycle between this pool and the soil available P pool (where P is in the form of PO_4^{3-}, $H_2P0_4^-$ and HPO_4^- in soil solution and is available to plants) through immobilisation and mineralisation. The mineral soil P pool is comprised of primary and secondary phosphate minerals, in this form P can be converted to available P by solubilisation. The final soil P pool comprises of P adsorbed by clay surfaces, calcium oxides and sesquioxides in the soil, alterations to soil chemistry and increase or decrease P desorption from this pool (Moody et al., 2013). These processes are sensitive to soil alteration through harvesting or nutrient application, with mineralisation rates controlled by microbial activity (principally phosphate solubilising microorganisms) (Hume et al., 2018).

Phosphate solubilising microorganisms, including mycorrhizal fungi, improve solubilisation of soil phosphorus and can also enhance mineralisation processes. These organisms can convert organic, inorganic and mineral P into available P (Rawat et al., 2021). The mechanism of solubilisation and mineralisation of P by PSMs is the release of low molecular weight organic acids and phosphatase enzymes. Hydroxyl and carboxyl groups from the organic acids can chelate the cations bound to phosphate, thereby converting it into soluble forms. Phosphatases enzymes result in the decomposition and mineralisation of soil organic P pools by catalysing the hydrolysis of both esters and anhydrides of phosphoric acid (Khan et al., 2009). Total ecosystem P is dependent on nutrient inputs from mineral weathering, atmospheric deposition and fertiliser application (Sohrt et al., 2017). The plant community composition will determine the proportional distribution of P between soil, microbial biomass and plant biomass P pools. In many nutrient poor upland soils the available P pool is insufficient to meet the nutritional requirement of seedlings during establishment, limiting growth (Taylor, 1991). The available P pool can be increased by direct P application or managing soil to stimulate P cycling processes which favour increased desorption, mineralisation and solubilisation (often described as biofertilisation) (Bhardwaj et al., 2014).

Soil Moisture Regime and Drought Stress

Soil texture and SOC content contributes to drought resilience. Seedlings established on freely drained sandy soils, without an iron pan, are at greater risk of acute drought stress as available water will more rapidly decline below threshold levels during periods of low precipitation, compared to loamy or clay soils (Beauchamp et al., 2016; Locatelli et al., 2021; Vanguelova et al., 2018). Species suitability modelling shows that growth stress due low water availability in common commercial conifer species is primarily limited to podzolic soils in upland areas (Wilson et al., 2001). These soils are often also phosphorus (P) deficient and nutrient poor. Water logging, and subsequent root suppression and soil anaerobicity, can limit establishment success in poorly drained soils, however this issue can be mitigated through planting design (Nisbet, 2001).Seedlings are most sensitive to drought stress caused by low rainfall during the early phases of establishment. During this initial period following out-planting root structure has not yet developed with roots concentrated within the planting hole. Cultivation creates locally low soil water availability in disturbed soil, further contributing to drought risk (Wood et al., 2003).

Gleyed forest soils can also be limited by soil moisture regime, where high rainfall and imperfect drainage conditions can cause waterlogging. This causes anaerobic soil conditions and prevents root growth (suppressing nutrient acquisition). Drainage is required in these soil to mitigate these conditions (Paterson, 1999). Seedlings established on imperfectly drained soils, which generally have a high clay content, can be prone to drought stresses due to the high wilting point and unavailable water in these soils (Pereira & Pallardy, 1989). This can result in similar available water as some sandy soils during periods of drought.

1.2.3 Productivity Issues in Forest Establishment

Soil Nutrient Decline

During establishment and subsequent growth to maturity, phosphorus, and other nutrients move from soil nutrient pools and are immobilised in plant biomass (resulting in a decline in soil nutrient availability) (Sohrt et al., 2017). Nutrients held within needle biomass are cycled back into soil through leaflitter and decomposition stabilises soil nutrients following canopy closure (Figure 1-1) (Prescott, 2002). Forest harvesting disrupts the stable nutrient cycles of mature woodland and results in rapid nutrient loss from the ecosystem nutrient pool. The removal of timber during harvesting directly results in nutrient loss, due to direct export of nutrients immobilised in wood. Further nutrient loss occurs as brash decomposes (Callery et al., 2015) (Callery et al., 2015). The phosphorus cycle is particularly impacted during harvesting and cultivation through lateral P movement and loss in runoff (Hume et al., 2018; Stott & Mount, 2004). Nutrient and base cation removal during harvesting results in a loss of nutrient and base cations, which in acid sensitive areas may enhance the acidification of forest soils and watercourses. This has both environmental and nutrient cycling implications (particularly in increased adsorption of available P in podzolic soils). The slow rate natural input of lithological P into the ecosystem P pool, through mineral weathering, is insufficient to mitigate harvesting P losses on low P soils (where underlying geology has resulted in a low P soil) (Taylor, 1991).

Soil P decline due to forest harvesting is widely recognised as a critical issue for future forest productivity, due to the incremental decline in total ecosystem P pool with each forest rotation (Hume et al., 2018). The effect of this decline on productivity will be most pronounced in forests where soil nutrient regime (particularly P) is poor and already a limiting factor to growth. This decline in soil nutrients will increase environmental stresses on seedlings and potentially decrease resilience to other stress factors (Gull et al., 2019; Suzuki et al., 2014). Degradation of the total ecosystem N pool through rotations is likely to be less acute due to higher input from N fixation and atmospheric deposition. Forest management practices can exacerbate net soil nutrient decline, however high disturbance harvesting methods have been found to increase available soil nutrients in some cases, due to increased mineralisation rates, despite decreasing the total nutrient pool (Thiffault et al., 2011; Elena Vanguelova et al., 2010; Yamulki et al., 2021). Extended fallow periods, to combat *Hylobius abietis*, have also been attributed to increased P loss and soil nutrient decline (O'Driscoll et al., 2011). Low impact silvicultural systems and continuous cover forestry may limit ecosystem P loss during harvesting cycles, although implementation of these systems is often limited by operation and site constraints. Novel solutions are required to mitigate soil nutrient decline and maintain productivity in established forests on nutrient poor soils.

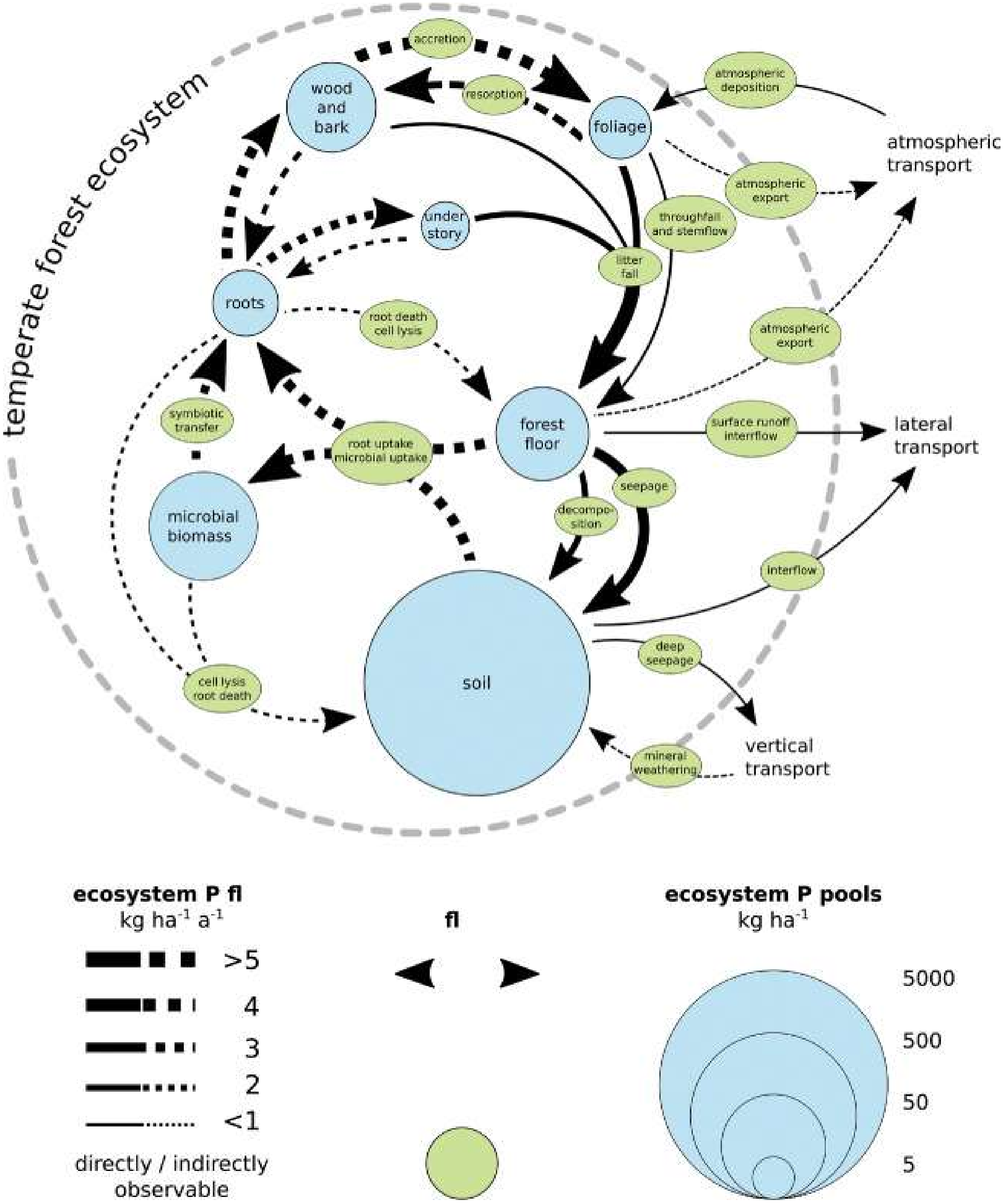

Figure 1-1- A schematic representation of the phosphorus (P) cycle in temperate forests. Pools and fluxes are scaled to their average size. In a nutrient poor forest soil, the total soil P pool can be <1000 kg ha^{-1}. Source: Sohrt et al. (2017).

Climate Change

Forests and forestry are vital to meet NetZero targets, however they also will be required to adapt to climate change. Climate models indicate that extended low rainfall periods will occur causing an increasing frequency, intensity and duration of drought conditions, with severity compounded by warmer temperatures (Atkinson et al., 2022; Ovenden et al., 2021; Rahiz & New, 2013). In recent years low rainfall has resulted in substantial losses during early establishment and reduced growth rates as seedlings become stressed (Green & Ray, 2009). Plant stress can be considered as physiological responses to abiotic and/or biotic stress factors. The exposure to stress factors at a high intensity and/or for long duration can exceed the limits of seedling stress responses and can cause permanent damage or death when a threshold is exceeded (Kranner et al., 2010). Most commercial conifer species are poorly adapted to drought stress and pine species (*Pinus* spp.) are generally the most suitable for planting on drought prevalent sites in the U.K. These negative stress effects are often described as distress, whereas positive effects, where seedling stress response thresholds are not exceeded, are described as eustress (hardening) (Kranner et al., 2010). The adaption of forest management practices to drought will be required to maintain productivity and establishment success. Planting design, species selection and silvicultural system improvements have been proposed as parameters which could be adapted to improve drought resilience. Novel localised soil amelioration methods, such as biochar have not yet been considered (Atkinson et al., 2022)

Increase stress response due to abiotic factors and a warming climate increase the risk of damage to forests from pests and diseases (Atkinson et al., 2022). Species often have resistance and resilience to exotic invasive pathogens which can rapidly decimate forests, for example the effects on widespread mortality and subsequent phytosanitary felling of larch (*Larix* spp.) caused by *Phytophthora ramorum.* Mitigation of soil moisture and nutrient related stresses could improve tree resilience to pests and diseases. Management interventions can improve overall forest resilience to pests and diseases through species diversification, thinning and felling design (Kelty, 2006).

1.2.4 Biochar Background

Background and Biochar Production

Biochar is a solid carbon rich substance produced by transforming biomass at high temperature low oxygen conditions. Biochar can further be defined according to use, requiring an environmental application rather than use as a fuel, which differentiates biochar from other pyrogenic carbon rich substances, such as charcoal (Lehmann & Joseph, 2015; Sohi et al., 2010). The agronomic value of biochar is widely accepted, and pyrogenic carbon has been used to improve soils for millennia. Early exploitation of pyrogenic carbon for soil improvement can be observed in the "*terra preta*" soils found in the Amazon basin, where repeated slash and burn cycles has created carbon rich soils which have improved nutrient retention and soil productivity (Glaser et al., 2001). Natural wild fires have also contributed to high concentrations of pyrogenic carbon in soils across the world (Jones et al., 2019). These soils often have higher available nutrients, increased water retention and enhanced tree yield, compared to soils with no pyrogenic carbon (Licht & Smith, 2020). Pyrogenic deposits resulting from wildfires are particularly prevalent in semi-natural conifer woodlands, where tree species are adapted to regular fire cycles. This natural phenomenon is a vital part of nutrient cycling and forest regeneration, highlighting the particular potential fit of biochar application in similar ecosystems (Gale & Thomas, 2021).

Biochar is typically produced by slow pyrolysis. The process of pyrolysis is the thermochemical breakdown of organic compounds at high temperature and low oxygen, increasing aromaticity and concentrating some nutrients (particularly phosphorus) (Mašek et al., 2018). Biochar function following application to soil is highly dependent on feedstock and pyrolysis conditions (Schmidt et al., 2021). The principal soil parameters, including resultant plant productivity and growth, which are influenced by biochar application are discussed in this section, along with the relative effects according to biochar properties.

Biochar and Soil

Plant Productivity and yield

The agronomic benefits of biochar application have been widely studied. Biochar has been found to improve crop yield and productivity. A meta-analysis by Jeffery et al. (2017)

presented that crop yield improvements due to biochar application were highly dependent on soil nutrient regime and soil pH. Biochar application on low nutrient acidic soils (pH <5) showed the highest yield increase (40%), whereas nutrient rich or neutral soils showed no significant yield improvements from biochar application. As agricultural soils are extensively managed to maintain a neutral pH and high soil nutrient regimes, Jeffery et al. (2017) state that biochar does not improve crop yields in temperate regions. This does not however consider biochar application in acidic forest soils, where soil pH and nutrient levels are generally low.

Meta-analyses by Ye et al. (2020) and Dai et al. (2020) also found that biochar application results in a mean increase to crop yields. High variability in the effect of biochar on yield was identified. Dai et al. (2020) found that biochar application results in a mean yield increase of 16%, and also identified positive biochar effects in neutral soils. Ye et al. (2020) identified the potential of biochar application in combination with fertiliser application. Mean yield improvement following biochar co-application with fertiliser was calculated at 16%. There is considerable evidence that biochar can be used to improve crop yield. However the effect of biochar on plant growth is highly variable according to biochar properties, soil type and agricultural or silvicultural system (Schmidt et al., 2021). Expected yield improvements should not be extrapolated to distinct biochar use systems, particularly establishment forestry where there has been little research to date.

A meta-analysis by Jeffery et al. (2011) found that the effect of biochar on soils and crop productivity is highly variable and dependent on soil and biochar properties. The study suggested that the main mechanisms for crop yield improvements resulting from biochar application, may be liming (in acidic soils) and ameliorating limiting soil water properties. Plant yield improvements have also been attributed to the native nutrient content of biochar (where certain nutrients from the feedstock material are concentrated during pyrolysis). High nutrient feedstock derived biochars have been shown to improve plant yield through the direct addition of micro and macro-nutrients to the soil (Ippolito et al., 2020). The comparatively low nutrient concentration of biochar, compared to conventional mineral and organic fertilisers, prevent biochar from replacing other nutrient applications and biochar can effectively be combined with other nutrient sources to improve overall fertilisation efficacy (Dietrich et al., 2020).

Root and rhizosphere development

Biochar has been consistently shown to stimulate root growth and improve root structure. A meta-analysis by Xiang et al. (2017) found that biochar application increased root surface area and root length by 39% and 52% respectively. Total root biomass was also shown to increase by an average of 32%. Biochar application resulted in an increase in root tip numbers (17%), further improving plant soil interaction. Biochar application only results in modest increases to root diameter (9%), showing a weak initial response in root secondary growth. This is likely due to the reduction in secondary growth as an P acquisition strategy and the lag to secondary growth following primary growth rate increases (Strock et al., 2018). These root trait improvements indicate an improvement in phosphorus (P) acquisition and water uptake capacity, and this is likely a mechanism which promotes crop yield improvements. Whilst the strongest root response to biochar has been observed in annual plants, root trait and growth enhancement following biochar application has also been observed in woody perennial plants (Lei et al., 2022; Robertson et al., 2012; Xiang et al., 2017). The roots of conifer seedlings have been shown to preferentially forage for biochar patches and proliferate root growth upon contact (Lei et al., 2022). This root response to biochar was found to be a result of P foraging, with root proliferation constrained to P replete biochar. The specific surface area, water-holding capacity and electric conductivity are biochar properties which have also been suggested to contribute to the positive root response (Schmidt et al., 2021). Xiang et al. (2017) concluded that biochar can increase plant phosphorus acquisition, through promotion of root growth.

Mycorrhizal fungi have also been shown to actively forage for phosphorus held within biochar particles and acquire this phosphorus, which is then allocated to host plants (Hammer et al., 2014). Biochar can increase mycorrhizal abundance and proliferation in proximity to biochar particles, however high dose (>10 t ha^{-1}) rates can suppress mycorrhizae and limit plant P acquisition (Warnock et al., 2010). Mycorrhizal hyphae have also been shown to colonise internal biochar spaces, however this is highly dependent on biochar properties (Jaafar, 2014). Mycorrhizal fungi drive soil nutrient cycling process and the effect of biochar on mycorrhizal fungi will likely have indirect effects on soil nutrient availability and plant acquisition (Bolan, 1991; Warnock et al., 2007; Wurzburger et al., 2017). To date there has been little research on the interaction between ectomycorrhizal fungi or endomycorrhizal fungi, and biochar, including in forest ecosystems. Early research suggests that ectomycorrhizal fungi can enhance seedling N and P uptake in biochar treated soil, however the interaction with biochar particles has not been studied (Robertson et al., 2012; Verma & Reddy, 2020).

Biochar has been shown to increase to mycorrhizal and root growth following application, enhancing interaction between the rhizosphere and the charosphere (Hammer et al., 2014; Lei et al., 2022; Xiang et al., 2017). Biochar application can improve the ability of plants to uptake soil nutrients, through roots and symbiotic mycorrhizae. This acquisition of nutrients from the soil available nutrient pools according to plant root traits and mycorrhizal symbiosis has been termed “acquisition efficiency” and “nutrient appropriation capacity” (Campos et al., 2018). In this thesis the combination of acquisition efficiency and available P in the rhizosphere has been termed nutrient accessibility, which can be defined as the soil available nutrients which can be acquired by the plant through roots and associated mycorrhizal fungi.

Phosphorus and Nitrogen

Phosphorus (P) and nitrogen (N) are the principal macronutrients which limit forest yield on nutrient poor sites. P is increasingly becoming the dominant limiting nutrient in U.K. forest establishment, partly due to increased atmospheric N deposition in some areas (Cannell et al., 1998; EI Vanguelova et al., 2010; Vanguelova & Pitman, 2019). A meta-analysis of the effects of biochar on soil P pools found that biochar application, without additional P fertiliser, increased the soil available P pool by an average of 45% and increased P in microbial biomass by 48% (Gao et al., 2019). Biochar increases soil available P by directly adding available P held within biochar (native P content) to the soil and by stimulating the cycling of inaccessible P into available P. The indirect effect of biochar on available P through a number of mechanisms, namely; increasing root exudates, limiting P adsorption by sesquioxides, stimulating microbial activity, stimulating mineralisation of the organic P pool (Ali et al., 2020; Attiwill & Adams, 1993; Gao et al., 2019; Zhang et al., 2019). Biochar can be loaded with additional N and P, or applied with mineral or organic fertilisers to further improve biochar nutrient content. This has been found to have the potential to further increase crop yields (Dietrich et al., 2020; Yu et al., 2018).

Biochar has been shown to temporarily decrease available N (NO_3-N, NH_4-N), as it is adsorbed to biochar surfaces. Gao et al. (2019) found a mean 12% reduction in topsoil NO_3-N and an 11% reduction in NH_4-N concentration. Plants are able to retrieve the nitrate captured in biochar particles (Haider et al., 2020). When pure biochar, without nutrient enhancement, is applied in extreme quantities to the soil, an initial reduction in plant available N due to adsorption from the soil can be expected. This could be detrimental to seedling growth and survival during the critical early establishment phase. The N is not lost when captured by biochar particles and can be released over more extended plant growth periods (Hagemann et al., 2017). Low dose targeted applications are unlikely to alter soil available N stocks and some microbial processes stimulated by biochar application may increase nitrogen mineralisation (Schmidt et al., 2021). Biochar is effective at immobilising heavy metal contaminates in soils, decreasing soil N_2O emissions and limiting NO_3-N leaching and runoff (Borchard et al., 2019; Chen et al., 2018). These are not prevalent issues in forest establishment sites and are therefore not discussed in detail.

Plant Available Water

In order for biochar to alter the water properties of bulk soil, high application rates are required (>10 t ha^{-1}) (Conte & Schmidt, 2017). However, the high porosity and low bulk density of biochar has the potential to locally improve soil water holding capacity and soil available water (Razzaghi et al., 2020). The effect of biochar on soil water properties is highly dependent on the physical, and to a lesser extent, chemical properties of biochar (Edeh & Mašek, 2022; Edeh et al., 2020). Edeh and Mašek (2022) concluded that particle size, specific surface area and porosity of biochar are the main biochar parameters which influence soil water dynamics. Optimal biochar properties for improving soil water holding capacity and soil water availability varied according to soil texture. Low particle size (<2 mm) and high specific surface area and porosity are most effective in sandy soils, conversely, larger particle sizes and with high specific surface area are effective at improving soil water properties in clayey soils. In sandy soils biochar can benefit plant available water by increasing field capacity, whereas in clayey soils the biochar can improve soil water properties by increasing plant available water, due to a decrease in wilting point, and improving drainage (aerating the soil and reducing water-logging) (Edeh et al., 2020). This highlights the requirement to specify biochar properties to target soils.

As with soil nutrient availability and accessibility, plant water acquisition in response to biochar application is a synergy between direct improvements to soil water properties and indirect improvements to uptake potential due to increased root and mycorrhizal development. In light of this distinction soil water properties are discussed as water availability and water accessibility in the context of this thesis.

Soil Biota

Biochar application has been found to increase microbial biomass, as discussed in previous paragraphs, which affects nutrient cycling, mineralisation and available soil nutrients (Lehmann et al., 2011). Bamminger et al. (2014) found that biochar increased microbial biomass and diversity, however community composition shifted to from bacteria towards higher fungi, suggesting that the positive effect of biochar on fungal. There has been limited studies on the effect of biochar on soil fauna. Some studies have shown that earthworms avoid soils with a high biochar composition (10% mass dose), however this represents extreme doses and no effect on earthworm populations has been observed at doses <40 t ha^{-1} (Li et al., 2011). The physical and chemical properties determine the effect of biochar on soil biota, along with soil type and application rate. Zhang et al. (2018) found that low temperature biochar addition in low pH soils greatly increased ratios of fungi to bacteria, which is particularly relevant to the use of biochar in a forestry context. This, however, contradicts finding by Pingree et al. (2022) who found that the stimulatory effect on microbial biomass was not observed in boreal forest soils. They suggest that the enhanced plant growth in response to biochar addition observed in boreal environments (Robertson et al., 2012) is likely due to other mechanisms, such as direct nutrient supply from biochar or amelioration of soil pH. More research is clearly required to support this hypothesis.

Soil Carbon Storage

Biochar is recognised as a leading emerging greenhouse gas removal (GGR) technology, with the potential to deliver emission reductions of 3.4–6.3 $PgCO_2e$, half of which constitutes direct CO_2 removal (carbon stabilised during pyrolysis and stored in biochar) (Lehmann et al., 2021; Woolf et al., 2018). Biochar directly increases persistent soil carbon due to the biochemical stability of biochar carbon, which is resistant to degradation in the soil (Gross et al., 2021; Wang et al., 2016). Biochar consists of labile (~3%) and recalcitrant (~97%) carbon pools. The recalcitrant carbon content of biochar is highly dependent on feedstock and pyrolysis temperature, woody feedstocks pyrolyzed at high temperatures provide the greatest potential for long-term carbon storage (Crombie et al., 2013; Leng & Huang, 2018). At high temperatures chemical aromaticity increases, which is an established indicator for the recalcitrance of organic matter (Lützow et al., 2006), and the molar ratio of hydrogen to carbon can provide a simple index of aromaticity and therefore carbon recalcitrance (Haumaier & Zech, 1995).

Chemical aromaticity is an established indicator for the recalcitrance of organic matter fractions in soil, where biochar occupies an extreme position in the aromaticity spectrum (Leng & Huang, 2018). The large range in potential longevity requires a simple static indicator of stability that can be attached to a longevity of specific magnitude and certainty (Leng et al., 2019). The molar ratio of hydrogen to carbon provides a simple index of aromaticity, carbon in aromatic molecules containing none of the hydrogen (or oxygen) present in the starting biomass. Biochar can display graphitic components but aromatization is always incomplete, although H:C can approach 0.1. Some standards for biochar have proposed H:C < 0.4 to indicate carbon storage. Calibrated against available short-term data extrapolated using a two-pool model (dividing biochar carbon into recalcitrant and labile pools) suggests 70% storage over 100 years (95% confidence interval) or 50% storage at a H:C threshold of 0.7) (Budai et al., 2013). The stability of biochar recalcitrant carbon is also affected by soil factors, which can influence the rate of biochar weathering, however biochar application to soil is found to have a net positive effect on soil carbon (Fang et al., 2015).

The labile carbon pool of biochar can stimulate microbial activity, which can prime the degradation of native soil carbon, which also accelerates decomposition of biochar labile carbon (Zimmerman et al., 2011). Priming is an imperfectly defined term that is sometimes used to describe more general effects of one material on the degradation of another, not related to carbon. Introduction of nitrogen, phosphorus or alkalinity to the soil can promote decomposition of soil organic matter (Gao et al., 2019). The effect of biochar on soil carbon priming can be positive or negative, depending on biochar properties and soil type (Wang et al., 2016). Negative priming refers to a slowing of background decomposition processes in soil resulting from the application of recalcitrant carbon, such as biochar. The negative carbon priming effect can increase the net carbon benefit of biochar application in certain scenarios. Biochar with a high proportion of recalcitrant carbon, such as high temperature wood derived biochar, has been found to negatively prime soil carbon, with this effect most pronounced at low doses and in clay soils (Butterly et al., 2018; Wang et al., 2016). The effects of biochar on soil labile carbon decomposition is highly complex and difficult to predict, the enhanced development of the rhizosphere with biochar application can further indirectly increase soil carbon, nitrogen and phosphorus priming synergistically, and therefore the detrimental effects of increased carbon priming needs to be considered in the context of increased available soil nutrients (Wang & Tang, 2017).

Biochar application can also increase belowground biomass, particularly mycorrhizal and root biomass (Prendergast-Miller et al., 2014; Xiang et al., 2017). This will increase the biological carbon pool in soils and could further contribute to the net soil carbon storage of biochar. Aboveground yield and growth increases from biochar application will also increase the carbon in biomass (Ye et al., 2020). The end-use of this carbon is vital in assessing the carbon storage contribution of biochar through biomass accumulation improvements. Utilising crop residues, from biochar treated plants, as a feedstock can convert biomass sequestered carbon for long-term storage. This can improve circularity and help close nutrient loops (Yang et al., 2021).

1.2.5 Biochar In Forestry

The potential for biochar application in forest systems for soil enrichment and carbon storage is widely recognised (McElligott et al., 2011; Page-Dumroese et al., 2016), however there has been limited research into the response of tree seedlings to biochar application, and no previous studies which have investigated targeted biochar application at establishment on nutrient limited restock establishment sites. The prevalence of fire in natural forest ecosystems has provided the earliest indication of the potential benefits of biochar on seedling growth. Naturally formed charcoal, from forest fires, has been found to promote growth of conifer seedling with nutrients transferred through mycorrhizal fungi, when mixed with soil (Wardle et al., 1998). Biochar has the potential to improve forest soils, and tree nutrition, by stimulating root development, increasing soil available nutrients and improving soil water properties.

was An early study on pyrogenic carbon in forest establishment by Makoto et al. (2010) simulated natural charcoal accumulation in forest soils. Targeted charcoal application, simulating natural deposition, was found to deliver P nutrition to larch (*Larix* spp.) seedlings which was enhanced by ectomycorrhiza. This study tested a narrow set of parameters and did not investigate nutrient transfer mechanisms. Biochar was further found to improve lodgepole pine seedling growth (*Pinus contorta* var. *latifolia*) only when co-applied with fertiliser (Robertson et al., 2012). This is likely due to the soil properties and biochar selection, highlighting the importance of biochar specification. The potential of specified biochar, produced to address limiting soil properties in forest establishment has not been previously investigated.

When applied to forest soils, biochar has been shown to increase soil porosity, soil moisture retention, and reduce soil bulk density. Soil chemical properties are also improved, including increased pH, soil organic carbon (SOC), cation exchange capacity (CEC) and the soil available P pool (Gundale et al., 2016; Li et al., 2018). The effect of biochar on soil properties in forest soils is consistent with observations made in agricultural soils, and similar growth responses might be expected. A meta-analysis by Thomas and Gale (2015) indicated that there is a strong tree growth response to biochar application, with a mean 41 % increase in biomass accumulations This response was found to be most pronounced during the establishment phase (principal studies are discussed in the previous paragraph). A combined pot and field study by Wrobel-Tobiszewska et al. (2014) showed that biochar directly altered soil nutrient dynamics, increasing macro nutrients. This study found no clear seedling response to biochar, however *Eucalyptus nitens* was selected, and the tested forest system does not represent U.K. forestry. The potential of biochar to improve soil properties and seedling establishment was further examined by Dietrich and MacKenzie (2018) and by Dumroese et al. (2018), where was identified as a potential replacement for peat in nursery production. In this study biochar was found to improve soil available nutrients and increase seedling growth in some soil mixes on reclaimed cover soils from oil sand regions. Previous research shows that there is clear potential for biochar use in forest establishment, however targeted research specific to a U.K. context is required.

There has been limited previous research investigating the effect of biochar on the seedling drought responses, through measuring photochemistry, in forest establishment. Pyrogenic carbon has been found to improve soil moisture retention and seedling water use efficiency in pine seedlings (Licht & Smith, 2020)As discussed in previous sections, biochar can directly and indirectly improve soil water availability and plant accessibility. Biochar has been shown to limit plant stress responses to drought factors in trees (Mattheck et al., 2021). Lyu et al. (2016) found that biochar application with biochar could increase seedling acclimation to drought stress through alterations to photochemistry. Previously, there has been no research which holistically assesses seedling response following biochar application to multiple abiotic stress factors, or discusses the synergies between biochar soil amelioration, seedling response and forest carbon balance.

The requirement for further research into biochar utilisation in forest establishment has been recently identified (Amonette et al., 2021; Schmidt et al., 2021; Trazzi et al., 2018). This thesis provides mechanistic and systems research into biochar utilisation in U.K. upland forestry. By creating a biochar use system and specified biochar that is tailored to addressing the challenges facing establishment on nutrient poor sites, the potential of biochar in mitigating soil nutrient decline and drought stresses can be investigated over an appropriate timeframe and some of the key research gaps can be filled.

Chapter 2

Materials and Methods Overview

This chapter details the production process and properties of the treatments that were applied to seedlings in the experimental work described in Chapter 3, Chapter 4, Chapter 5 and Appendix 2. The selection of tree species used in this study is also discussed. Lastly, this chapter describes the potential scenario for biochar use in U.K. forestry that was identified as a potentially effective system of biochar production and use in the forestry and wood processing industries. This biochar production and use system and scenario was considered when developing the biochars used in this study and in determining biochar application methods. Experimental methods and data collection are detailed in the relevant chapters.

2.1 Biochar Specification and Production

This section discusses the development of the specified biochar used in experimental work and also describes other treatments. The production process that was used to manufacture the biochar used in the experimental work is also detailed. The potential scale-up scenario and system design for industry adoption is also included, as this influenced feedstock selection.

2.1.1 Biochar Specification

Biochar function in soil is highly dependent on the physical and chemical properties of biochar. The interaction between biochar-soil-plant will determine any agronomic, or silvicultural, benefit to the crop. The efficacy of distinct biochar types (which can be categorised according to physical and chemical properties) on amelioration soil deficiencies can vary from strong negative effects to strong positive effects depending on the type of biochar applied. Correctly pairing biochar properties with the target soil properties which limit crop growth can directly improve yield, productivity or influence crop resilience to abiotic stress factors. Biochar can also be developed to have properties which improve the soil environment, enhancing rhizosphere development and nutrient cycling.

Several key target biochar properties were identified for creating a specified biochar for use in improving conifer establishment on nutrient phosphorus (P) poor planting sites. These properties were then matched with available feedstock, considering circularity and sustainability, and pyrolysis conditions to create a viable specified biochar product for use in U.K. forestry which could be practically implemented.

Specified biochar was created to address the following target functions;

i) *Deliver available P* – high available P feedstock

ii) *Improve water retention and availability* – high porosity and low bulk density and low hydrophobicity

iii) *Enhance mycorrhizal fungi* – Moderate nutrient content, high porosity at multiple scales

iv) *Store stable carbon* – High stable carbon content

Specified biochar was developed with the aim of meeting the target functions outlined above. The specified biochar was produced from feedstock which consisted of the outer layer of wood. This feedstock is produced as a sawmill co-product during the ring debarking process. During this process the outer 2 – 5 cm of the tree is removed, including the vascular cambium. The vascular cambium contains high concentrations of available P, relative to the rest of woody biomass (up to 20x greater than sapwood) and exhibits ideal physical properties for improving soil water properties (Edeh et al., 2020). This feedstock also contains phloem tissue, accounting for <20% of the feedstock. The phloem is nutrient rich and energy rich, containing mobile forms of carbon (principally sucrose) and phosphates (Dominguez & Niittylä, 2021). The co-product has been described as the vascular cambial zone, comprising 80% wood (including the vascular cambium) and 20% bark. As the sawmills in northern U.K. principally utilise Sitka spruce (*Picea sitchensis* (Bong.) Carr) timber, the feedstock was from this species.

Specified biochar was produced by slow pyrolysis. A nominal HTT (highest treatment temperature) of 550°C was used. This pyrolysis temperature was selected to balance carbon storage with pH and adsorption. Increasing pyrolysis temperature improves carbon stability (and longevity), whilst high temperature pyrolysis (<700°C) will result in high alkalinity (which limit nutrient availability) and a decline in adsorption capacity (Crombie et al., 2013; Gai et al., 2014; Neina, 2019). This also enabled comparison in experiments to a control biochar produced at the same temperature (standard biochars produced at the UKBRC use pyrolysis temperatures of 550°C and 750°C nominal HTT). The resultant specified biochar produced from vascular cambial zone feedstock at 550°C nominal HTT was termed Vascular Cambial Zone Derived Biochar, or VCZ biochar.

2.1.2 Biochar Production

All biochar was produced using the "Stage III", pilot scale, reactor at the U.K. Biochar Research Centre (UKBRC), University of Edinburgh. The unit is a continuously fed rotary kiln manufactured by Ansac Ltd. The kiln is comprised of a 2.8 m heating tube angled at 0.5°, with a rotation speed of 1-7 rpm. Biomass input ranges from 50 kg hr^{-1} to ~30 kg hr^{-1} for SWP feedstock and VCZ feedstock (variable density) respectively. The kiln is screw fed via an elevated biomass hopper. Vapours produced during pyrolysis were combusted in the afterburner and exhaust gases were discharged. Biochar was collected into nitrogen purged metal drums and sealed. Temperature and pressure were monitored along the heat tube to ensure correct pyrolysis temperature and the complete pyrolysis of feedstock biomass.

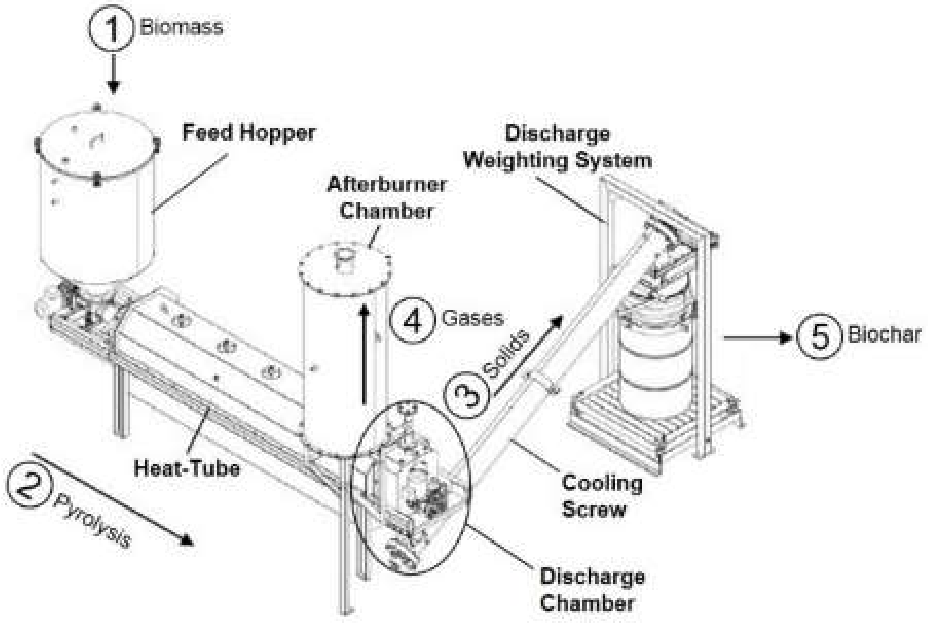

Figure 2-1- Illustration, not to scale, of Stage III pyrolysis unit at the UKBRC, which was used to produce the biochar used in Chapter 3, Chapter 4, Chapter 5 and Appendix 2. Source: Buss (2016).

Other Biochar and Application Materials

In order to test the efficacy of the specified VCZ biochar, a low nutrient and low porosity (high bulk density) biochar was used as the control (reference) biochar. Softwood pellet derived biochar, produced at the same temperature as VZ biochar (550°C nominal HTT) was selected. Soft-wood pellet derived biochar (SWP) was prepared according to methods which match the production of standard biochar SWP 550, a material widely adopted by the research community and readily available from UKBRC (Mašek et al., 2018). The SWP biochar was produced from mixed softwood pellets (Puffin Wood Fuels, Insch, Scotland) using the Stage III pyrolysis previously mentioned.

Inorganic mineral derived P fertiliser application treatments used granular triple superphosphate fertiliser. Granular TSP (42 - 50% P_2O_5) was sourced from Yara Uk Ltd.

A high available P biochar was developed in order to contain a comparable biochar mass dose to SWP biochar and comparable available P dose to conventional inorganic mineral derived P fertiliser. The biochar physical structure, and other properties, were kept consistent to SWP biochar to allow direct investigation of the effect of biochar on P accessibility. SWP biochar was treated, post pyrolysis, with addition P to create a high available P infused biochar which was termed SWP+P. To create SWP+P biochar, the SWP biochar was infused with dissolved P solution with a concentration of 13.2 mol P l^{-1} prepared from commercial TSP fertiliser (46% P_2O_5, Progreen Ltd, Bourne, England). The infusion was undertaken (1:2 w/v) over a period of 72 hours to ensure even distribution through the biochar particles. Processed SWP+P biochar was air dried for 72 hours.

Treatment properties, including VCZ biochar are displayed in Table *3-1*.

Table 2-1- Selected chemical (*top*) and physical (*bottom*). Surface area and micropore volume are assessed from values for comparable biochar feedstock and temperatures reported by (Downie et al., 2012). Methods for other properties are detailed in Chapter 3.

		VCZ	**SWP**	**SWP+P**	**TSP**
pH		10.0 ± 0.01	7.42 ± 0.02	3.60 ± 0.02	2.74 ± 0.40
NH$_4^+$-N (mg g^{-1})	mg g^{-1}	< 0.01	< 0.01	< 0.01	< 0.01
NO$_3^-$-N (mg g^{-1})	mg g^{-1}	< 0.02	< 0.02	< 0.02	< 0.02
Available P (mg g^{-1})	mg g^{-1}	3.68 ± 0.49	0.432 ± 0.014	164 ± 10	598 ± 4
Fe (mg kg^{-1})	mg g^{-1}	22.9 ± 11.3	66.4 ± 5.7	29.1 ± 0.5	189 ± 3
Al (mg kg^{-1})	mg g^{-1}	21.4 ± 10.4	42.4 ± 3.6	148 ± 8	387 ± 6
Ca (g kg^{-1})	mg g^{-1}	12.4 ± 5.0	2.71 ± 0.05	19.7 ± 0.5	53.2 ± 0.9
Cd (mg kg^{-1})	mg g^{-1}	0.06 ± 0.01	0.05 ± 0.01	1.49 ± 0.04	7.61 ± 0.20
		VCZ	**SWP**	**SWP+P**	
Bulk density (BD)	g cm$^{3-1}$	0.19	0.32	0.45	
Total ash	%	4.5	2.5	26.8	
Surface area	m^2 g^{-1}	470	25	24	
Micropore volume	cm^3 g^{-1}	0.17	0.02	0.02	

2.1.3 Circularity and Sustainability

The development of VCZ biochar considered production sustainability and circularity. This is particularly important in scenarios where biochar is being applied to soil for carbon storage. Limiting transport distance is vital when incorporating a LCA approach to certify carbon storage. Utilising feedstock from timber processors is an obvious fit, allowing relatively close proximity to nutrient limited planting sites. Timber processors, particularly sawmills produce significant quantities of low-value (compared to sawn timber) assortments of biomass which could be used for biochar feedstock. High efficiency sawmills convert ~47% of timber into co-products. The vascular cambial zone assortment is a minor component of co-products, accounting for ~5%.

During 2021, 15,300 ha of restock establishment were planted (Forest Research, 2022). If biochar was adopted in all new planting at a rate of 25 g seedling^{-1} less than 1000 t of biochar would be required. In order to produce the required biochar ~10,000 t green feedstock would be required. This represents ~10% of total co-product production of a single large sawmill. The biochar use system which was investigated in this project can sustainable be implemented using sawmill co-products. The low volume required for adoption will likely have minimal impact on other co-product markets and will not significantly increasing competition or inflate price for co-product biomass. Locating pyrolysis systems at sawmills has the further advantage of effective utilisation of excess heat, which can be used for the kiln drying process. This would reduce consumption of biomass (where bioenergy is used) or fossil fuels.

The proposed biochar production and use system provides a scalable, sustainable scenario for general adaption by the forest industry for low dose targeted application. This enables cycling of nutrients from high nutrient rich sites to nutrient poor planting sites and can also recycle a proportion of harvested nutrient back into forest soils (Figure 2-2). Despite the high retainment of phosphorus during pyrolysis, coupled with the high P of the vascular cambial zone, overall recycling of harvest P is <5% at the dose rates tested. Blanket biochar doses can provide higher total P recycling but is not feasible due to operational and environmental constraints. The scale-up scenario for industry adoption of blanket applications would require ~30% of total U.K. co-product production to be allocated as a biochar feedstock (at 10 t ha-1, this would require 1.53 Mt of green feedstock) (Forest Research, 2022). This would necessitate a fundamental change to the wood processing industry and also impact the energy sector.

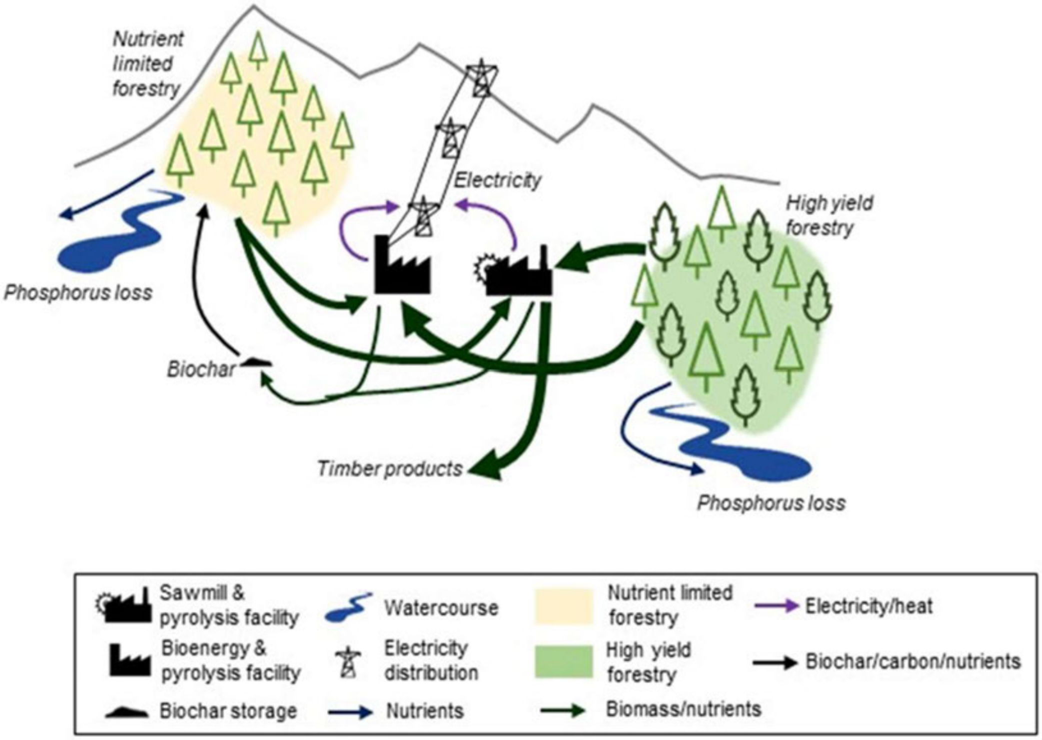

Figure 2-2- Biochar production and utilisation system scenario in U.K. forestry. Nutrients are cycled, and recycled, into nutrient limited forestry using low-value sawmill co-products to produce a high value specified biochar which can mitigate soil phosphorus loss, soil carbon loss and improve tree establishment.

2.2 Species Selection

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Scots pine (*Pinus sylvestris* L.) were selected as the principal species of study (Chapter 3 and 4). These species are the most extensive in the U.K., account for approximately 23% (Sitka spruce) and 11% (Scots pine) of total forest area (Savill, 2019), and are essential to U.K. timber production. Scots pine is the only native conifer tree to Britain with commercial significance. It is a light demanding pioneer species, which is well suited to nutrient poor free draining acid soils. Scots pine is adapted to grow in P limited environments, where other commercial conifer species would have poor establishment success and is considered as tolerant to nutrient deficiency (Savill, 2019). Scots pine seedlings typically have vigour growth to canopy closure (as a pioneer species), after which annual increment declines compared to other commercial species to normal maximum yield of 14 m^3 ha^{-1} yr^{-1} (Matthews, 2016). Scots pine can grow well in low rainfall climates (<1000 mm yr^{-1}) and is widely considered as drought tolerant. As a native species, Scots pine can also be found as dominant species in the Caledonia Forest which is a vital protected ecosystem. Scots pine was selected to allow extrapolation of results to Caledonian pine woodland creation, which is constrained to nutrient poor soils. All Scots pine seedings were grown from seed sourced from Forest Enterprise Ferndown seed orchard, which is a clonal seed orchard and considered the industry standard (Lee, 1999).

Sitka spruce is the primary timber species in the U.K. due to high yield, broad site suitability and tolerance to exposure. Sitka spruce can achieve a normal maximum yield of 24 m^3 ha^{-1} yr^{-1}. Despite the extensive site suitability across the U.K., Sitka spruce is susceptible to phosphorus deficiency and requires nutrient addition. It is considered a moderately nutrient demanding species, which is often planted in a self-thinning mixture with Scots pine as a nurse species (Kelty, 2006). On heather dominated planting sites, Sitka spruce can suffer from nitrogen deficiency from high interspecific competition (Weatherell, 1953). Sitka spruce is also a water demanding species which does not grow well in dry climates (<1000 mm yr^{-1}). It is highly sensitive to drought stress and has been highlighted as a species which will have a reduces planting range in the U.K. with climate change (Atkinson et al., 2022). The Sitka spruce forest reproductive material used in the study was from vegetatively propagated (Chapter 4) and seed orchard (Chapter 3) planting stock. Vegetatively propagated material was from full sibling clones sourced from Newton Forest Research Nursery, Moray. Seed orchard reproductive material was grown from seed sourced from A14, A17 & A18 orchards. The selection of Scots pine and Sitka spruce provided contrasting species to test the impact of biochar in a P deficient soil.

In addition to Scots pine and Sitka spruce, Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) was selected as a third species of study (Chapter 3). Douglas fir is a common conifer species in the U.K. (accounting for ~2% of the total forest area). It is often favoured on nutrient rich planting sites due to the high-quality timber of this species. All Douglas fir seedlings were grown from seed using Moray seed orchard stock. Details on seed orchard provenance mix can are detailed in Forestry Commission Bulletin 129 (Fletcher & Samuel, 2010). The species can achieve high yield on fertile, aerated freely drained soils (up to a normal maximum yield of 24 m^3 ha^{-1} yr^{-1}). It is considered as a nutrient demanding species, similar to Sitka spruce, however Douglas fir has a narrower site requirement than the other selected species (Savill, 2019). Douglas fir is unsuitable for planting on sites with moist soils (soil moisture regime), moderate exposure to wind and cool accumulated temperature (Pyatt et al., 2001). The selection of this species allows comparison of species suited to soil nutrient regimes which are poor (Scots pine) and moderate/rich (Sitka spruce and Douglas fir), and species suited to soil moisture regimes which are dry (Scots pine and Douglas fir) and moist (Sitka spruce). The use of single tree orchard sourced seedlings limits the genetic phenological differences between individuals.

2.3 Data Handling & Software

Specific data analysis is detailed in each chapter. In the completion of this study a variety of software packages were used. Data analysis was originally principally conducted using R; however SPSS was also used. ArcGIS was used for rhizosphere analysis (Appendix 2), using a novel method which was developed in this study, and ImageJ was used for microscopy image analysis (Chapter 5). Data was held in Excel and converted to csv. as needed. The statistical tests and methods are described in the relevant chapter. All data was backed-up in multiple locations and version control was in place.

Chapter 3

Impact of biochar on conifer seedling growth and nutrient delivery in phosphorus deficient conditions: A controlled environment study

3.1 Abstract

The agronomic application of biochar has been widely studied. Biochar has been shown to improve crop productivity and nutrition by directly delivering nutrients and improving soil nutrient cycling. The potential silvicultural applications of biochar have not been widely studied in the context of nutrient deficient forest soils. This study assesses the role of low dose targeted biochar for early establishment tree nutrition in Doulas fir (*Pseudotsuga menziesii* (Mirbel) Franco), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Scots pine (*Pinus sylvestris* L.) seedling, grown in a phosphorus (P) limited growing environment and compared distinct biochar types against conventional inorganic mineral derived P fertilisers at a range of application rates. A large-scale artificial growing environment pot experiment was conducted to assess the physiological response to biochar application. Specified vascular cambial zone derived biochar (VCZ), developed specifically to improve soil available P and enhance root development, was found to significantly increase above ground biomass accumulation in early establishment across all species compared with no application, although optimal dose rate was species specific. Inorganic mineral derived P fertilisers were found to suppress root biomass development and were found to cause increased mortality during year 1. Biochar was found to effectively increase seedling nutrition, ameliorating nutrient deficient growing conditions. Inorganic mineral derived P fertilisers were ineffective at improving crop nutrition due to the reduced ability of the rhizosphere to access nutrients within the soil, as a result of suppression and avoidance mechanisms. The results of this study highlight the potential of novel fertilisers in improving early growth in the establishment phase, in turn expanding the range of viable species which can be established on nutrient poor environments.

3.2 Introduction

Plantation forests established on nutrient-poor soils are widespread in northern Europe and the United Kingdom. Forest soils with poor to very poor soil nutrient regimes limit forest yield in most temperate environmental conditions, particularly in freely drained soils (Miller, 1990). Historical afforestation on nutrient-poor soils was widespread, relying on the addition of fertiliser to boost productivity and in some cases facilitate establishment (Miller, 1981). This has created a legacy of plantation forests which are situated on nutrient-poor soils (Chapter 1). Challenges underpinning fertiliser application to restock establishment sites on nutrient-poor soils have resulted in the decline of widespread fertiliser application, in favour of silvicultural solutions. Under certain circumstances, this can result in reduced establishment rates due to the deficiency of key nutrients. Soil nutrient availability is one of the primary environmental factors which limit tree growth on peaty soils and upland mineral soils (Taylor, 1991). Tree nutrition is influenced by both soil nutrient availability, but also the ability of seedlings to access these available nutrients through root systems and mycorrhizal associations (which can be described as nutrient accessibility).

Phosphorus deficiency (P) is frequently observed in conifer species established on soils with a poor or very poor nutrient status. This is particularly pronounced in free draining weathered acidic organo-mineral soils which are widespread in upland areas of the U.K. Available P has a high affinity to soil Fe^{3+} & Al^{3+}, which immobilises soil available P and reduces the accessibility of additional available P which is applied during forest fertilisation (Bueis et al., 2019). Low phosphorus soil conditions can lead to a physiological response by tree seedlings. Seedlings can adapt to P-deficient conditions by altering root architecture, root proliferation and root elongation. Studies (Lei et al., 2022; Strock et al., 2018; Zou et al., 2014) have found that spatial heterogeneity in available P can increase conifer root proliferation in P-replete patches. Under P stress, plants have been observed to actively forage for soil available P by adapting root growth to favour primary root growth over secondary growth, increasing total root length (Strock et al., 2018). Root responses to low P-deficient conditions are particularly pronounced in the early vigorous growth stage (White et al., 2013). The suppression of root foraging from high available P application reduces root development, except around fertiliser granules, until available P concentration in soil or growing media stabilises through P cycling and uptake(Ahmed et al., 2016).

Research into upland forest fertilisation in the U.K has historically focused on blanket applications of nutrients on soils which have been identified as nutrient poor or nutrient limited. Seedling foliar analysis has emerged as the primary method for quantitative non-destructive assessment of seedling nutrient status and to identify nutrient deficiency, although visual inspection is predominantly used in forest management up to pole stage. Threshold values for foliar nutrient content of the principal commercial conifer species, which are the standard values still used, were established by Binns et al. (1980) and are shown in Table 3-1. Renou-Wilson and Farrell (2007) have shown that foliar analysis can effectively be used to monitor nutrient deficiency changes in response to phosphorus fertilisation. In addition to the three primary macro-nutrients in soil, which can lead to nutrient deficiency crops established on upland soils, certain micro-nutrients have also been found to limit tree growth on certain U.K. soils (Taylor, 1991). Copper deficiency is particularly prevalent in crops established on podzolic soils with a low pH and low soil organic matter (Turvey & Grant, 1990). Deficient foliar copper concentrations are considered as <2.5 mg kg^{-1} (Hagen-Thorn & Stjernquist, 2005).

Table 3-1 – Foliar nutrient concentrations for key commercial conifer species in the U.K., with deficient and optimum thresholds shown for nitrogen, phosphorus and potassium. All units are expressed as mg g^{-1} oven dry. Modified from Binns et al. (1980); Savill et al. (1997); Taylor (1991).

		Nitrogen		Phosphorus		Potassium	
Species		Def.	Opt.	Def.	Opt.	Def.	Opt.
Picea sitchensis	Sitka spruce	<12	>15	<1.2	>1.8	<5	>7
Pinus sylvestris	Scots pine	<11	>14	<1.2	>1.4	<3	>5
Pseudotsuga menziesii	Douglas fir	<12	>15	<1.8	>2.2	<6	>8

Inorganic mineral derived P fertilisers, including granular triple superphosphate (TSP), rapidly decrease the pH of soils and growing media following application. The extent of pH reduction is highly dependent on P source and soil/growing media pH, with TSP fertiliser resulting in the greatest decline in pH (Schefe et al., 2007). This is predominantly due to changes in cation concentration, due to both the influx of cations, and the resultant changes in solution pH (Savini et al., 2006). The high solubility of TSP can result in rapid localised deceases in soil/growing media pH, which subsequently dissipate as ions disperse through lateral movement or plant uptake. In P-deficient acidic forest soils, which have a starting pH 4 - 5.5, this rapid temporary pH decrease, following TSP fertiliser application, can have a negative impact on seedling development. Plant accessible P is limited in highly acidic conditions as P sorption increases with elevated soluble Al, which can also inhibit plant growth through Al toxicity (Haynes & Mokolobate, 2001; Qin et al., 2010). Whilst there is little direct evidence on the effects of P fertiliser on pH induced conifer seedling mortality, studies have found that in extreme acid conditions pH is sufficient to result in seedling mortality due to meristem tissue death (MacDonald & Ducsay, 1997; Percy, 1986; Zhang et al., 2021).

The relationship between spatial temporal available P distribution and seedling root development contributes to P fertilisation efficiency (Rashid et al., 2022). The impact of P fertilisation on root development has the potential to negatively influence seedling growth following exhaustion of applied P fertiliser if root development is reduced. As crops mature a small proportion of P plant uptake is directly provided from applied P sources, with the principal P source coming from bulk soil P reserves (Johnston et al., 2014). In crops which develop small ineffective root systems potential P uptake is limited and either growth is reduced, or additional P application is required. Root development also directly impacts the uptake and availability of other micro and macro nutrients, with clear synergies between forest soil nutrient cycles (Fisk et al., 2014).

The limitations associated with inorganic mineral derived P fertilisers, highlight the need for a sustainable alternative for use in forest establishment. Rock-derived phosphorus fertiliser can be considered a finite resource and current fertilisation practices are not sustainable and may result in global P scarcity (Scholz et al., 2013). Closing P cycling loops through the uptake of organic-derived fertilisers in agriculture and forestry may provide a sustainable alternative to inorganic mineral-derived fertiliser (Vaneeckhaute et al., 2015). Biochar, produced by the chemical transformation of biomass at high temperature and low oxygen, is widely accepted as an effective carbon negative soil improver (Lehmann et al., 2021; Štrubelj, 2022; Woolf et al., 2010). There is compelling evidence that biochar can have a beneficial effect on key soil parameters which influence crop growth and nutrient availability (Schmidt et al., 2021). The extent of agronomic, and likely silvicultural, benefits of biochar on plant soil interactions is highly dependent on biochar properties, application dose and environmental factors (Gao et al., 2019; Xiang et al., 2017). Specified biochar application to seedlings during planting has the potential to improve establishment fertilisation, both as a direct nutrient source and by favourably altering the rhizosphere enhancing the nutrient availability and nutrient accessibility from the bulk soil.

Examining the physiological response of conifer seedlings to biochar and conventional P fertilisers in a controlled environment experiment, using a homogenised bespoke growing media, can help identify the principal underpinning mechanisms and factors which influence seedling establishment on nutrient limited soils. The purpose of this study is to; *i*) examine the growth response of selected conifer species to biochar soil inputs *ii*) investigate the foliar nutrient concentration of seedlings according to crop inputs to identify foliar nutrient deficiency *iii*) assess the below ground biomass development of seedlings according to available phosphorus input and the subsequent impact on above ground growth.

3.3 Methods

3.3.1 Experimental Design and Site

Experiment Site

The experiment was conducted at Forest Research Northern Research Station, Easter Bush (EH25 9SY). The experiment was setup April 2019 and end of experiment sampling was completed February 2021. Seedlings were grown for two growing seasons. Due to the number of seedlings and required growth a straight poly-tunnel was used. The experiment was located in 15 m x 20 m poly-tunnel with a semi-controlled environment. Seedlings were individually potted and place on weed control fabric over a freely drained aggregate base.

Experimental Treatments

The response of Sitka spruce (*Picea sitchensis*), Douglas fir (*Pseudotsuga menziesii*) and Scots pine (*Pinus sylvestris*) seedlings to soil amendment treatments was tested in this study. Biochar treatments were specified according to available P content, to supply high available P softwood pellet derived P infused biochar (SWP+P)), moderate available P vascular cambial zone derived biochar (VCZ) and low softwood pellet derived biochar (SWP) treatments (detailed in Chapter 2). Three doses were tested for each selected biochar and the targeted triple superphosphate P fertiliser application (TSP. Surface phosphate fertiliser (SPH) application was applied at a single dose rate, using TSP fertiliser. A control (CON) treatment with no soil amendment was also included. In total there were 14 application treatments for each species, giving a total number of 42 treatments.

Application Rates

Three dose rates for each biochar type and for TSP fertiliser application were applied. A single dose rate for surface phosphate (SPH) was used, which matched the intermediate targeted TSP dose (7 g seedling^{-1}). All dose rates are expressed on a per seedling basis (g seedling^{-1}). SWP and VCZ biochars were applied at 15 g, 25 g and 40 g seedling^{-1} dose rates. Biochar was applied on a volume basis, delivering the required mass dose. SWP+P dose rate was calculated to deliver an equal mass dose of biochar, plus the additional mass of infused P (and associated elements, mainly; Ca, Al and Cd). SWP+P mass dose was applied at 19 g (15 g biochar + 4 g TSP fertiliser), 31.66 g (25 g biochar + 6.87 g TSP fertiliser) & 50.66 g (40 g biochar + 10.67 g TSP fertiliser). In the results, application dose rates of SWP+P biochar is stated as the biochar component dose rate (15 g, 25 g & 40 g). The three application doses of TSP (4g, 7 g and 10 g) delivered comparable available P to seedlings when compared to SWP+P application rates (Table *3-2*). The intermediate SWP + P, intermediate TSP and SPH dose rate was based on the recommended dose of P fertilisation for conifer seedlings in P deficient soils (Taylor 1991). The stated application rate delivers 60 kg available phosphorus ha^{-1}, which equates to 24 g available P per tree (requiring 39.87 g TSP per seedling). When this rate is adjusted for targeted application and growing medium volume, to maintain equivalent TSP:growing medium ratio.

Table 3-2 – Total available P applied to seedlings, according to treatment and dose. Calculated by multiplying available P concentration (mg g^{-1}) and dose rate (g).

Treatment	Application rate (g $seedling^{-1}$)	Available P concentration (mg g^{-1})	Total available P (mg $seedling^{-1}$)
Control (CON)	n/a	n/a	n/a
Surface phosphate (SPH)	7	598 ± 4	4186
Triple superphosphate (TSP)	4	598 ± 4	2392
	7	598 ± 4	4186
	10	598 ± 4	5980
Biochar (SWP)	15	0.432 ± 0.014	6.48
	25	0.432 ± 0.014	10.80
	40	0.432 ± 0.014	17.28
Biochar (VCZ)	15	3.68 ± 0.49	55.20
	25	3.68 ± 0.49	92.00
	40	3.68 ± 0.49	147.20
Biochar (SWP+P)	15	164 ± 10	2460
	25	164 ± 10	4100
	40	164 ± 10	6560

Experiment Setup and Design

The experiment layout utilised a two-block randomised design. There were 42 single treatment plots per block, with a randomised plot layout. Each plot consisted of 10 individually potted seedlings arranged in two five pot rows, with no gap between pots in each plot. There was a total of 20 seedlings per treatment (10 per plot). This gave a total of 840 individually potted seedlings. A 50 cm buffer was used between plots, preventing any canopy interaction.

Bare root seedlings were graded prior to planting, ensuring that seedling length within one S.D of the mean length of a measured sub-sample for each species. 30 seedlings were measure (one ten tree bundle from each planting bag) to establish the mean seedling length for each species. The mean seedling lengths were 28.5 cm (Douglas fir, 64.2 cm (Sitka spruce) and 23.7 cm (Scots pine). Sitka spruce seedlings were vegetatively propagated, and Scots pine were from seed. Seedlings were visually assessed and discarded if signs of poor health (low root mass, needle discolouration, needle loss etc.) was observed. Seedlings were undercut prior to nursery uplift, decreasing root length and variability root biomass between seedlings. Seedlings were then planted into 3 L individual pots using a custom mixed peat based growing media supplied by Sinclair Ltd. The peat-based growing media had no added nutrients and the pH was adjusted to 4.5. Prior to planting, the peat based growing media was homogenously mixed with washed quartz sand at a volume rate of 70% peat growing media and 30% quartz sand. Mixing ensured that available P was evenly distributed in the growing media. Key chemical properties are shown in Table 3-3. Biochar treatments and the targeted inorganic mineral derived P fertiliser treatments (TSP) were applied directly to seedling roots during planting as the growing media was added on a volume basis calibrated to each mass dose. All soil amendment treatments contained available P with a concentration at least one order of magnitude greater than the surrounding growing media, creating localised P-replete conditions. The surface applied inorganic mineral derived P fertiliser treatment (SPH) was evenly applied to the growing media surface immediately following planting.

Table 3-3 - Selected properties of growing media prior to quartz sand addition.

	Growing Media	
	mg g^{-1}	Total per pot
pH	4.41 ± 0.02	4.41
NH_4^+-N	1.41 ± 0.06	592.20
NO_3^--N	1.04 ± 0.02	436.80
Available P	0.046 ± 0.020	19.32
Fe	0.0649 ± 3.0	27.26
Al	0.00952 ± 0.54	4.00
Ca	0.135 ± 0.007	56.70
Cd	0.00004 ± 0.01	0.02

3.3.2 Biochar Production

The three biochars applied in this study were SWP “reference biochar” (low in P), VCZ “specified biochar” (derived from vascular cambium, naturally higher in P) and SWP+P “processed biochar” (high in P, artificially enhanced P content). All three biochars used were derived from woody biomass feedstock and produced by the UK Biochar Research Centre (UKBRC; University of Edinburgh, Edinburgh, UK). The equipment used was the Stage III system, a horizontal continuous feed pyrolysis kiln, manufactured by Ansac Pvt Ltd (Bunbury, Australia). The feedstock was pyrolyzed at a nominal highest heating temperature (HTT) of 550 ± 5°C with a heating rate of 78°C min^{-1}. Mean residence time in the kiln was 12 min, with 3.9 min at HTT. Details of the Stage III pyrolysis system can be found in Mašek et al. (2018).

The feedstock for VCZ biochar was the product of ring debarking of *Picea sitchensis* (Sitka spruce) (Rathnayake et al., 2021). This is a sawmill process that removes the outer 5 mm of timber logs, yielding 80% wood and 20% bark by volume, incorporating the vascular cambium. The VCZ feedstock from this study was obtained from a sawmill in south-east Scotland (BSW Ltd, Petersmuir, Scotland).

Soft-wood pellet derived biochar (SWP) was prepared according to methods which match the production of standard biochar SWP 550, a material widely adopted by the research community and readily available from UKBRC (Mašek et al., 2018). The SWP biochar was produced from mixed softwood pellets (Puffin Wood Fuels, Insch, Scotland) using the Stage III pyrolysis previously mentioned. To provide comparison with Processed biochar that was soaked in P solution and dried afterwards, the SWP biochar was soaked in deionized water for 72 hours and oven dried at 50°C to provide Reference biochar in the present study.

To create SWP+P biochar, the SWP biochar was infused with dissolved P solution with a concentration of 13.2 mol P l^{-1} prepared from commercial TSP fertiliser (46% phosphate, Progreen Ltd, Bourne, England). The infusion was undertaken (1:2 w/v) over a period of 72 hours to ensure even distribution through the biochar particles. Processed SWP+P biochar was air dried for 72 hours.

Inorganic mineral derived P fertiliser application treatments were used to compare biochar treatments to conventional fertilisation practices and targeted available P application. Surface phosphate was applied (SPH) using the same TSP fertiliser used in targeted triple superphosphate treatments. Granular TSP (42 - 50% P_2O_5) was sourced from Yara Uk Ltd. The selected chemical properties of all treatment materials are displayed in Table 3-4.

Table 3-4 – Selected chemical composition of treatment applications.

	VCZ	**SWP**	**SWP+P**	**TSP**
pH	10.0 ± 0.01	7.42 ± 0.02	3.60 ± 0.02	2.74 ± 0.40
NH_4^+-N (mg g^{-1})	< 0.01	< 0.01	< 0.01	< 0.01
NO_3^--N (mg g^{-1})	< 0.02	< 0.02	< 0.02	< 0.02
Available P (mg g^{-1})	3.68 ± 0.49	0.432 ± 0.014	164 ± 10	598 ± 4
Fe (mg kg^{-1})	22.9 ± 11.3	66.4 ± 5.7	29.1 ± 0.5	189 ± 3
Al (mg kg^{-1})	21.4 ± 10.4	42.4 ± 3.6	148 ± 8	387 ± 6
Ca (g kg^{-1})	12.4 ± 5.0	2.71 ± 0.05	19.7 ± 0.5	53.2 ± 0.9
Cd (mg kg^{-1})	0.06 ± 0.01	0.05 ± 0.01	1.49 ± 0.04	7.61 ± 0.20

3.3.3 Experiment Management

Daily experiment management was conducted by Forest Research nursery staff. Fluctuations in internal temperature was controlled using poly-tunnel ventilation. The experiment was kept weed free. All seedlings were sprayed with Gazelle SG® MAPP 13725 pesticide (to treat green aphid) twice per year. Powdery mildew was found in the Scots pine seedlings during year 2. This was not extensive, and a decision was made to leave untreated. Application of fungicide risked influencing soil fungi communities, which could affect nutrient dynamics. Seedlings were watered approximately every second day and watering intensity/frequency was controlled by nursery staff to ensure that growing media was kept above wilting point. Soil was watered to field capacity (~30% volumetric water content). Irrigation was stopped when field capacity was reached to minimise leaching and lateral nutrient loss out of the pots. Soil moisture was maintained at within a soil moisture range where water was available to seedlings to minimise moisture stress. Soil water was maintained above 10% (volumetric water content). Volumetric water content was assessed using a handheld Campbell Scientific soil moisture sensor. This experiment did not assess the impact of biochar on soil water properties. Poly-tunnel temperature and seedling watering was recorded daily.

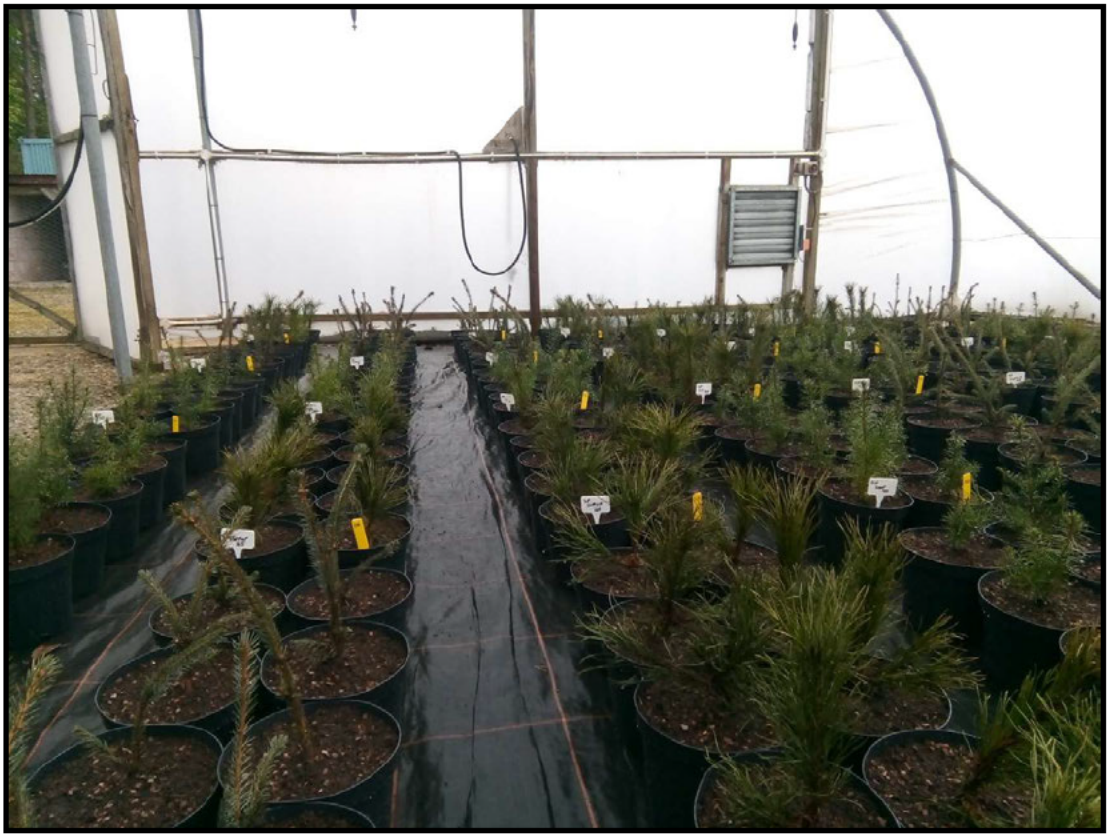

Figure 3-1- Part of the experiment (a portion of Block 2) can be seen in this image, highlighting experiment layout, plot buffers and trees size/condition at planting. Image was taken April 20219.

3.3.4 Data Collection and Measurement

This section details the data collected during this experiment. This includes growth measurements (used to assess seedling morphology), chlorophyll fluorescence, needle sampling and end of experiment biomass. Biochar was retrieved for use in the microscopy analysis detailed in Chapter 5.

Seedling Growth and Biomass

Data were collected following the end of each growing season (November), during seedling winter dormancy, and at the start of the experiment, immediately following planting. Seedling mensuration was conducted following planting and experimental setup during April 2019. Subsequent measurements were conducted during October 2019 and April 2020. Final mensuration and destructive biomass sampling were completed between January and February 2021. Data were collected for two growing seasons (2019 and 2020). All April measurements were completed before new growth was observed. October measurements were conducted during seedling winter dormancy. Seedling morphology, chlorophyll fluorescence and needle chemical composition was measured. Morphological measurements were taken which can be used to assess seedling quality and establishment performance. The week of budburst foe each seedling was recorded in Year 1, inspecting seedlings on a weekly basis.

Biannual growth measurements included leader length (cm), lateral length (cm), seedling height (cm) and root collar diameter (cm). Survival and tree condition was also recorded. Leader length was measured from the upper surface of the highest branch of the top branch whorl to the top of the apical bud. Where two leaders were present, both lengths were measured, and the longest length was recorded. Seedling height was measured using a vertically orientated 3 m measuring rod. Height was taken from the top of the root collar to the seedling apical bud. Seedling stem angle did not exceed 10° from vertical in any seedlings and height was not corrected for seedling lean. Root collar diameter was measured using 150 mm digital callipers with a resolution of 0.01 mm. As the root collar of seedlings is not uniformly circular, the widest root collar diameter was measured. The three longest lateral branches in the top branch whorl were measured and the mean lateral length was recorded for each seedling. Length was taken from the upper stem-branch confluence to the apical bud, along the upper surface of the lateral branch. The soft new growth of lateral branches was delicately straightened during measurement.

Chlorophyll Fluorescence

Chlorophyll fluorescence parameters of photosystem 2 were measured using a pulse modulated Waltz Mini PAM II fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The measured parameters are;

F_v/F_m - Maximum quantum efficiency of PSII photochemistry

ϕPSII - PSII operating efficiency: the quantum efficiency of PSII electron transport in the light.

Dark adapted measurements were taken using dark leaf clips (part no. 2030-B). Only needles on the uppermost lateral branch whorl were used, ensuring that the most recent year of needle growth were measured. Care was taken to orientate needles to completely cover the clip aperture. In order for dark adaption to occur, the dark leaf clips were affixed to seedlings 30-40 minutes prior to measurements.

Biomass and Root Sampling

From each treatment plot three trees were randomly selected for assessment. Selected trees were firstly photographed from a fixed position prior to destructive sampling. Above ground biomass was separated from below ground biomass by cutting seedling stems at the root collar. Above ground biomass for each selected seedling was sampled to provide dry weight measurements for needle biomass, lateral branch biomass and stem biomass. Lateral branches, including needles, were removed at the stem and collected in aluminium foil trays for each sample tree individually. A 3 g (wet weight) needle sample was separately collected from new needle growth on the seedling leader. Stem biomass, including needles, was cut and added to separate aluminium foil trays. All biomass samples were oven dried at 70°C for 48 hrs (Alomar et al., 2003; Ramsumair et al., 2014). Needle biomass for each sample seedling was collected by removing dry needles from the stem and laterals, then aggregated. Dried biomass sample mass was determined to give total needle, lateral and stem biomass data. The dried 3g (w/w) needle samples were measured for dry weight, and moisture content (MC %) was calculated using measured wet weight and dry weight (Equation 3-1); these samples were bagged and retained for foliar chemical analysis.

$$MC_w\ (\%) = MCw\ = \left(\frac{(mw - md)}{mw}\right) \times 100$$

Equation 3-1 – Moisture content on wet basis (MC_w (%)) calculated from needle wet mass (*mw*) and dry mass (*md*).

Following the removal of aboveground seedling biomass at the root collar, the roots were removed from pots and loose growing media was gently removed from root systems manually in order to assess belowground root biomass. Root systems were then washed with running water, ensuring that root biomass was completely free of growing media. Individual root systems were independently dried in aluminium foil trays at 70°C for 48 hours. During the root washing process some fine roots (<3 mm diameter) became detached. These were collected and dried with the cleaned root ball in order to ensure that all root biomass was measured. Dried root biomass was measured using an analytical lab balance.

During root removal biochar samples were retrieved from the growing media, 5 g wet-weight samples were collected. Biochar samples were sieved through a 2 mm aperture soil sieve to remove growing media and sand. Following sieving retained material was collected. Biochar samples were dried for 48 hours at 70°C prior to chemical analysis. At this time five biochar particles were selected in each seedling, for analysis by scanning electron microscopy and optical microscopy (detailed in Chapter 5). Growing media were sampled following the removal of seedling root systems, 4 g dry-weight samples were collected for each seedling. For all sample trees, growing media samples were collected from the centre of the pot, 5 cm in height from the pot base. Material was passed through a 1 mm aperture soil sieve, removing any biochar, root or sand particles. Growing media samples were dried for 48 hours at 70°C prior to chemical analysis.

Root structure was assessed following measurement of root biomass. Seedling root balls were rehydrated in water by soaking for a period of 24 hrs, ensuring that all roots were pliable. The previously cleaned seedling root systems were spread onto a white ceramic tile background. Root structure was photographed using a fixed position camera. The camera was fixed 1 m directly above the centre of the ceramic background tile. This provided spatially comparable photographs, which included a fixed 1cm reference scale. Lateral root branching density within the treatment area was measured for each seedling by selecting the highest diameter root at 5 cm vertically below the root collar root. A 5 cm length of the selected root was assessed from this point and the number of lateral roots were counted and a mean density was calculated (expressed as lateral roots cm^{-1}).

Chemical Analysis

New leader needles were sampled individually from new growth close to the apical bud of each selected seedling and oven dried at 90°C before preparation for total P analysis. Part of fresh samples were used for NH_4^+-N, NO_3-N and available P analysis The needles were digested with concentrated H2SO4 and 30% H2O2 and analysed by autoanalyser (Bran Luebbe AA3, Seal Analytical, Norderstedt, Germany).

Each treatment material and the growing media was analysed before use. 1 kg of each material was sampled and homogenised using the cone and quartering method, and a 10 g pooled sample was then taken (Schumacher et al., 1990). As with needle biomass, part of the fresh samples was used for NH_4^+-N, NO_3-N and available P analysis. The rest of the samples were subsequently oven dried at 90°C and milled in a ball grinder before further P, Cu, Mg, Cd, Fe, Al, Ca analysis.

Chemical analysis of the growing medium and application treatments was undertaken to elucidate potential factors influencing biochar-soil-seedling interactions. For the pooled pre-planting samples, the extraction or digestion procedure was replicated 3 times (as technical replicates). The final samples at the end of experiment were only extracted or digested once without setting technical replicates. Extraction for NH_4^+-N, NO_3-N was undertaken using 1:10 w/v 1M KCl and analysed by autoanalyser (as above). Available P was analysed through water extraction (Prendergast-Miller et al. 2014) and subsequent analysis of the extracts by autoanalyser (as above). Total P, Cu, Mg, Cd, Fe, Al and Ca were then extracted by a modified dry-ash method for biochar. Samples were heated in a muffle furnace (500°C, 8 h) and placed into a steam bath upon cooling. Concentrated nitric acid (HNO^3) was added and evaporated to dryness, followed by 1:4 HNO_3 and $H2O_2$ which was evaporated to dryness. All residues were transferred through Whatman Grade 4 filter paper, using HNO_3 and deionized water, into a volumetric flask. The elemental composition of the filtrate was measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo-iCAP 6300, Thermo Electron, Waltham, USA). The pH of the growing medium was assessed for the experiment period using a soil: water ratio of 1:2 (McLean 1983).

3.3.5 Statistical Analysis

Data was analysed separately according to year and species. Inter year and interspecies analysis was also conducted, however there was high variability between variables and total yearly data was not normally distributed. Statistical analysis was fulfilled on growth data, chlorophyll fluorescence and biomass parameters separately for each species using analysis of variance. Each measured parameter was analysed with application treatment as fixed effect and block, replication and interaction terms treated as random effect. Biomass data and chlorophyll fluorescence data was first tested according to treatment groups for equal variance using Levene's test. This was used assess whether data fulfils the homogeneity of variance assumption required to accept Analysis of Variance (ANOVA). Treatment groups showed homogeneity of variance (*p*>0.05) following Levene's test for equal variance and no transformations were required. One-way and two-way (with foliar chemical composition as a covariable) ANOVA statistical tests were used. Post-hoc analysis was used to determine treatment groupings; the Tukey test was selected for this purpose. Multiple linear regression was used to assess the proportion of variation between biomass measurements (dependent variable) that can be explained using this model for dose rates, treatment type and available P delivery (independent variables). Normality of residuals was tested using Shapiro-Wilk test. Where ANOVA obtained significant F-values, pairwise comparisons were tested using Tukey HSD test, adjusted for type I error. The variation between and within treatment groups was compared using the coefficient of variation (CV), which is a statistical measure of the relative dispersion of data points in a data series around the mean. The relationship between treatment dose and measured dependent variables was tested using a simple linear regression analysis. Residuals were tested in order to determine whether assumptions required for regression analysis have been met. Correlation between root and above ground biomass was also assessed for each treatment independently using Pearson correlation coefficient. Inter-species comparisons were analysed by calculating seedling biomass difference from the mean biomass in the CON treatment for each species separately in order to compare treatment efficacy across species. Results were reported in figures using an abbreviated code structure based on species/treatment/dose (for example SSCON00) (Table 3-5).

Table 3-5 – Description of codes and abbreviations used in results figures.

Species		Treatment		Application rate	
Code	Description	Code	Description	Code	Description
SS	Sitka spruce	CON	Control	00	No application
DF	Douglas fir	SWP	SWP biochar	04	g seedling^{-1}
SP	Scots pine	VCZ	VCZ biochar	07	
		S+P	SWP+P biochar	10	
		TSP	TSP fertiliser	15	
				25	
				40	

3.4 Results

Biomass and foliar analysis results for each species are reported separately, and differences between each species seedling response are briefly discussed in Section 4.5.

3.4.1 Sitka spruce (*Picea sitchensis*)

Sitka spruce - Above Ground Biomass

Seedling above ground biomass was found to varied across treatments (Table 3-6). No significant difference in the mean total aboveground biomass (p=0.085), stem biomass (p=0.183), lateral biomass (p=0.453) was found across treatments in Sitka spruce seedlings. Total above ground biomass was close to significant (p=0.86) between treatments, particularly comparing moderate and high VCZ doses to control treatments, with higher total above ground biomass in seedlings treated with 25 g seedling^{-1} & 40 g seedling^{-1} VCZ biochar. The dose rate for each treatment showed no linear relationship with needle biomass, stem biomass or aboveground biomass (R^2<0.1), except for treatment with VCZ biochar. Results show that little variance can be explained using a linear regression model for needle biomass (R^2=0.117) and above ground biomass (R^2=0.155) of seedlings treated with VCZ biochar, with a weak increase in biomass with increased dose (needle biomass [y=1.64+0.03*x] and above ground biomass [y=12.37+0.23*x]).

Treatment had a significant effect (p=0.013) on total needle biomass, although post-hoc analysis revealed that this was only between 40 g seedling^{-1} VCZ and 10 g seedling^{-1} TSP treatments (p=0.022). This difference can primarily be attributed to observed old needle loss and poor needle retention (a symptom generally associated with K deficiency (Taylor, 1991)) in high available P treatments. There was a very weak positive relationship between needle biomass and the dose rate of biochar treatments. The highest amount of variation between doses which could be explained using a linear regression model was found in SWP+P biochar (R^2=0.357, y=3.59+0.08*x), followed by SWP biochar (R^2=0.222, y=3.68+0.08*x) and VCZ biochar (R^2=0.155, y=4.96+0.1*x) respectively

Table 3-6 – Summary ANOVA table for end of experiment Sitka spruce biomass accumulation results according to treatment applications and doses. The biochar treatments were softwood pellet) SWP), vascular cambial zone (VCZ) and phosphorus infused softwood pellet (SWP+P) biochar. Above ground (A.G.) woody biomass is derived from the sum of lateral and stem values, and above ground (A.G.) total biomass also includes needle biomass. Means, standard error and ANOVA Tukey groupings are displayed (*n* = *6*).

		Biomass (g seedling^{-1})					
Treatment	Application rate (g seedling^{-1})	Needle	Lateral	Stem	A.G. Woody	A.G. Total	Root
Control (CON)	n/a	5.21 (0.57)^{ab}	1.86 (0.26)	6.03 (1.26)	7.89 (1.44)^{a}	13.10 (1.97)	9.82 (1.02)
Surface phosphate (SPH)	n/a	5.41 (0.75)^{ab}	1.47 (0.85)	6.01 (0.35)	7.47 (1.16)^{a}	12.88 (1.83)	9.65 (1.08)
Triple superphosphate (TSP)	7	7.30 (1.40)^{ab}	2.54 (0.60)	8.44 (1.67)	10.98 (2.27)^{ab}	18.28 (3.62)	13.02 (2.35)
	10	4.12 (0.85)^{a}	1.93 (0.54)	5.98 (1.10)	7.91 (1.62)^{a}	12.03 (2.44)	8.34 (1.92)
Biochar (SWP)	15	5.03 (0.72)^{ab}	2.12 (0.45)	6.92 (0.90)	9.04 (1.29)^{ab}	14.07 (1.99)	10.64 (1.44)
	25	5.67 (0.84)^{ab}	2.49 (0.89)	6.18 (1.31)	19.87 (3.13)^{cd}	15.17 (2.26)	10.37 (1.71)
	40	7.12 (0.61)^{ab}	2.03 (0.28)	7.79 (1.23)	9.82 (1.40)^{ab}	16.95 (1.89)	11.73 (1.83)
Biochar (VCZ)	15	6.18 (1.09)^{ab}	2.05 (0.38)	6.32 (0.86)	8.36 (1.18)^{a}	14.54 (2.21)	10.26 (1.52)
	25	8.16 (1.20)^{ab}	2.49 (0.34)	9.50 (0.87)	24.82 (2.33)^{d}	20.16 (1.94)	12.83 (1.36)
	40	8.91 (1.44)^{b}	2.82 (0.43)	8.93 (1.33)	11.76 (1.60)^{acb}	20.66 (2.27)	12.58 (2.27)
Biochar (SWP+P)	15	4.81 (0.37)^{ab}	1.46 (0.15)	6.72 (0.89)	15.59 (2.10)^{bcd}	18.40 (1.36)	9.67 (0.80)
	25	5.79 (0.56)^{ab}	1.95 (0.14)	9.45 (1.25)	11.40 (1.36)^{abc}	17.19 (1.88)	9.67 (0.80)
	40	6.94 (0.72)^{ab}	2.85 (0.52)	8.33 (0.73)	11.18 (1.03)^{ab}	18.12 (1.57)	11.95 (1.20)
	F	2.381	1.007	1.459	8.912	1.709	0.884
	P	0.013	0.453	0.163	<0.001	0.85	0.567

Superscripted lowercase letters (^{abcd}) indicate significant differences ($p<0.05$) and groupings from ANOVA with post-hoc Tukey test.

Values in parenthesis are standard errors of mean.

Mean above ground woody biomass accumulation differed across treatments (*p*<0.001) (Figure 3-2). Mean total woody biomass was significantly higher than control treatments in low dose SWP+P and moderate dose VCZ and SWP biochar applications. Unlike other biomass parameters, high dose rates (40 g seedling^{-1}) of SWP and VCZ caused a relative decline in mean total biomass compared to 25 g seedling^{-1} doses. Total woody biomass in high dose biochar treatments was comparable to low (15 g seedling^{-1}) dose rates and to non-biochar control treatments. When the ratio of above ground woody biomass to needle biomass is tested; SWP+P 15 g seedling^{-1}, SWP 25 g seedling^{-1} and VCZ 25 g seedling^{-1} have a significantly (*p*<0.001) higher proportion of woody biomass allocated over needle biomass, compared to all other treatments. Other differences between treatments were not significant.

Despite the high woody biomass accumulation (F=19.45) in seedlings subjected to these treatments, no obvious signs of leaf litter loss or reduction in needle length (a symptom of P deficiency) was observed. There was no linear relationship between dose rate and woody biomass in any biochar treatment (R^2<0.1). There was a positive linear correlation between above ground woody biomass and needle biomass (R^2>0.650), except in SWP 40 g seedling^{-1} biochar (R^2=0.549), VCZ 25 g (R^2=264) and VCZ 40 g (R^2=0.541) treatments. As the woody biomass and needle biomass response of seedlings to certain biochar treatments is distinct, it can be observed that biochar can alter seedling growth traits and morphological development.

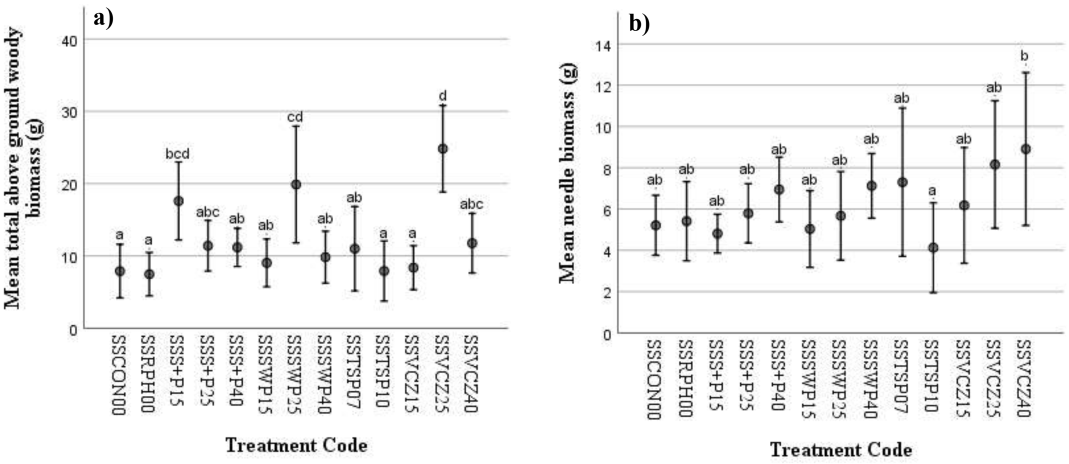

Figure 3-2 – Mean a) total above ground woody biomass and b) needle biomass for all treatments. ANOVA post-hoc Tukey groupings are displayed. There is sig. dif. between treatments ($p<0.05$), with results shown in Table 3-6 ($n=6$).

Sitka spruce - Root Biomass and Morphology

There was no significant difference ($p=0.567$) in below ground biomass between treatments, however different growth traits may be present. A strong linear relationship between root biomass and above ground biomass was found in all treatments excluding moderate (25 g seedling^{-1}) and high dose (40 g seedling^{-1}) SWP+P applications (Figure 3-3). In these SWP+P treatments root biomass showed no observable relationship to above ground biomass. ($R^2<0.1$). Despite the high available P content in TSP treatments, there was a strong positive linear relationship between seedling root biomass and above ground biomass ($R^2>0.8$).

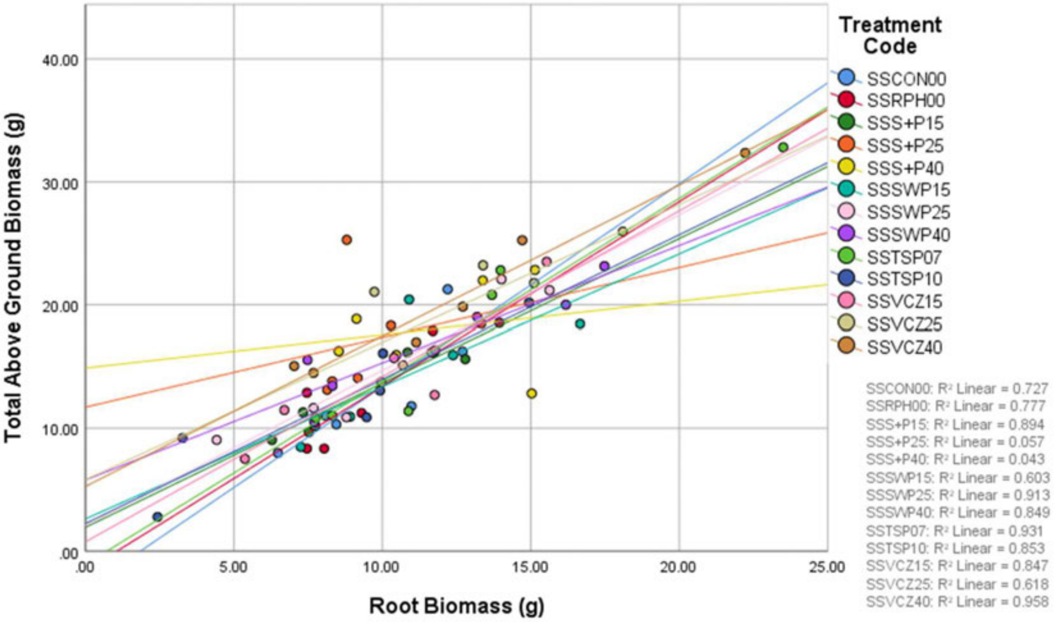

Figure 3-3 – Scatter plot of Sitka spruce root biomass (g) and above ground biomass (g) for end of experiment sample seedlings grouped according to treatment. Linear regressions are included with R^2 for each treatment group, *n=6*.

There was a significant reduction ($p<0.001$) in lateral root and secondary root development from primary roots close to the root collar of seedlings in targeted high available P treatments (SWP+P 40 g seedling^{-1}, TSP 7 g seedling^{-1} and TSP 10 g seedling^{-1}). Root death was observed in TSP treatments in primary and secondary roots which were present in bare root stock at time of planting (Figure 3-4). Extensive death of fine roots (<2 mm) was observed, with suppression of un-lignified first and second order roots distributed across the root system. The death of lignified fine roots, in addition to roots with diameters >2 mm, was observed in areas where lignified roots were in direct contact with TSP granules.

Figure 3-4 – Root structure of Sitka spruce seedlings at the end on year 2. A healthy root system (*left*), from seedling planted with SWP 15 g treatment, has observable fine roots and dense lateral roots close to the root collar (which has some adventitious roots present). This is contrasted to the root system of seedlings treated with a 10 g TSP dose. The root structure present at planting is highlighted by the red circle, noting the absence of fine roots and lateral branching. Scale (cm) is displayed.

Sitka spruce - Seedling Growth Dynamics & Survival

Growth rate was significantly higher than the control in some biochar treatments ($p<0.001$), despite no significant difference in above ground biomass accumulation ($p>0.05$). This can partly be attributed to the high proportional variation in bare root seedlings and the relatively low, when compared to other species, mass accumulation across treatments. Vegetatively propagated Sitka spruce seedlings were used in this study, which have distinctive seedling form. Seedlings produced from this method have a low frequency of lateral branching and therefore new growth in the initial establishment phase following out-planting accounts for a low proportion of total biomass. VCZ biochar application significantly increased year two growth rates, compared to CON, TSP and SPH treatments. Moderate VCZ biochar application (25 g seedling^{-1}) resulted in the highest year 1 and year 2 growth rates, significantly higher than the control treatment ($p<0.001$). All biochar treated seedlings showed no decline in growth rate between year 1 and year 2. The CON treatment showed relatively high seedling growth rate during year 1, however this collapsed in year 2, with a mean seedling growth rate of <10 cm yr^{-1}. Inorganic mineral derived P fertilisers had a variable effect on seedling growth, with high variation in year 1 and year 2 growth. Some seedlings showed poor year 1 growth, recovering in year 2. Conversely, some seedlings treated with inorganic mineral derived P fertilisers exhibited strong year 1 growth which subsequently slowed during year 2. This variable effect was correlated to root biomass. Inorganic mineral derived P fertiliser treated seedlings which had highly suppressed root system showed poorer year 1 growth and seedlings with stronger root development in these treatments had stronger year 1 growth.

Seedling survival decreased in seedlings treated with high available P applications. Targeted TSP application had significantly higher mortality than all other treatments ($p<0.0001$). SWP+P, which has comparable available P dose to TSP had higher seedling survival than TSP, showing that infused P biochar can mitigate the negative effects of high available P fertiliser. SWP, VCZ and CON had mortality rates below <5 %., suggesting that inorganic mineral derived P fertilisers directly contributed to the increased seedling mortality in these treatments, rising to between 20% and 85%.

Sitka spruce - Foliar Composition and Nutrient Deficiency

Foliar analysis results show significant differences in the concentration of key plant nutrients (P ($p<0.001$), K ($p=0.002$), N ($p=0.013$) & Cu ($p<0.001$)) and toxicants (Cd ($p<0001$). These results are summarised in Table 3-7. Needle P concentration was higher than the control treatment (CON) in high dose (40 g seedling^{-1}) VCZ and SWP+P treatments, with TSP fertiliser applications also showing elevated needle P concentrations. Seedlings treated with high doses (40 g seedling^{-1}) of SWP and VCZ biochar had significantly higher ($p<0.05$) needle K concentration than SPH, CON and low dose (15 g) SWP treatments. High dose (40 g seedling^{-1}) VCZ and high dose (10 g seedling^{-1}) TSP amendment resulted in significantly increased ($p=0.016$ and $p=0.013$) needle Cu concentration, when compared to the control (CON) treatment. Inorganic mineral derived P fertiliser has a high Cd content, which in turn has resulted in elevated needle Cd concentration in treatments containing inorganic mineral derived P. TSP, SPH and high dose rates of SWP+P significantly ($p<0.05$) increase needle P concentration compared with amendments which contain no inorganic mineral derived P. Lower doses of SWP+P (15 g seedling^{-1} and 25 g seedling^{-1}) do not lead to a significant increase in foliar Cd ($p>0.05$) concentration.

When needle P concentration is used to extrapolate total seedling needle P, accepting that this is only indicative as needle P concentration is variable depending on needle age, there is a significant difference between treatments (F=4.445, *p*=0.001). Several high available P treatments (VCZ 40 g, SWP+P 40 g and TSP 7 g) have a significantly higher calculated total needle P than control treatments.

Variations in needle nutrient content (including Cd) across dose rates could not be accounted for using a linear regression model ($R^2<0.250$) for any treatments. Mean needle Cd and P concentration was significantly higher (*p*<0.001) in 40 g SWP+P treatments compared to other SWP+P treatments. Needle Cu concentration within TSP treatments was significantly (*p*=0.032) higher than low dose application when applied at a dose rate of 10 g seedling^{-1}. Whilst intra-application dose rate shows limited significant differences in foliar chemistry, inter-application comparisons suggest that higher biochar application results in increased foliar micro and macro-nutrients.

Table 3-7 –. Mean foliar nutrient levels with standard error. The biochar treatments were softwood pellet) SWP), vascular cambial zone (VCZ) and phosphorus infused softwood pellet (SWP+P) biochar. ANOVA Tukey groupings are displayed for each treatment. Mass units are expressed as mg kg^{-1} of oven dry needles or as per cent oven-dry weight (%).

		Foliar Nutrient Concentration				
Treatment	Application rate (g seedling^{-1})	Nitrogen (N) (mg g^{-1})	Phosphorus (P) (%)	Potassium (K) (%)	Copper (Cu) (mg kg^{-1})	Cadmium (Cd) (mg kg^{-1})
Control (CON)	n/a	13.87 (0.41)^{bc}	0.169 (0.004)^{a}	0.979 (0.093)^{a}	0.973 (0.041)^{a}	0.075 (0.004)^{a}
Surface phosphate (SPH)	n/a	12.12 (0.65)^{ab}	0.294 (0.011)^{bc}	0.902 (0.050)^{a}	1.237 (0.075)^{abc}	0.331 (0.050)^{bc}
Triple superphosphate (TSP)	7	11.51 (0.86)^{ab}	0.379 (0.045)^{cd}	1.094 (0.034)^{ab}	1.086 (0.016)^{a}	0.314 (0.022)^{bc}
	10	10.47 (0.71)^{a}	0.292 (0.029)^{bc}	1.061 (0.037)^{ab}	2.196 (0.191)^{c}	0.322 (0.062)^{bc}
Biochar (SWP)	15	13.25 (0.32)^{bc}	0.215 (0.008)^{ab}	1.139 (0.091)^{a}	1.170 (0.138)^{abc}	0.130 (0.005)^{a}
	25	13.00 (0.45)^{abc}	0.252 (0.015)^{ab}	1.211 (0.035)^{ab}	1.632 (0.190)^{abc}	0.139 (0.028)^{a}
	40	12.47 (0.40)^{b}	0.253 (0.005)^{ab}	1.389 (0.071)^{b}	1.556 (0.192)^{abc}	0.114 (0.005)^{a}
Biochar (VCZ)	15	15.15 (0.28)^{c}	0.203 (0.008)^{ab}	1.210 (0.075)^{ab}	1.801 (0.427)^{abc}	0.111 (0.006)^{a}
	25	14.84 (0.43)^{bc}	0.214 (0.008)^{ab}	1.229 (0.030)^{ab}	1.267 (0.141)^{abc}	0.083 (0.030)^{a}
	40	15.15 (0.37)^{c}	0.272 (0.019)^{b}	1.200 (0.004)^{b}	2.172 (0.097)^{bc}	0.106 (0.018)^{a}
Biochar (SWP+P)	15	12.79 (0.66)^{ab}	0.221 (0.010)^{ab}	0.903 (0.046)^{ab}	1.279 (0.484)^{abc}	0.215 (0.022)^{ab}
	25	13.97 (0.67)^{bc}	0.241 (0.009)^{ab}	1.159 (0.133)^{ab}	1.138 (0.082)^{ab}	0.220 (0.029)^{ab}
	40	12.68 (0.82)^{ab}	0.409 (0.034)^{d}	1.390 (0.114)^{ab}	1.772 (0.435)^{abc}	0.431 (0.044)^{c}
	F	2.381	11.789	4.870	3.930	15.241
	p	0.013	<0.001	<0.001	0.002	<0.001

Superscripted lowercase letters indicate significant differences (p<0.05) and groups from ANOVA with post-hoc Tukey test.

Values in parenthesis are standard errors of mean, *n=3*.

3.4.2 Douglas fir (*Psuedotsuga menziesii*)

Douglas fir - Above Ground Biomass

All biomass data showed significant differences between treatments ($p<0.05$). These results are displayed in Table 3-8 and Figure 3-5. Mean needle biomass was highest in low dose (15 g seedling^{-1}) VCZ and moderate dose (25 g seedling^{-1}) SWP biochar application, with significantly greater biomass than targeted TSP treatments and the control treatment (CON) ($p<0.001$). The low needle biomass in TSP and comparatively high coefficient of variability (46.7%, mean centred) can be attributed to the observed needle loss of old needle growth (Figure 3-8), which was also recorded to a lesser extent in SWP+P treatments. Stem biomass was significantly higher in low dose (15 g seedling^{-1}) VCZ treated seedlings when compared to the control treatment (CON) and low dose (7 g seedling^{-1}) TSP ($p=0.039$ & $p=0.028$). No significant differences between treatments were identified.

The dose rate of VCZ biochar appeared to have negative linear relationship with woody biomass accumulation. This negative linear relationship was significant (F=6.533, $p=0.021$) for stem biomass, despite the low proportion of variability explained by a linear regression model ($R^2=0.660$). Increasing the dose rate of VCZ biochar appeared to have a weak negative effect ($R^2=0.290$) on stem biomass, however mean stem biomass at higher doses still exceeded the mean biomass of control treatments (CON, TSP and SPH), although this was not found to be significant (p>0.05). Above ground woody biomass showed a clear negative linear correlation with VCZ dose (F=30.998, $p<0.001$). No other treatment application showed any significant linear relationship between dose rate and above ground biomass ($R^2<0.25$, $p>0.05$). Despite some significant relationships between growth responses and treatment does, there was no significant differences between treatment dose rates within application treatments ($p>0.005$). Low dose (15 g seedling^{-1}) VCZ biochar, and to a lesser extent moderate dose (25 g seedling^{-1}) SWP biochar, were found to be the most effective fertilisation interventions for Douglas fir seedlings. These treatments increased above ground biomass development compared to conventional P fertilisers and no application (CON).

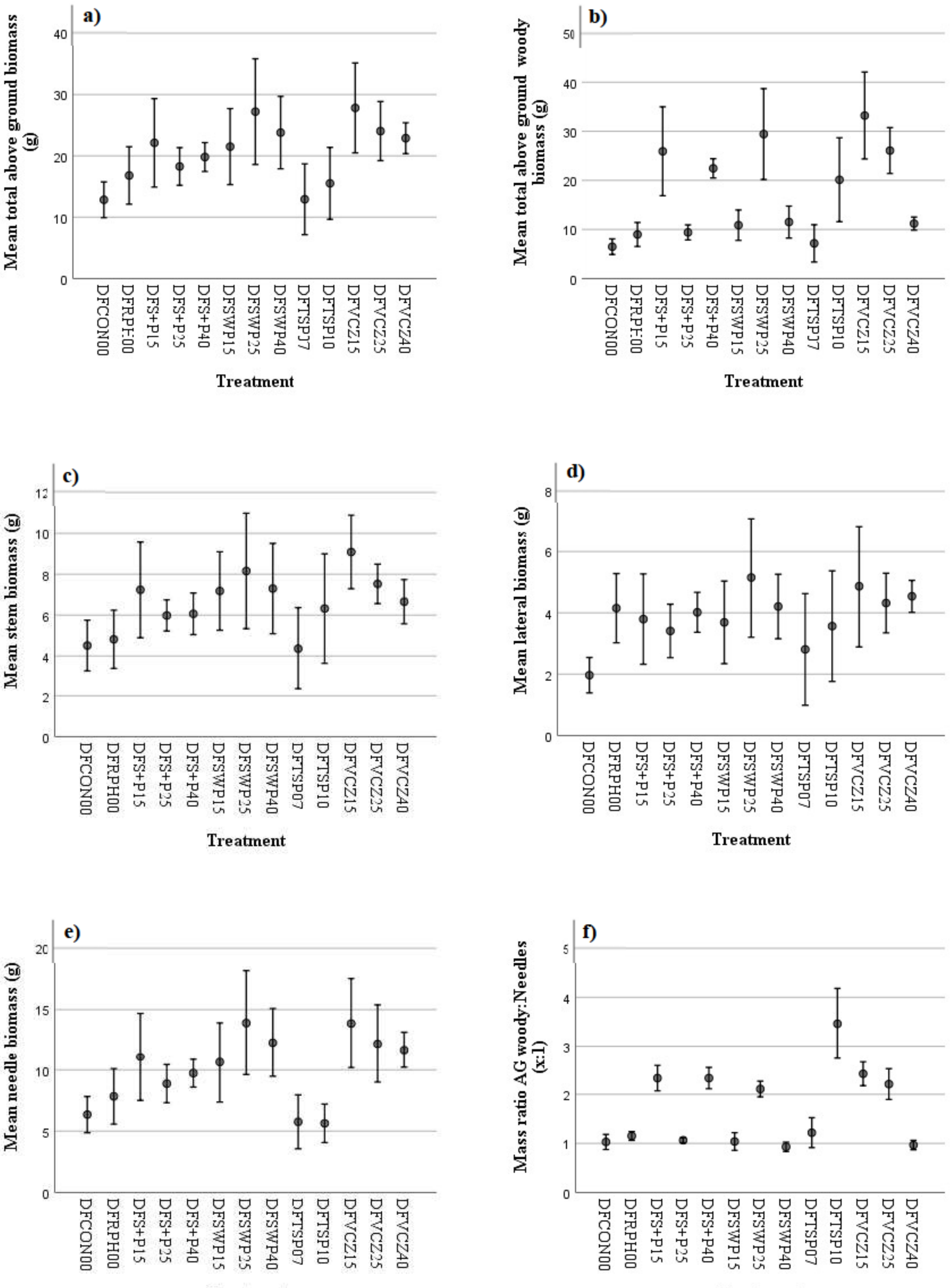

Figure 3-5 – End of experiment above ground biomass for sampled Douglas fir seedlings. Showing mean biomass (g) for; a) total above ground biomass, b) total woody above ground biomass, c) stem biomass, d) lateral biomass and e) needle biomass. The ratio between woody above ground biomass and needle biomass (expressed as x g A.G woody biomass: 1 g needle biomass) is included (f). S.E is displayed. All response variable shows sig. dif. between treatments ($p<0.05$), $n=6$.

Table 3-8 – Summary ANOVA table for end of experiment biomass accumulation for Douglas fir. All biomass results show treatment mean (g seedling^{-1}) and standard error in parenthesis. The biochar treatments were softwood pellet) SWP), vascular cambial zone (VCZ) and phosphorus infused softwood pellet (SWP+P) biochar. Biomass ratio results are expressed as the ratio between *x* above ground biomass (AG) to 1 below ground biomass (BG) and *x* above ground biomass (AG) to 1 needle biomass (BG). Summary ANOVA results and post hoc Tukey groupings are also displayed, *n=6*.

		Biomass (g seedling^{-1})						Biomass ratio	
Treatment	Application rate (g seedling^{-1})	Needle	Stem	Lateral	A.G. Woody	A.G. Total	Root	Ratio AG:BG biomass	Ratio AG woody biomass: Needles
Control (CON)	n/a	6.356 (0.74)^{ab}	4.51 (0.62)^{a}	1.97 (0.29)	6.48 (0.79)^{a}	12.83 (1.46)^{a}	14.71 (2.71)^{ab}	0.95 (0.09)^{a}	1.03 (0.08)^{a}
Surface phosphate (SPH)	n/a	7.83 (1.13)^{abc}	4.82 (0.71)^{ab}	4.15 (0.56)	8.97 (1.22)^{ab}	16.8 (2.34)^{ab}	15.12 (1.69)^{ab}	1.11 (0.07)^{ab}	1.15 (0.05)^{a}
Triple superphosphate (TSP)	7	5.76 (1.10)^{ab}	4.36 (1.01)^{a}	2.81 (0.91)	7.16 (1.9)^{ab}	12.92 (2.89)^{a}	8.81 (1.73)^{a}	1.46 (0.10)^{bc}	1.22 (0.15)^{a}
	10	5.63 (0.78)^{a}	6.32 (1.34)^{ab}	3.57 (0.90)	20.12 (4.28)^{bcd}	15.52 (2.94)^{ab}	10.24 (2.1)^{ab}	1.55 (0.08)^{bc}	3.46 (0.36)^{c}
Biochar (SWP)	15	10.64 (1.64)^{abc}	7.18 (0.96)^{ab}	3.69 (0.67)	10.87 (1.55)^{abc}	21.51 (3.1)^{ab}	14.51 (2.03)^{ab}	1.48 (0.06)^{bc}	1.04 (0.09)^{a}
	25	13.90 (2.14)^{c}	8.16 (1.41)^{ab}	5.15 (0.97)	29.43 (4.63)^{c}	27.21 (4.31)^{b}	16.12 (2.51)^{ab}	1.71 (0.09)^{c}	2.11 (0.08)^{b}
	40	12.29 (1.40)^{bc}	7.30 (1.10)^{ab}	4.21 (0.53)	11.51 (1.63)^{abc}	23.79 (2.95)^{ab}	15.52 (3.04)^{ab}	1.65 (0.13)^{c}	0.93 (0.05)^{b}
Biochar (VCZ)	15	13.86 (1.84)^{c}	9.09 (0.90)^{b}	4.87 (0.99)	33.21 (4.43)^{c}	27.82 (3.67)^{b}	19.26 (3.02)^{b}	1.49 (0.13)^{bc}	2.42 (0.12)^{b}
	25	12.20 (1.60)^{bc}	7.53 (0.48)^{ab}	4.32 (0.49)	26.08 (2.34)^{c}	24.04 (2.41)^{ab}	14.24 (1.54)^{ab}	1.71 (0.10)^{c}	2.21 (0.16)^{b}
	40	11.69 (0.73)^{abc}	6.66 (0.54)^{ab}	4.54 (0.26)	11.19 (0.66)^{abc}	22.88 (1.26)^{ab}	15.45 (0.98)^{ab}	1.49 (0.04)^{bc}	0.97 (0.05)^{a}
Biochar (SWP+P)	15	11.10 (1.80)^{abc}	7.24 (1.17)^{ab}	3.80 (0.74)	25.93 (4.53)^{c}	22.13 (3.61)^{ab}	14.9 (2.69)^{ab}	1.50 (0.08)^{bc}	2.33 (0.13)^{b}
	25	8.88 (0.78)^{abc}	5.98 (0.38)^{ab}	3.41 (0.44)	9.39 (0.77)^{abc}	18.27 (1.54)^{ab}	12.7 (1.49)^{ab}	1.47 (0.10)^{bc}	1.06 (0.03)^{a}
	40	9.73 (0.57)^{abc}	6.06 (0.51)^{ab}	4.02 (0.33)	22.46 (0.98)^{cd}	19.81 (1.18)^{ab}	12.38 (0.45)^{ab}	1.6 (0.09)^{c}	2.33 (0.11)^{b}
	F	4.659	2.376	1.592	11.588	1.709	1.542	5.736	33.933
	p	<0.001	0.013	0.116	<0.001	0.85	0.048	<0.001	<0.001

Superscripted lowercase letters indicate significant differences ($p<0.05$) and groups from ANOVA with post-hoc Tukey test.

Values in parenthesis are standard errors of mean.

Douglas fir - Root Biomass and Morphology

Below ground biomass showed significant difference between treatments (F=1.542, *p*=0.04) and clear differences in root growth traits were also observed (Table 3-8). Low dose VCZ (15 g seedling^{-1}) resulted in significantly higher root biomass than the intermediate TSP treatment (7 g seedling^{-1}) and high dose TSP application also resulted in lower root biomass (however not statistically significant). Treatments which delivered targeted high available P application (all TSP and SWP+P applications) limited root development in some seedlings, with the lowest individual seedling root biomass measurements belonging to these treatments. There was also clear evidence of root death and root hair development suppression. Seedlings treated with high available P doses also showed weak tap root development and a clear decrease in lateral rooting (Figure *3-6*), similar to Sitka spruce,

TSP and SWP+P treated seedlings had significantly (p<0.001) lower lateral roots density in the targeted treatment area compared to other treatments. There were no other observable differences in the root morphology of SWP, VCZ or CON treatments, excluding mycorrhizal structures (detailed in Chapter 5). Intra-treatment variation was also highest in the TSP applications and low dose SWP+P (15 g seedling^{-1}), showing variable recovery from the initial period of root suppression (Table 3-9). There was no evidence of any linear relationship between dose and root biomass (R^2<0.250).

Table 3-9 – Treatment root biomass mean centred coefficient of variation in Douglas fir seedlings, showing total intra-treatment variation.

Group	Mean	Std. Deviation	Coefficient of Dispersion	Coefficient of Variation Mean Centred
DFCON00	14.707	6.626	0.364	45.1%
DFSPH00	15.117	4.134	0.206	27.3%
DFS+P15	14.902	6.600	0.363	44.3%
DFS+P25	12.702	3.645	0.249	28.7%
DFS+P40	12.378	1.105	0.061	8.9%
DFSWP15	14.505	4.973	0.259	34.3%
DFSWP25	16.123	6.157	0.251	38.2%
DFSWP40	15.515	7.450	0.432	48.0%
DFTSP07	8.812	4.227	0.448	48.0%
DFTSP10	10.240	5.153	0.461	50.3%
DFVCZ15	19.258	7.398	0.324	38.4%
DFVCZ25	14.237	3.775	0.182	26.5%
DFVCZ40	15.450	2.393	0.113	15.5%
Overall	14.150	5.452	0.323	38.5%

Figure 3-6 – End of experiment root structure of suppressed TSP (7 g) treated seedling (*right*) and non-suppressed root system (*left*). Both seedlings had comparable aboveground biomass. Clear decrease in lateral root development and avoidance of root growth around treatment area in TSP treated seedling. Seedlings had comparable aboveground biomass (within 20%).

A strong linear relationship (R^2>0.7) between root biomass and above ground biomass was found in all treatments, excluding low (25 g seedling^{-1}) VCZ and high dose (40 g seedling^{-1}) SWP+P applications (Figure *3-7*). This can be attributed to a very narrow range of values in the case of SWP+P 40 g and due to abnormally high root development in some VCZ 15 g seedlings. All other VCZ, TSP, SWP and SWP+P applications showed a similar relationship between below ground and above ground biomass, with no difference between regressions. The control treatment (CON) showed a significantly lower regression slope which suggests that the nutrient delivery of fertilisation treatments increases above-ground growth relative to below ground.

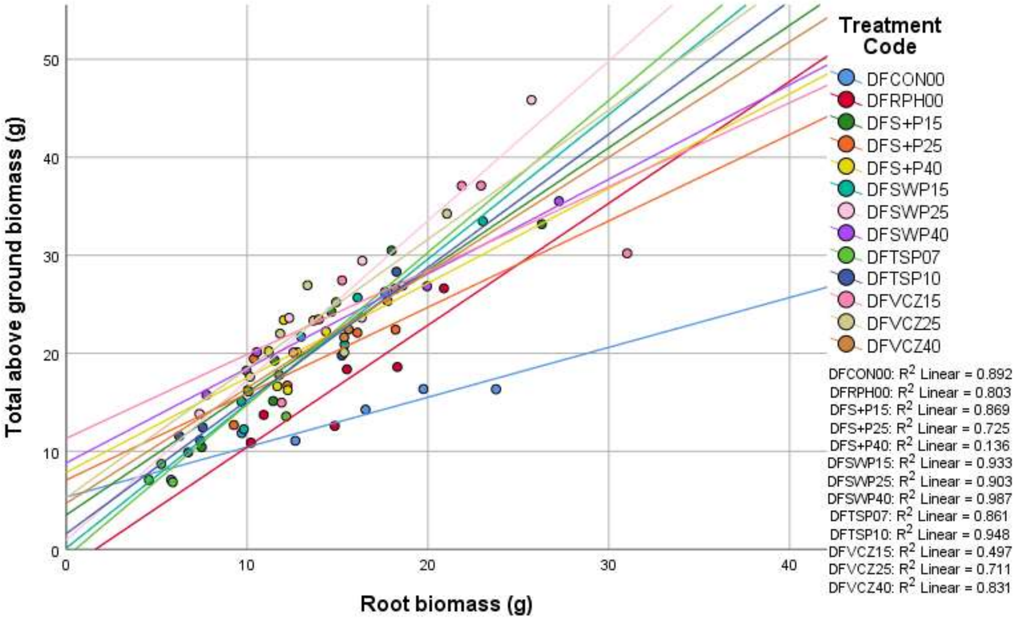

Figure 3-7 – Scatter plot of Douglas fir root biomass (g) and above ground biomass (g) for end of experiment sample seedlings grouped according to treatment. Linear regressions are included with R^2 for each treatment group, *n=6*.

Douglas fir (*Psuedotsuga menziesii*) - Seedling Growth Dynamics & Survival

Year 1 and year 2 seedling growth was found to be significantly higher in all VCZ biochar treatments compared to the CON, SPH and TSP treatments (p<0.001). Low dose (15 g seedling^{-1}) VCZ application resulted in the greatest year 1 and year 2 growth rate, outperforming all other biochar and inorganic mineral derived P fertiliser treatments (p=0.036). As with other species, all biochar treatments showed no decline in growth rate between year 1 and year 2.

The CON treatment showed relatively strong growth during year 1, however this collapsed in year 2, with mean seedling growing <10 cm. Mineral P fertilisers had a variable effect on seedling growth, with high variation in year 1 and year 2 growth. Some seedlings showed poor year 1 growth, recovering in year 2. Conversely, some seedlings treated with mineral P fertilisers exhibited strong year 1 growth which subsequently slowed during year 2. This variable effect was correlated to root biomass. Mineral P treated seedlings which had a highly suppressed root system showed poorer year 1 growth and seedlings with stronger root development in these treatments had stronger year 1 growth.

Douglas fir seedlings exhibited the same survival response across application treatments as Sitka spruce and Douglas fir seedlings, however overall mortality rates were consistently lower. Targeted TSP application had significantly higher mortality than all other treatments ($p<0.0001$). SWP+P, which has comparable available P dose to TSP had higher seedling survival than TSP, showing that infused P biochar can mitigate the negative effects of high available P fertiliser. SWP, VCZ and CON had mortality rates below <5 %. High available P treatments, and high dose rates of these treatments, resulted in <50% mortality, which is considerably lower than both Sitka spruce and Scots pine (60%-80%). Douglas fir is the most nutrient demanding species tested and is adapted for growth in nutrient rich soils. High dose available P fertiliser application appeared to have a lesser impact on year 1 seedling survival.

Douglas fir - Foliar Composition and Nutrient Deficiency

Treatment application had a significant effect ($p<0.05$) on needle P, K, Cu and Cd concentration. Foliar concentration results are displayed in Figure 3-9 & Table 3-10. Treatment was found to significantly affect foliar needle P concentration ($F=5.818$, $p<0.001$), showing that P application to growing media will result in increased seedling P uptake compared to the CON treatment. Moderate and high dose application of VCZ (25 g seedling^{-1} & 40 g seedling^{-1}) and targeted TSP fertiliser (7 g seedling^{-1} & 10 g seedling^{-1}), along with SPH fertiliser application, resulted in elevated needle P concentration compared to the control treatment. All treatments, excluding CON, showed needle P concentrations above the deficiency threshold. Seedlings of the CON treatment, where no additional P was added, had marginal mean needle P concentration with some seedlings exhibiting deficient needle P concentrations (P (%) <0.25). Total seedling needle P content is significantly higher in all VCZ treatments ($p<0.001$) compared to CON and low dose (4 g seedling^{-1}) TSP treated seedlings, due to the higher needle biomass. Treatment dose rate had a weak linear relationship with needle P concentration in VCZ (R^2=0.387), although not significant (*p*=0.067).

Needle K concentration, whilst significant (F=2.561 *p*=0.018), was similar in general across treatment groups, as indicated by the low F statistic. Only moderate (25 g seedling^{-1}) SWP+P and high (40 g) SWP applications had significantly higher needle K concentrations than CON and moderate (25 g seedling^{-1}) SWP treatments. CON treatment seedlings had marginal needle K concentration (~0.8 %), with some seedlings having deficient needle K concentration. There was no observable relationship between treatment dose and needle K concentration (R^2>0.250). High available P treatments (TSP, SWP+P and SPH) did not reduce end of experiment needle K concentration, however these seedlings showed clear symptoms of K deficiency, which occurred in the first year of growth (Figure 3-8). Loss of older needles and death of sideshoots in Douglas fir seedlings, caused by severe potassium deficiency is shown in (Figure 3-8). Needle foliar N concentration was correlated with needle biomass. There was high intra-treatment variation, with no difference between treatment (*p*>0.05). Mean needle N concentration was deficient in CON, TSP and SWP+P treatments and optimal in VCZ treated seedlings.

Figure 3-8 – Example of end of experiment sample seedlings exhibiting clear K deficiency in TSP treated seedlings (*left*), compared to health VCZ seedlings (*right*).

Mean needle Cd concentration was elevated in inorganic mineral derived P treatments which contain high levels of Cd, although only SPH was significantly higher (F=6.788, *p*<0.001) than low available P treatments (including VCZ and CON). This suggests that seedlings can effectively intercept surface applied Cd as it is mobilised when soluble P is released. Needle Cu concentration was marginal in most biochar and inorganic mineral derived P treatments, and was deficient in SPH, CON and SWP+P (40 g seedling^{-1}). VCZ, SWP and TSP application resulted in significantly higher mean needle Cu concentration than SPH treatment (F=11.156, $p<0.001$). This suggests that surface applied P fertiliser (SPH) reduces Cu uptake, compared with targeted application (TSP), despite no difference in root biomass. Other micro-nutrients which were analysed were above optimal foliar concentration and therefore were not limiting to seedling growth.

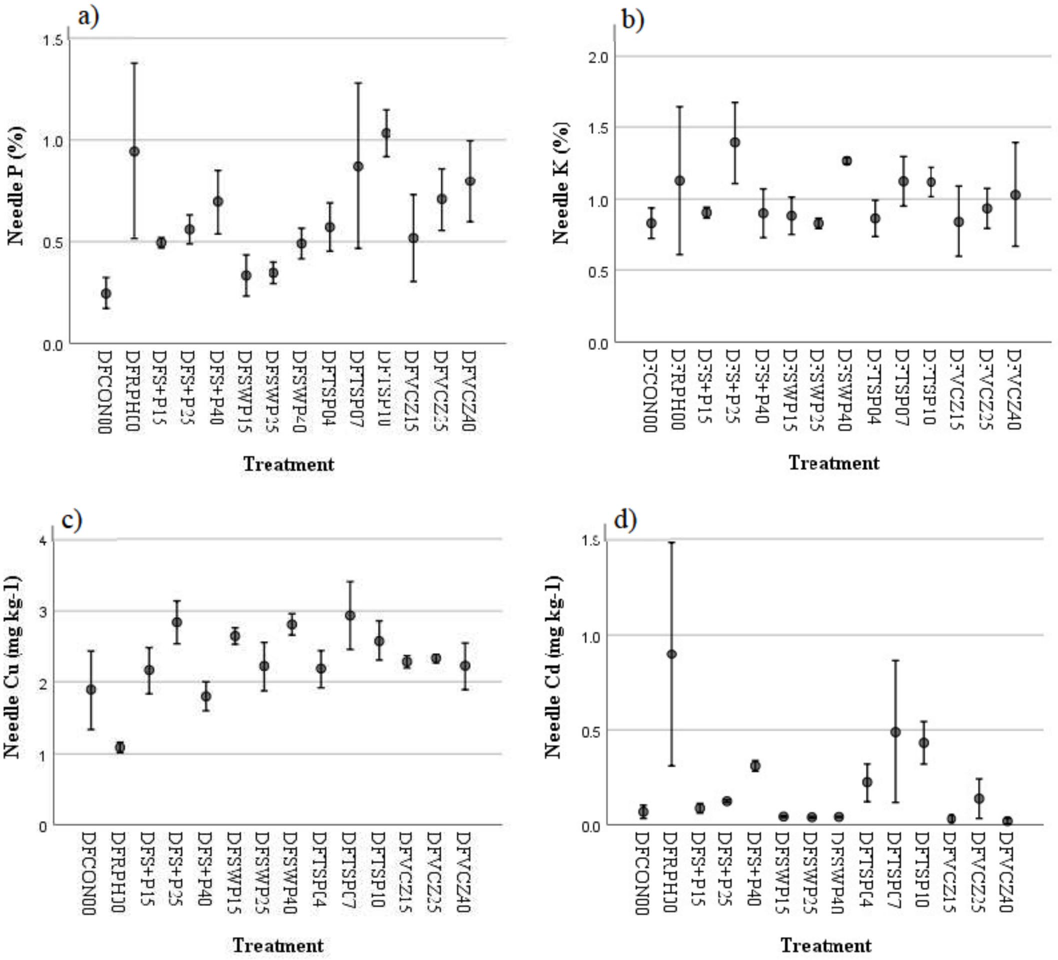

Figure 3-9 – Mean foliar analysis nutrient concentrations at end of experiment for Douglas fir, including standard error. Cd and Cd concentration are expressed as mg kg^{-1} oven dry weight and K and P as percentage of dry weight (%).

Table 3-10 - Mean foliar nutrient levels with standard error. The biochar treatments were softwood pellet) SWP), vascular cambial zone (VCZ) and phosphorus infused softwood pellet (SWP+P) biochar. ANOVA Tukey groupings are displayed for each treatment. Mass units are expressed as mg kg^{-1} of oven dry needles or as per cent oven-dry weight (%).

		Foliar Nutrient Concentration				
Treatment	Application rate (g seedling^{-1})	Nitrogen (N) (mg g^{-1})	Phosphorus (P) (%)	Potassium (K) (%)	Copper (Cu) (mg kg^{-1})	Cadmium (Cd) (mg kg^{-1})
Control (CON)	n/a	11.56 (2.61)	0.248 (0.039)^{a}	0.833 (0.053)^{a}	1.891 (0.277)^{bc}	0.067 (0.017)^{a}
Surface phosphate (SPH)	n/a	12.23 (2.70)	0.944 (0.214)^{cd}	1.128 (0.261)^{ab}	1.089 (0.037)^{a}	0.897 (0.292)^{b}
Triple superphosphate (TSP)	4	10.54 (3.66)	0.570 (0.059)^{abcd}	0.866 (0.063)^{ab}	2.183 (0.133)^{bcde}	0.221 (0.051)^{a}
	7	11.01 (3.17)	0.871 (0.202)^{cd}	1.123 (0.086)^{ab}	2.937 (0.235)^{e}	0.488 (0.186)^{ab}
	10	10.95 (3.24)	1.032 (0.057)^{d}	1.118 (0.051)^{ab}	2.582 (0.141)^{cde}	0.432 (0.055)^{ab}
Biochar (SWP)	15	13.45 (1.87)	0.335 (0.050)^{ab}	0.884 (0.065)^{ab}	2.653 (0.057)^{de}	0.042 (0.002)^{a}
	25	12.47 (2.68)	0.348 (0.026)^{ab}	0.832 (0.018)^{a}	2.218 (0.173)^{bcde}	0.038 (0.003)^{a}
	40	13.68 (1.95)	0.491 (0.037)^{ab}	1.265 (0.012)^{b}	2.814 (0.074)^{de}	0.040 (0.001)^{a}
Biochar (VCZ)	15	15.01 (2.67)	0.517 (0.106)^{abc}	0.842 (0.124)^{ab}	2.279 (0.044)^{bcde}	0.031 (0.009)^{a}
	25	14.98 (2.59)	0.707 (0.076)^{abcd}	0.935 (0.069)^{ab}	2.329 (0.034)^{bcde}	0.135 (0.051)^{a}
	40	15.12 (2.01)	0.796 (0.100)^{bcd}	1.029 (0.182)^{ab}	2.222 (0.166)^{bcde}	0.018 (0.007)^{a}
Biochar (SWP+P)	15	12.10 (1.98)	0.495 (0.013)^{ab}	0.906 (0.019)^{ab}	2.162 (0.165)^{bcd}	0.085 (0.013)^{a}
	25	12.23 (2.45)	0.559 (0.035)^{ab}	1.393 (0.143)^{b}	2.843 (0.148)^{de}	0.122 (0.003)^{a}
	40	13.54 (2.21)	0.695 (0.079)^{abc}	0.902 (0.085)^{ab}	1.797 (0.100)^{ab}	0.313 (0.014)^{a}
	F	1.154	5.818	2.561	11.156	6.788
	p	0.104	<0.001	0.018	<0.001	<0.001

Superscripted lowercase letters indicate significant differences ($p<0.05$) and groups from ANOVA with post-hoc Tukey test.

Values in parenthesis are standard errors of mean, n=3.

3.4.3 Scots pine (*Pinus sylvestris*)

Scots pine - Above Ground Biomass

Targeted biochar application significantly increased seedling aboveground biomass (p<0.001). (Figure 3-10 & Table 3-11). VCZ biochar had the greatest woody biomass growth increase and was significantly higher than the CON or TSP treatments. High dose VCZ (40 g seedling^{-1}) application resulted in significantly higher (p=0.021) lateral biomass than CON and TSP treatments, which may have had supressed lateral development due to K deficiency. Stem biomass in low dose and high dose VCZ biochar (15 g seedling^{-1} and 25 g seedling^{-1} respectively) treated seedlings was significantly higher (p=0.001) than low dose (4 g seedling^{-1}) TSP application. Above ground woody biomass was significantly higher (p<0.001) in Scots pine seedlings treated with moderate and high (25 g seedling^{-1} & 40 g seedling^{-1}) VCZ and low (15 g seedling^{-1}) dose SWP+P and SWP biochar application, compared to CON and TSP treatments.

High dose VCZ biochar treatment resulted in higher (p<0.001) above ground biomass than CON and inorganic mineral derived fertiliser (TSP and SPH) applications. High dose (40 g seedling^{-1}) VCZ and low dose (15 g seedling^{-1}) SWP biochar application increased needle biomass compared to SPH and TSP (4 g seedling^{-1}) treatment (p=0.001), although this can partially be attributed to the needle loss observed in high available P treatments. No linear relationship (R^2<0.25) was found between biomass and dose rate in any treatment or biomass variable. High dose (40 g seedling^{-1}) consistently improved above ground biomass development and was identified as the most effective fertilisation treatment for Scots pine seedling establishment in nutrient poor conditions, outperforming no application (CON) and conventional P fertiliser.

Table 3-11- Summary ANOVA table for Scots pine end of experiment biomass accumulation according to treatment applications and doses. The biochar treatments were softwood pellet) SWP), vascular cambial zone (VCZ) and phosphorus infused softwood pellet (SWP+P) biochar. Above ground (A.G.) woody biomass is derived from the sum of lateral and stem values, and above ground (A.G.) total biomass also includes needle biomass. Means, standard error and ANOVA Tukey groupings are displayed (n = 6).

		Biomass (g seedling^{-1})					
Treatment	Application rate (g seedling^{-1})	Needle	Lateral	Stem	A.G. Woody	A.G. Total	Root
Control (CON)	n/a	9.78 (1.45)^{ab}	2.54 (0.40)^{a}	7.33 (0.96)^{abc}	9.87 (1.35)^{a}	19.66 (2.61)^{a}	5.30 (0.65)^{ab}
Surface phosphate (SPH)	n/a	7.54 (0.55)^{a}	2.81 (0.22)^{ab}	7.47 (0.6)^{abc}	10.28 (0.78)^{ab}	17.82 (1.04)^{a}	7.24 (0.72)^{abc}
Triple superphosphate (TSP)	4	8.63 (1.29)^{a}	2.42 (0.50)^{a}	6.26 (1.09)^{a}	8.68 (1.50)^{a}	17.32 (2.66)^{a}	6.47 (0.73)^{abc}
	7	7.58 (1.86)^{ab}	2.17 (0.62)^{a}	7.02 (1.70)^{ab}	9.18 (2.19)^{a}	16.76 (4.04)^{a}	4.29 (0.99)^{a}
Biochar (SWP)	15	10.29 (2.43)^{ab}	3.94 (0.91)^{ab}	9.82 (1.02)^{abc}	21.62 (2.36)^{cd}	24.05 (3.30)^{ab}	7.87 (1.47)^{abc}
	25	14.46 (2.10)^{ab}	4.31 (0.40)^{ab}	10.16 (0.65)^{abc}	14.47 (0.85)^{abc}	28.93 (2.81)^{ab}	8.95 (0.89)^{bc}
	40	12.37 (3.26)^{ab}	3.77 (0.76)^{ab}	8.97 (1.38)^{abc}	12.74 (2.10)^{ab}	25.11 (5.19)^{ab}	7.81 (1.30)^{abc}
Biochar (VCZ)	15	13.06 (1.33)^{ab}	4.81 (0.86)^{ab}	11.96 (1.63)^{bc}	16.77 (2.41)^{abcd}	29.83 (2.59)^{ab}	9.27 (0.60)^{bc}
	25	13.37 (0.86)^{ab}	3.51 (0.34)^{ab}	11.29 (0.70)^{abc}	23.44 (1.45)^{d}	28.17 (1.71)^{ab}	8.64 (0.56)^{abc}
	40	16.46 (1.67)^{b}	6.15 (1.67)^{b}	12.52 (0.97)^{b}	18.66 (1.76)^{bcd}	35.12 (2.85)^{b}	9.88 (1.30)^{c}
Biochar (SWP+P)	15	17.09 (1.87)^{b}	4.04 (0.69)^{ab}	10.22 (1.23)^{abc}	23.84 (2.36)^{d}	31.35 (3.53)^{ab}	9.58 (0.81)^{bc}
	25	11.56 (1.44)^{ab}	4.35 (0.72)^{ab}	9.60 (0.72)^{abc}	13.95 (1.39)^{abc}	25.51 (2.67)^{ab}	7.90 (0.85)^{abc}
	40	9.09 (1.30)^{ab}	3.24 (0.28)^{ab}	8.83 (0.71)^{abc}	14.31 (1.50)^{abc}	21.16 (2.22)^{ab}	7.11 (0.78)^{abc}
	F	3.186	2.213	3.192	8.949	3.755	3.094
	p	0.001	0.021	0.001	<0.001	<0.001	0.002

Superscripted lowercase letters indicate significant differences ($p<0.05$) and groups from ANOVA with post-hoc Tukey test.

Values in parenthesis are standard errors of mean, n=6.

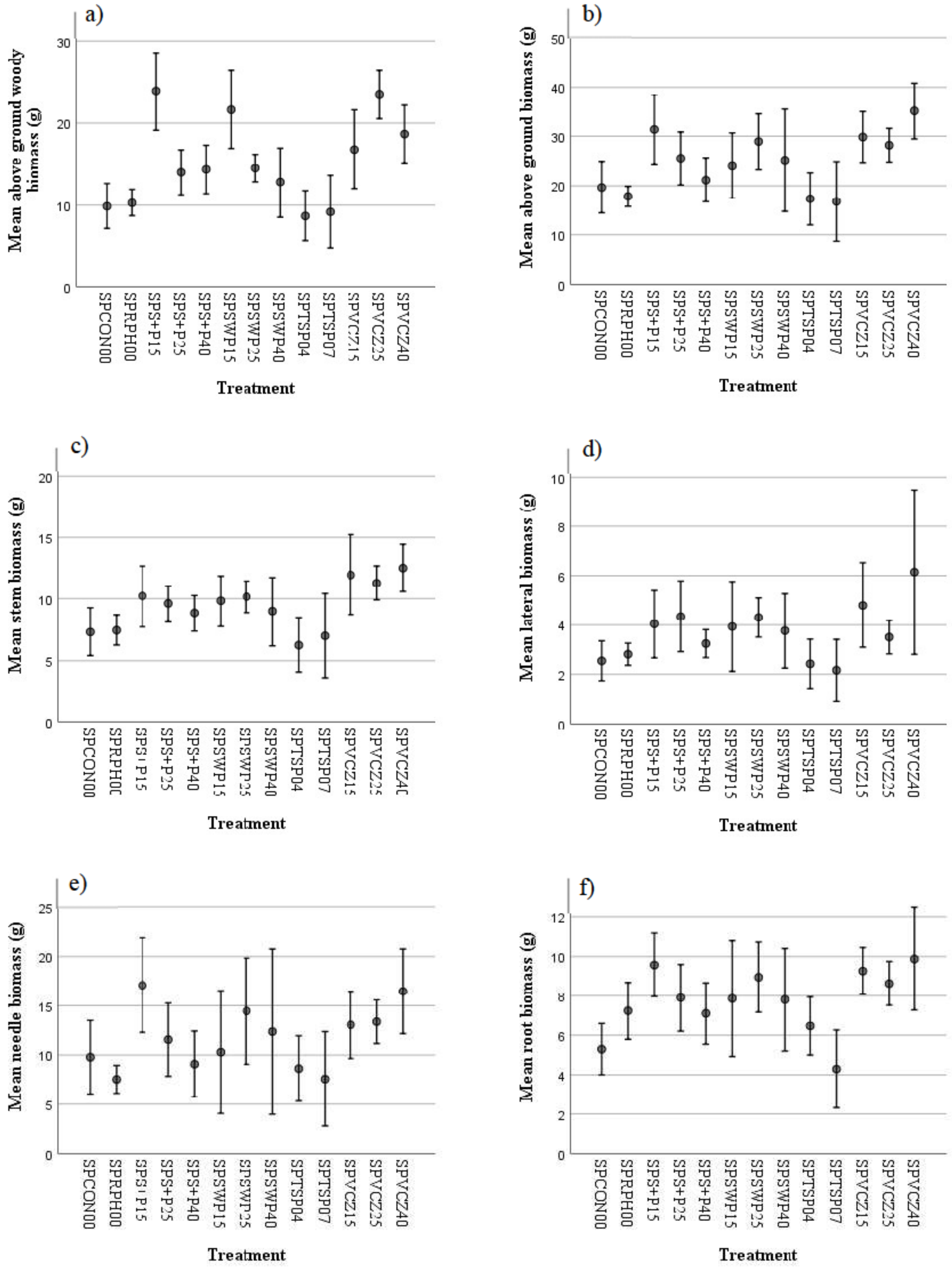

Figure 3-10- Mean end of experiment biomass accumulation measurements for Scots pine. Including a) above ground woody biomass, b) above ground biomass, c) stem biomass, d) lateral biomass, e) needle biomass and f) root biomass. Biomass is expressed as mean oven dry weight (g seedling^{-1}) with standard error (S.E.), *n=6*.

Scots pine - Root Biomass and Morphology

All biochar treatment applications resulted in higher mean root biomass than CON or TSP treatments. SWP (25 g), VCZ (15 g seedling^{-1} & 40 g seedling^{-1}) and SWP+P (15 g seedling^{-1}) treated seedlings had significantly (p=0.002) higher root biomass than TSP (7 g seedling^{-1}). Increased dose rate in high available P treatments (TSP and SWP+P) had a negative impact on root biomass, with higher doses suppressing root development. SWP biochar application resulted in high variation in Scots pine root biomass, with VCZ biochar showing consistently low intra-treatment variation (Table 3-12). There were no linear relationships observed between dose and root biomass ($R^2<0.25$). SWP+P root biomass decreased at moderate and high dose rates (25 g seedling^{-1} & 40 g seedling^{-1}).

Table 3-12 - Treatment root biomass mean centred coefficient of variation in Scots pine seedlings, showing total intra-treatment variation.

Group	Mean	Std. Deviation	Coefficient of Dispersion	Coefficient of Variation Mean Centred
SPCON00	5.297	1.586	0.200	29.9%
SPSPH00	7.238	1.772	0.198	24.5%
SPS+P15	9.575	1.976	0.154	20.6%
SPS+P25	7.903	2.077	0.200	26.3%
SPS+P40	7.105	1.910	0.231	26.9%
SPSWP15	7.865	3.603	0.344	45.8%
SPSWP25	8.953	2.184	0.148	24.4%
SPSWP40	7.807	3.188	0.286	40.8%
SPTSP04	6.470	1.798	0.157	27.8%
SPTSP07	4.292	2.419	0.514	56.4%
SPVCZ15	9.266	1.460	0.101	15.8%
SPVCZ25	8.637	1.367	0.116	15.8%
SPVCZ40	9.877	3.189	0.250	32.3%
Overall	7.714	2.648	0.267	34.3%

Seedling lateral root density in the treatment area was significantly lower ($p<0.001$) in all high available P treatments (SWP+P, TSP and SPH), compared to VCZ, SWP and CON treatments. This result clearly highlights the suppression of root development in the high available P and low pH conditions associated with P fertilisers. VCZ biochar application resulted in higher lateral root density than the CON (*p*=0.023), low dose (15 g seedling^{-1}) SWP (*p*=0.0.34) and moderate dose (25 g seedling^{-1}) SWP (*p*=0.029) applications. Specified VCZ biochar increases Scots pine seedling lateral root development in P deficient conditions. Root structure in Scots pine seedlings was observably different in high dose available P treatments (Figure 3-11). Evidence of root death was observed in SWP+P and TSP treatments.

Figure 3-11 – Root structure of Sitka spruce seedlings at end on year 2. A healthy root system (*left*), from seedling planted with SWP 15 g treatment, has observable fine roots and dense lateral roots close to the root collar (which has some adventitious roots present). This is contrasted to the root system of seedling treated with a 10 g TSP dose. The root structure present at planting is highlighted by the red circle, noting the absence of fine roots and lateral branching. Scale (cm) is displayed. Seedlings had comparable above ground biomass (with 20%).

Root biomass showed a strong linear relationship with above ground biomass (R^2>0.70) in TSP, SWP (40 g seedling^{-1}) and VCZ (25 g seedling^{-1}) (Figure 3-12). There was no linear relationship (R^2<0.25) between root biomass and aboveground biomass in Scots pine seedlings treated with SWP (15 g seedling^{-1}) and no application (CON). There was no significant difference in treatment linear regression (*p*>0.05), however VCZ biochar (15 g seedling^{-1} & 25 g seedling^{-1}) showed a weaker relationship and above ground biomass increase with root biomass increase than other treatments (*p*=0.069).

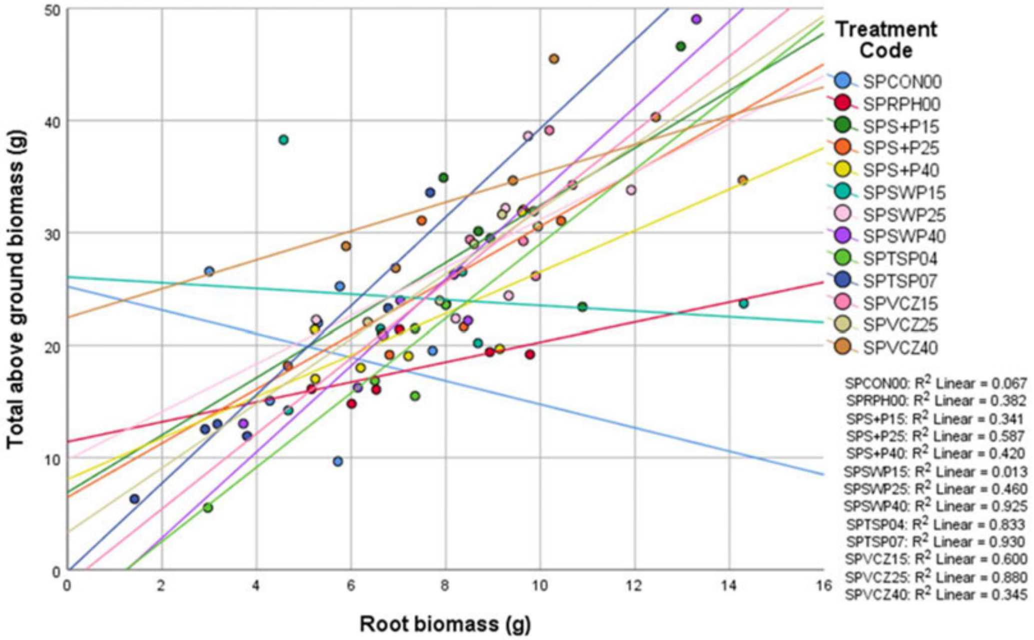

Figure 3-12 - Scatter plot of Scots pine root biomass (g) and above ground biomass (g) for end of experiment sample seedlings grouped according to treatment. Linear regressions are included with R^2 for each treatment group, *n=6*.

Scots pine - Seedling Growth Dynamics & Survival

Scots pine seedling year 1 and year 2 growth rates and year 1 survival were significantly different across treatments (*p*<0.001) and growth years (*p*<0.001). As with Sitka spruce and Douglas fir, treatments resulted in the same seedling growth response over the first two growing seasons. VCZ biochar application significantly increased year two growth rates, compared to CON, SWP, TSP and SPH treatments. All biochar treatments (excluding SWP+P) showed no decline in growth rate between year 1 and year 2. High dose VCZ application (40 g seedling^{-1}) resulted in significantly higher growth than all other (excluding other VCZ doses) treatments, outperforming CON growth by an increase of <100%. Low dose (15 g seedling^{-1}) SWP biochar showed modest year 2 growth improvements over the CON treatment (*p*=0.46). The CON treatment showed relatively strong growth during year 1, however this collapsed in year 2, with mean seedling growing <10 cm. Inorganic mineral derived P fertilisers had a variable effect on individual seedling growth rate, with high variation between individual seedling growth rate in year 1 and year 2 growth. Some seedlings showed poor year 1 growth, recovering in year 2. Conversely, some seedlings treated with inorganic mineral derived P fertilisers exhibited strong year 1 growth which subsequently slowed during year 2. Again, this variable effect was correlated to root biomass. Inorganic mineral derived P treated seedlings which had highly suppressed root systems showed poorer year 1 growth and seedlings with stronger root development in these treatments had stronger year 1 growth.

As with other tested conifer species, seedling survival decreased in seedlings treated with high available P applications. Targeted TSP application had significantly higher mortality than all other treatments ($p<0.001$). SWP+P, which has comparable available P dose to TSP had higher seedling survival than TSP, showing that infused P biochar can mitigate the negative effects of high available P fertiliser. SWP, VCZ and CON had mortality rates below <5 %, suggesting that inorganic mineral derived P fertilisers directly contributed to the increased seedling mortality in these treatments, rising to between 20% and 95%. Scots pine seedlings were highly sensitive to high dose (10 g seedling^{-1}) targeted TSP application, which resulted in a 10% survival rate in these treatment plots.

Scots pine - Foliar Composition and Nutrient Deficiency

Mean needle nutrient concentrations are shown in Figure 3-14 & Table 3-13. Mean needle P concentration was lowest in the CON treatment (0.091%). Needle P was below the deficient nutrient concentration level (0.12%), showing that seedling needle P is at a level which can limit growth in CON and SWP (15 g seedling^{-1} & 40 g seedling^{-1}) treatments. Needle P concentration was only significantly different between SPH and CON treatments (F=3.714, $p=0.002$). Total needle P content (calculated from needle biomass and needle P concentration) was significantly higher in VCZ applications ($p<0.001$) than TSP and CON treatments. Needle K concentration was above the optimal threshold level (>0.5%) in all treatments, except SPH, and no treatments were deficient (<0.3%). There was a significant difference in mean needle K concentration across treatments (F=2.708, $p=0.016$), however only SWP and VCZ biochar (25 g seedling-1 & 40 g seedling^{-1}) had significantly elevated levels compared to SPH. High dose available P treatments exhibited clear symptoms of K deficiency (needle loss and decreased lateral development) during the first year of the experiment (Figure 3-13). These symptoms were still visible at the end of the experiment, despite a clear recovery of K uptake.

Needle N concentration was not deficient in any treatments, and needle N concentration in VCZ (40 g seedling^{-1}) was significantly higher than CON and SPH treatments (p<0.05).

Figure 3-13- End of experiment Scots pine seedlings treated with 4 g seedling $^{-1}$ TSP (*right*) and 40 g seedling $^{-1}$ VCZ (*left*). Health seedling (*left*) can be illustratively compared to seedling exhibiting legacy K deficiency symptoms (*right*).

Scots pine seedings treated with high available P treatments (excluding low dose SWP+P) and CON treatments had low needle Cu concentrations (<2 mg kg^{-1}). Biochar was found to elevate needle Cu concentration and prevent seedling Cu deficiency, particularly with VCZ application. High dose VCZ and moderate dose SWP biochar treated seedlings had significantly higher needle Cu concentration than inorganic mineral derived P fertiliser applications and the CON treatment (F=6.171, p<0.001). Dose rate had no clear relationship with needle Cu concentration. High available P treatments, which contain a high Cd content, resulted in significantly (p<0.001) increased needle Cd concentration. Cd needle content was highly dependent on Cd dose rate. The VCZ 15 g seedling $^{-1}$ needle cadmium content was highlighted as an unusual result, as total needle Cd exceeds VCZ and growing media Cd content.

Table 3-13- Mean foliar nutrient levels with standard error. The biochar treatments were softwood pellet) SWP), vascular cambial zone (VCZ) and phosphorus infused softwood pellet (SWP+P) biochar. ANOVA Tukey groupings are displayed for each treatment. Mass units are expressed as mg kg-1 of oven dry needles or as per cent oven-dry weight (%).

		Foliar Nutrient Concentration				
		Nitrogen (N)	Phosphorus (P)	Potassium (K)	Copper (Cu)	Cadmium (Cd)
Treatment	Application rate (g seedling^{-1})	(mg g^{-1})	(%)	(%)	(mg kg^{-1})	(mg kg^{-1})
Control (CON)	n/a	11.51 (2.17)^{a}	0.091 (0.006)^{a}	0.505 (0.046)^{ab}	1.486 (0.049)^{ab}	0.045 (0.003)^{ab}
Rock phosphate (SPH)	n/a	11.25 (2.10)^{a}	0.207 (0.046)^{b}	0.376 (0.052)^{a}	1.626 (0.120)^{ab}	0.166 (0.029)^{bcd}
Triple superphosphate (TSP)	4	12.51 (2.33)^{ab}	0.128 (0.010)^{ab}	0.594 (0.059)^{ab}	1.374 (0.124)^{a}	0.228 (0.050)^{cd}
	7	12.65 (1.67)^{ab}	0.130 (0.006)^{ab}	0.492 (0.016)^{ab}	1.458 (0.043)^{ab}	0.235 (0.051)^{d}
Biochar (SWP)	15	12.47 (1.42)^{ab}	0.104 (0.006)^{a}	0.627 (0.038)^{ab}	1.702 (0.069)^{abc}	0.072 (0.005)^{ab}
	25	12.98 (2.07)^{ab}	0.130 (0.001)^{ab}	0.720 (0.009)^{b}	2.394 (0.275)^{c}	0.072 (0.019)^{ab}
	40	12.41 (1.87)^{ab}	0.108 (0.007)^{a}	0.504 (0.105)^{ab}	2.160 (0.127)^{bc}	0.078 (0.013)^{ab}
Biochar (VCZ)	15	13.87 (2.30)^{ab}	0.113 (0.005)^{a}	0.602 (0.033)^{ab}	1.955 (0.222)^{abc}	0.064 (0.013)^{ab}
	25	13.54 (2.11)^{ab}	0.114 (0.003)^{a}	0.541 (0.055)^{ab}	2.085 (0.129)^{abc}	0.028 (0.002)^{a}
	40	14.91 (1.45)^{b}	0.125 (0.009)^{a}	0.676 (0.058)^{b}	2.396 (0.174)^{c}	0.058 (0.006)^{ab}
Biochar (SWP+P)	15	13.41 (2.41)^{ab}	0.153 (0.019)^{ab}	0.508 (0.012)^{ab}	2.179 (0.086)^{bc}	0.105 (0.011)^{abc}
	25	12.14 (1.95)^{ab}	0.120 (0.009)^{a}	0.663 (0.107)^{ab}	1.756 (0.088)^{abc}	0.126 (0.023)^{abcd}
	40	13.47 (1.64)^{ab}	0.162 (0.010)^{ab}	0.599 (0.034)^{ab}	1.650 (0.1380)^{ab}	0.229 (0.009)^{cd}
	F	2.861	3.664	2.708	6.171	9.297
	p	0.010	0.003	0.016	<0.001	<0.001

Superscripted lowercase letters indicate significant differences ($p<0.05$) and groups from ANOVA with post-hoc Tukey test.
Values in parenthesis are standard errors of mean, n=3.

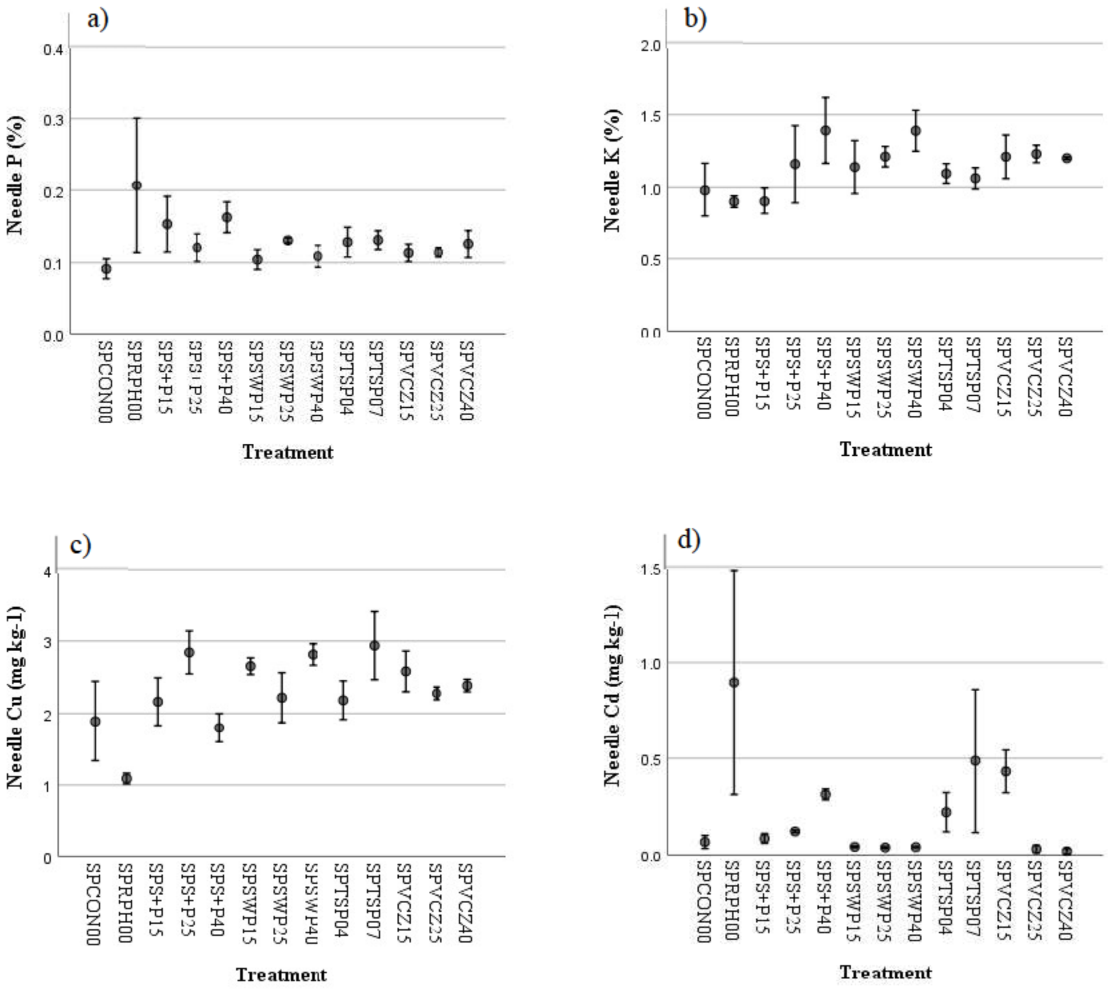

Figure 3-14- Mean foliar analysis nutrient concentrations at end of experiment for Scots pine, including standard error. Cd and Cd concentration are expressed as mg kg-1 oven dry weight and K and P as percentage of dry weight (%).

3.4.4 Inter-species comparison

There was no difference in the CON corrected above ground total biomass accumulation to VCZ biochar application (for all does rates) when comparing species (p>0.05) (Section 9.1.1). Low dose VCZ biochar application resulted in higher above ground woody biomass accumulation (in relation to the CON treatment) in Douglas fir seedlings, with a lower rate in Sitka spruce (p<0.05). There was no difference in control corrected above ground woody biomass for VCZ application rate of 25 g seedling^{-1} and 40 g seedling^{-1}. There was no significant differences (p>0.05) in the time of bud burst between treatments for each species, however there was a significant difference in the time of bud burst across species (p<0.001), with Scots pine flushing earlier than Sitka spruce and Douglas fir. Bud burst was only assessed in Year 1.

F_v/F_m (Maximum quantum efficiency of PSII photochemistry) was found be significantly higher in Sitka spruce seedling treated with VCZ biochar, compared to CON, TSP and SWP+P treated seedlings during year one (p<0.05). This result was consistent in Douglas fir. Scots pine seedlings exhibited no differences in F_v/F_m measurements across treatments. VCZ biochar resulted in consistent F_v/F_m between years, whereas other treatments had higher F_v/F_m in year 2. Chlorophyll fluorescence data is included in Section 9.1.1.

3.5 Discussion

Biochar application was observed to have a complex effect on biomass development during the early establishment phase (years 1 to 5) of conifer seedling growth depending on biochar type, application dose and seedling species. Multiple mechanisms may be attributed to the differences in biomass development across treatments and the extent of these mechanisms varies across doses and species. Woody above ground biomass growth improvement or suppression, compared to CON treatment can be attributed to ameliorated or exacerbated nutrient deficiency, which in turn limits growth. Nutrient deficiency in seedlings is highly dependent on the properties of biochar or inorganic mineral derived fertiliser. Fertiliser addition directly impacts rhizosphere development and affects the ability of seedlings to uptake, or access, nutrients contained in soil. Biochar, and inorganic mineral derived fertilisers, can also alter soil ecology and soil chemistry in addition to direct nutrient delivery. Soil P pools can be altered following the addition of tested treatment, altering soil P availability, and further influencing seedling growth and morphology.

3.5.1 Nutrient Accessibility and Seedling Growth

Biochar amendment has been found to promote root proliferation, root foraging and mycorrhizal fungi abundance, capturing P from biochar surfaces (Hammer et al., 2014; Prendergast-Miller et al., 2014; Warnock et al., 2007). Hammer et al. (2014) & Jabborova et al. (2021) have shown that biochar nutrient content is positively correlated with mycorrhizal abundance and root density, however the highest nutrient content biochar tested was comparable to VCZ, with no P infused biochar assessed. The suppression of root development observed directly within treatment areas of high available P biochar (SWP+P) will limit the direct uptake of P from the interaction of the rhizosphere and charosphere. P will primarily become available to the plant through lateral movement of soluble phosphate ions PO_4^{3-} transferring from biochar internal spaces and desorption from biochar surfaces to the growing media (Almanassra et al., 2021; Son et al., 2021). This may partially explain why some seedlings treated with high doses of available P (SWP+P and TSP) have deficient needle P concentrations and low growth rates.

Mycorrhizal symbioses are recognised as having a vital role in plant nutrition, particularly in phosphorus uptake, with arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi showing the same P delivery function (Roy-Bolduc & Hijri, 2011; Treseder, 2004). Fungal biomass and mycorrhizal association in seedling roots were observed to be more prevalent in VCZ and SWP treated seedlings compared to mineral TSP treated seedlings. This supports the results of the study detailed in Chapter 5. The inoculate source of the mycorrhizal fungi was unknown and likely was from the homogenised growing media or existing associations in bare root seedlings. Mycorrhizal identification or DNA sequencing was not part of this study and mycorrhizal fungi community composition may not reflect forest soils. Mycorrhizal fungi can replace direct root P uptake in low available P conditions (Li et al., 2008). Rousseau and Reid (1991) found that ECM fungi development can slow the rate of P transfer from soil to pine seedlings, resulting in a delayed growth response. A meta-analysis by Treseder (2004) found that mycorrhizal abundance decreased 15% under N fertilisation and 32% under P fertilisation, as plants allocate carbohydrates to new vegetative growth and mycorrhizal fungi become C-limited (Johnson et al., 2015). This reduces the accessibility of nutrients from soil treated with inorganic mineral derived fertiliser. The enhancement of mycorrhizal abundance and root associations following biochar application increases the ability of seedlings to uptake nutrients from the wider growing media and from within biochar particles, improving nutrient accessibility (Gujre et al., 2021; Warnock et al., 2007).

Improvement to above ground biomass accumulation and a decrease in P deficiency stress in seedlings treated with VCZ biochar may be attributed to improved P uptake resulting from root proliferation and enhance mycorrhizal associations. Mycorrhizal associations have been found to be most effective at mediating macro-nutrient deficiency in P-limited soils (Hoeksema et al., 2010), which in turn can improve biomass accumulation (Mohammadi et al., 2011). High total growth rates in VCZ treated seedlings and higher biomass create an increases seedling P demand. Despite the limited growing media P pool in VCZ treated trees (with modest added available P), seedling P deficiency was not increased by the end of experiment. This suggests that VCZ enhanced P uptake efficiency and increased P accessibility, compared to CON and high available P treatments. Treatments did not appear to alter seedling phenology, with no differences in budburst across treatment applications. However, differences in phenological responses between species likely impacted the observed differences in seedling responses to biochar application. In particular, earlier budburst and quiescence in,Scots pine seedlings may have contributed to the higher observed mortality in high P treatments in Year 1. The soil pH may not have rebounded when quiescence and bud burst occurred in Scots pine, following the temporary decline associated with high P fertilisers, compared to the other tested species which are still in the dormancy phase. Seedling phenological differences in controlled environment studies do not represent outplanting conditions, with bur burst occurring earlier and frost damage prevented. This may have limited the effect of phenological differences on growth and survival results.

The root death observed across all species in high SWP+P and all targeted TSP treatments is likely due to the rapid localised decrease in growing media pH as applied TSP interacts with the growing media in the treatment area, which reduced the pH of growing media in the treatment area from 4.41 pH to 2.9 pH at one week after planting. Whilst plant cells can adapt to rapid acidification, through dynamic cell wall molecular restructuring (Arsuffi & Braybrook, 2017), extreme acid pH can sufficiently degrade cell wall molecular structure leading to tissue necrosis (Phyo et al., 2019). Root death caused by pythium root rot is common in glasshouse environments and is most prevalent in acidic conditions (Krasnow & Hausbeck, 2017). It is unlikely that pythium root rot contributed to the observed root death, as optimal pH for this pathogen is pH 4.5 – 6.5. Root death, coupled with decreased root hair and lateral root growth, limit root surface area, and reduces the ability of seedlings to uptake nutrients and water. In this experiment water was not limited, therefore no difference in drought stress was observed. The impact of high available P fertilisers and biochar on rhizosphere development likely contributed to the observed K deficiency in all species during year 1. The foliar K deficiency in high available P treatments suggests that the initial suppression of root development associated with high available P application will also impact the accessibility of other nutrients and result in short term nutrient deficiency which may be alleviated as the rhizosphere recovers. As the detrimental effects diminish as soluble P is released, root systems may partially recover, along with seedling K uptake.

Rhizosphere pH also regulates the rhizosphere priming effect (RPE), which in turn will impact on the availability of soil nitrogen (Wang & Tang, 2017). The decrease in rhizosphere pH from high available P applications could also limit, or reverse, the RPE. Targeted biochar amendment increases rhizosphere pH in acid soils and will enhance the RPE , which increases the microbial mineralisation of soil N to plant available N (Rousk et al., 2016). Organo-mineral forest soils, which have a large labile carbon pool, have increased N-cycling through microbial mineralisation stimulated by root exudation (one factor which contributes to the RPE) (Phillips et al., 2011). The high organic matter content of the growing media used in this study has the potential to contribute to variable growing media NH_4^+ dependent on root biomass. Biochar application has been shown to enhance seedling N uptake. Conversely, the supressed root development in seedlings treated with high available P applications can decrease the accessibility of N. These mechanisms and effects contribute to the improved seedling NH_4^+ uptake following VCZ biochar application, as soil organic nitrogen mineralisation is increased. This has the potential to improve seedling N uptake during the establishment phase, before soil N cycling moves from a low available N closed acquiring cycle to a higher available N open recycling cycle at canopy closure (Xia et al., 2021).

Copper uptake can be decreased by moderate P application (4.5 mg P kg^{-1}) (Kuziemska et al., 2021; Singh & Swarup, 1982). Elevated soil nitrogen can also lead to foliar copper deficiency in tree crops, where soil has low copper concentration (Brown & van den Driessche, 2005; Fox et al., 2007). Mycorrhizal associations, which are reduced by high inorganic mineral derived P application (Treseder, 2004) and increased by biochar amendment (Jabborova et al., 2021), has also been found to contribute to improved copper uptake by plants (Lehmann & Rillig, 2015).It can therefore be concluded that despite the low native Cu contained in P fertiliser and tested biochars, the impact of treatments on rhizosphere development influences the potential accessibility of growing media Cu to the seedlings and can result in increased Cu deficiency with inorganic mineral derived P fertiliser application and elevated Cu uptake in VCZ treatments.

Seedling nutrient deficiency, measured by foliar analysis nutrient concentration, impacts on needle photochemistry by limiting photochemical processes. The clear correlation between foliar P concentration and needle F_v/F_m (Maximum quantum efficiency of PSII photochemistry) suggests that in treatments with low biomass accumulation (and low foliar P) seedling growth was limited by P accessibility. In treatments where foliar P was not deficient, but growth rates were also low, the effect of biochar on other micro and macro nutrients is likely responsible for the enhancement of maximum quantum efficiency of PSII photochemistry (in Scots pine and TSP treatments). It also suggests that the suppression of root systems in TSP treatments effects seedling health (as indicated by a decline in F_v/F_m) despite the increased available P. This supports findings by Percival et al. (2008) & Parkhill et al. (2001) who identify the potential use of F_v/F_m as a diagnostic of nutrient stress in plants.

3.5.2 Nutrient Availability and Seedling Growth

The relatively high Al^{3+} and Fe^{3+} content, coupled with low native available P, in SWP biochar has the potential to locally decrease soil available P in the treatment area through adsorption of available P to Fe/Al oxides and the precipitation of available P, forming Fe/Al phosphates (Dai et al., 2017). Increased dose rate of SWP biochar application was not found to ameliorate P deficiency and high dose rates were less effective than low dose application in some treatments. Local immobilisation of the available P pool in soil solution due to the addition of Fe^{3+} & Al^{3+} in high dose SWP treatment is likely the underpinning mechanism.

VCZ biochar has a relatively high pH, creating a localised liming effect in acidic soils. Elevated pH in the treatment area around VCZ particles has been found to increase soil pH from pH 4.5 to pH 6.5-7 and supports findings by Dai et al. (2017) which show that biochar can ameliorate soil acidification. This creates optimal pH conditions in the rhizosphere for P and N availability and uptake (Gao et al., 2019; Neina, 2019; Penn & Camberato, 2019). Conversely, TSP and SPH application contributes to temporary local soil acidification in the rhizosphere, further limiting nutrient availability (excluding Fe). The low Fe^{3+} & Al^{3+} of VCZ biochar coupled with relatively high available P results in a direct source of available P, which is accessible to seedlings. Soil available P can precipitate on the Ca content of VCZ biochar, increasing the adsorbed phosphorus pool, which slowly releases for plant uptake. This adsorbtion, coupled with absorption within VCZ particles, limits lateral P loss through leaching (which is a considerable environmental challenge with conventional P fertilisers). Soil organic P is increased with VCZ biochar application, as mycorrhizal, microbial and root biomass is increased. This organic soil P is cycled by immobilization and mineralisation process, stabilising the total soil P pool by decreased leaching of soil solution P (Miransari, 2013) . Soil microorganism diversity and abundance can be enhanced with biochar application, which can improve plant P uptake and soil P retention (Bueis et al., 2019). Specified VCZ biochar can therefore stimulate soil biological fertilisation by increasing mycorrhizal fungal abundance, root growth and promoting rhizobacteria.

The high available P content of TSP, SWP+P and SPH is sufficient to decrease the organic P pool and will result in a high proportion of soil P loss and potential to only temporarily increase the soil P pool, particularly in soils with a low clay content or low in Ca, Fe or Al. Exhaustion of P in inorganic mineral derived P fertiliser is unlikely to occur in the first year following application due to precipitation of fertiliser available P to the high Ca content of these fertilisers. However, these fertilisers will influence soil P pools and P cycling immediately upon application. . High available P fertilisers have also been found to degrade the soil organic N pool due to stimulated rapid mineralisation. The resultant flux in NH_4^+ is likely to be favoured by herbaceous plants or lost through leaching. The soil N pool will slowly recover as above ground biomass develops and cycles N back into the soil through litterfall. Biochar can promote soil microbial communities, which inorganic mineral derived fertilisers suppress (Dangi et al., 2020; Palansooriya et al., 2019). Increased abundance of non-symbiotic N-fixing soil bacteria, such as *Azotobacter* spp., due to biochar application could locally increase soil NH_4^+, further improving N availability (Mierzwa-Hersztek et al., 2020).

Biochar can improve the availability of soil nutrients, in addition to directly delivering native available nutrients to the soil. Specified biochar may also stimulate the biological fertilisation of soils. These factors contribute to the increased growth of seedlings treated with biochar in this study. Despite the high concentration of available P delivered by inorganic mineral derived fertiliser, these fertilisers can have wider impacts on soil nutrient availability and nutrient cycling, decreasing relative efficacy.

3.5.3 Silvicultural Implications

Biochar application was found to out-perform inorganic mineral derived P fertilisers, improving early growth in the establishment phase and alleviate nutrient deficiency in seedlings grown in a nutrient limited acidic soil. Targeted VCZ biochar was the most effect fertilisation method, although the optimal application dose was species specific. Biochar, and fertiliser application, showed greatest growth improvements in Douglas fir seedlings. This result is expected as Douglas fir has higher nutrient demand and is less suitable to low nutrient soils than other tested species. Sitka spruce seedlings showed a strong growth response to biochar treatments, but biomass results were less conclusive. This can be attributed to the use of vegetatively propagated seedlings which have high biomass variability and low needle and lateral biomass. Targeted VCZ biochar application was found to increase Douglas fir and Sitka spruce growth to a rate comparable to conventionally planted Scots pine. Species site suitability can therefore be improved with the application of specified targeted biochar doses, increasing the potential for establishment species diversification. Forest fertilisation methods can be improved by considering novel fertilisation technologies which both deliver native nutrients and improve wider nutrient accessibility by improving rhizosphere development. Conventional P fertilisers can be effective at short term P delivery but were found to constrain the uptake of other nutrients due to rhizosphere suppression. Longer-term implications on growth caused by altered root morphology are likely without continued fertilisation to canopy closure. The effect of fertiliser management interventions on long term soil nutrient availability, nutrient accessibility, nutrient cycling, and seedling development should be considered synergistically. Chlorophyll fluorescence (particularly F_v/F_m) could be used as a practical and economic method to identify early nutrient deficiency in seedlings, prior to manifestation of visual symptoms or a decline in growth rate. This technology cannot be used to identify exact nutrients which are deficient, this would foliar analysis or soil analysis. Fertiliser application in a nutrient deficient forest establishment context alters complex plant-soil interactions and can have a positive or negative effect on the soil environment depending on dose rate, fertiliser type and application method.

3.6 Conclusion

Forest phosphorus (P) fertilisation practices are not effective in improving overall seedling growth but can provide a short-term nutrient source. High available P fertiliser applications have a profound impact on root development and morphology. Limited rhizosphere development can limit the accessibility of other key nutrients in soil to seedlings. Biochar was found to increase the accessibility of P, K and Cu, particularly in VCZ biochar. This directly increased aboveground biomass growth and accumulation, where soil nutrient content was the limiting factor to growth. Specified VCZ biochar, which contains moderate available P, increases root development, and enhances mycorrhizal fungi association (Chapter 5). VCZ biochar out-performed other tested fertilisation interventions. The results of this experiment highlight the potential of novel fertilisers in improving early growth in the establishment phase, in turn expanding the range of viable species which can be established on nutrient poor environments. This study has also provided mechanistic insights into P fertiliser nutrient accessibility (including macro and micronutrients) and the impact of P fertiliser on root development. Practical management implications highlight the potential of biochar in mitigating soil nutrient loss and preventing establishment failure.

3.7 Contributions

Forest Research's Technical Services Unit managed the experiment, including irrigation, temperature control and daily environmental data collection. Thanks must be given to Dave Clark, Nursery Manager. Dr Gail Jackson kindly provided use of a MINI-PAM II Fluorometer for part of the experimental work. Chemical analysis was completed by John Mormon, University of Edinburgh and Francois Bochereau, Forest Research. Prof. Ondrej Masek produced the biochar at the UKBCE Stage III facility. This experiment was made possible by the support of Mike Perks and Forest Research. Seedlings were provided by Forest and Land Scotland Newton Nursery. Destructive biomass sampling was assisted by Kaiyu Lei, without this help the replication in data collected at the end of experiment would have been poor. The contribution of Forest Research and University of Edinburgh staff must also be expressed, who allowed this experiment and end of experiment sampling to be conducted through the COVID-19 restrictions. The advice of Dr Saran Sohi was invaluable throughout this experiment.

Chapter 4

Biochar and seedling stress response in nutrient limited establishment environments: An out-planting field experiment

4.1 Abstract

Seedling growth response to biochar application was tested in a nutrient poor soil in an out-planting field experiment. A range of distinct biochar types were applied at planting to Sitka spruce (*Picea sitchensis*), and Scots pine (*Pinus sylvestris*) seedlings at planting on a restock establishment site were soil available P was expected to limit growth in the establishment phase (identified using site suitability modelling). Biochar application was found to improve early establishment seedling growth, compared to no application and inorganic mineral derived P fertiliser. Specified VCZ biochar showed the strongest seedling growth response. Seedling growth trends in response to application treatments provided insight into longer term impact of biochar on establishment. Growth rates show a gradual decline over the experimental period with no application, high available P treatments showed a highly variable growth rate across years (suggesting low resilience to abiotic stress factors) and VCZ treatments showed a consistent high growth rate. Chlorophyll fluorescence was used to assess seedling stress response to nutrient and water related stress factors. Biochar was found to limit the stress response of seedlings, suggesting improved resilience to abiotic stress factors. Targeted specified VCZ biochar application has been found to increase seedling growth and survival in the early establishment phase, in turn ameliorating soil nutrient deficiency and the resultant seedling stress response. Biochar, when correctly used, has the potential to help mitigate declining forest productivity caused by climate change and soil nutrient loss. The efficacy of biochar types are highly dependent on physical and chemical properties. This study demonstrated that practical biochar applications can be effectively introduced to the forestry sector to improve forest early establishment success, resilience and growth on nutrient poor planting sites. The results highlight the potential of biochar use in adapting forestry to increased climate pressures and soil P decline, increasing resilience to climate change through reducing abiotic stress factors.

4.2 Introduction

Modern forest management is facing increasing difficulties in establishing seedlings in both woodland creation and restocking on productive conifer clearfell sites, due to soil nutrient loss and climate change. As soil nutrient pools are degraded from rotational clearfell harvesting systems and periods of extreme water stress become more frequent, forest restock growth in the establishment phase will decline and the range of environmentally suitable species will become more limited on nutrient poor soils. Soil nutrient loss is being further exacerbated by some silvicultural practices. Fallow periods are being favoured over chemical application, which is increasing soil N loss from harvesting residues, highlighting the trade-offs between environmental management considerations. Alternative species are required to match sites which are becoming increasing limited by water deficits and low soil nutrient status (Ennos et al., 2020; Hornung et al., 1987). Maintaining forest productivity and establishment is vital in ensuring that total forest carbon stocks do not decline and that established woodlands remain net carbon sinks. Declines in forest yield and establishment success, which is considered as a combination of early growth and seedling survival (Thiffault et al., 2012), and increased time to canopy closure, is due to seedling responses to increased abiotic (e.g. drought) and biotic (e.g. peats and diseases) stress factors. The extent and array of abiotic stress factors affecting seedling establishment on nutrient poor planting sites can be primarily determined according to climate and soil. Seedling resilience to stress factors associated with climate and soil will decrease as the intensity of these stress factors increase with climate change and degradation of soil nutrient pools. Active management interventions will be required to mitigate these environmental stresses and maintain forest productivity (Moffat, 2003). Biochar, a carbon rich soil additive produced from the pyrolysis of biomass, has the potential to ameliorate stress factors linked to soil in forest systems, however there is a clear research gap on the use of biochar in restock establishment (Lehmann et al., 2021; Wang et al., 2020).

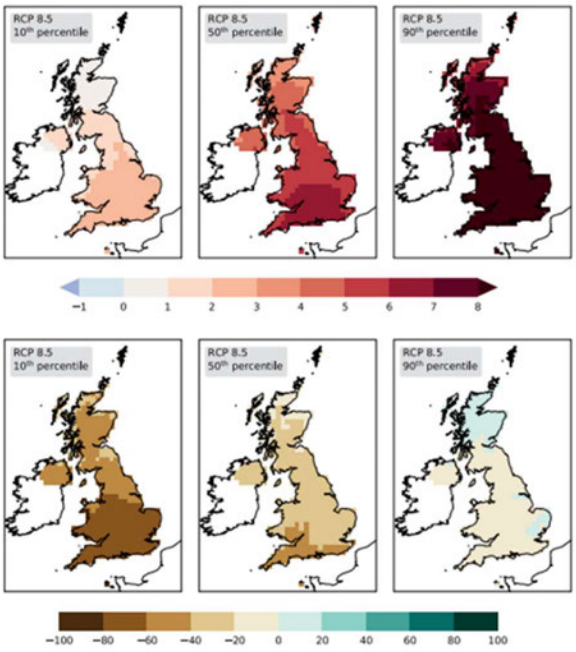

Figure 4-1 – Met office projected climate change under simulated scenarios showing summer temperature (top) and precipitation (bottom). Maps display temperature (°C) and precipitation (mm) change at 2080-2099 compared to 1981-2000 mean baseline. Percentile ranges of projections are shown in individual maps. Source: Forest Atkinson et al. (2022)

Climate models show that summer temperature in upland areas of the U.K are projected increase by 3-4ºC, (with projections ranging from 1-8°C) by the end of the century (Figure 4-1). Most projections also suggest that average summer rainfall is expected to decrease by 20-60 mm, although some projections suggest a modest increase to summer precipitation. Climate models also show that rainfall patterns are likely to significantly change over decadal timescales. This will result in extended low rainfall periods increasing the frequency, intensity and durations of drought conditions, with severity compounded by warmer temperatures (Ovenden et al., 2021; Rahiz & New, 2013). In recent years low rainfall has resulted in substantial losses during early establishment and reduced growth rates as seedlings become stressed (Green & Ray, 2009). Plant stress can be considered as physiological responses to abiotic and/or biotic stress factors. The exposure to stress factors at a high intensity and/or for long duration can exceed the limits of seedling stress responses and can cause permanent damage or death when a threshold is exceeded (Kranner et al., 2010). Most commercial conifer species are poorly adapted to drought stress factors and pine species (*Pinus* spp.) are generally the most suitable for planting on drought prevalent sites in the U.K. These negative stress effects are often described as distress, whereas positive effects, where seedling stress response thresholds are not exceeded, are described as eustress (hardening) (Kranner et al., 2010).

During the early phases of establishment seedlings are most sensitive to drought stress caused by low rainfall. During this initial period following out-planting root structure has not yet developed with roots concentrated within the planting hole. Cultivation can create locally low soil water availability in disturbed soil, further contributing to drought risk (Wood et al., 2003). Soil disturbance removes surface vegetation and improves soil structure, increasing root zone temperature. The removal of surface vegetation, and associated soil temperature increase, can accelerate surface evaporation and locally limit soil water availability. In cultivated soils vegetation cannot intercept precipitation and runoff can increase, reducing soil water absorption (Nisbet, 2001). The structural improvement of soil with cultivation reduces bulk density and increases porosity by disturbing soil, which is beneficial to stimulating soil nutrient cycles and creates improved conditions in the rhizosphere in some soil types. Cultivation usually creates a warmer dryer microclimate and soil environment which can enhance seedling growth but also increase the risk of increased soil water loss. Seedlings planted on cultivated clearfell restock sites can be considered as being particularly sensitive to drought related stress (Locatelli et al., 2021).

Soil texture and SOC content contributes to drought resilience. Seedlings established on freely drained sandy soils, without an iron pan, are at greater risk of acute drought stress as available water will more rapidly decline below threshold levels during periods of low precipitation, compared to loamy or clay soils (Beauchamp et al., 2016; Locatelli et al., 2021; Vanguelova et al., 2018). Species suitability modelling shows that growth stress due low water availability in common commercial conifer species is primarily limited to podzolic soils in upland areas (Wilson et al., 2001). These soils are often also phosphorus (P) deficient and nutrient poor. Water logging, and subsequent root suppression and soil anaerobicity, can limit establishment success in poorly drained soils, however this issue can be mitigated through planting design (Nisbet, 2001). Current management interventions to mitigate establishment drought impacts are limited to species/provenance selection, continuous cover forestry and site preparation (Atkinson et al., 2022). However, these management considerations are constrained by other environmental factors, such as soil nutrient loss. Altering localised soil conditions through biochar application has promise in facilitating the rapid enhancement of root development, root structure and mycorrhizal abundance, whilst also improving water holding capacity, which will increase plant available and accessible water in the rhizosphere (Lei et al., 2022; Warnock et al., 2007). However, there has been little research into the efficacy of biochar in alleviating water induced stress in a field context or as a possible measure in adapting forest management to climate change.

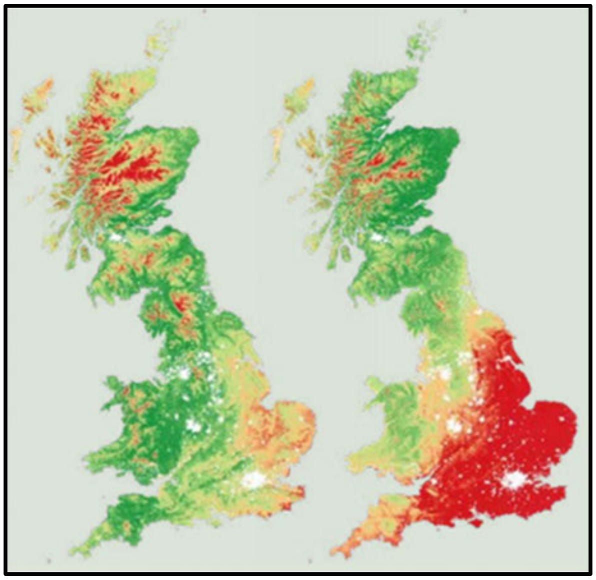

Figure 4-2- Climate suitability calculated using ESC model and median met office climate change projections for Sitka spruce establishment in 1961-1990 (*left*) and 2041-2060 (*right*). Suitability ranges from unsuitable (red), marginal (yellow) to very suitable (dark green). Source: Forest Atkinson et al. (2022).

Due to historic afforestation policy a legacy of forests on nutrient poor soils has emerged (Chapter 1). Freely drained podzolic soils, extensive across central and eastern areas of northern Britain, have limited phosphorus (P) pools and are vulnerable to soil nutrient loss (including N and C) during forest harvesting and cultivation (Hume et al., 2018). P stored within roots, brash and needles is vulnerable to loss through lateral transport (water) caused by mineralisation and transport of soluble P. Cultivation can also stimulate microbial activity, increasing the rate of mineralisation of organic P and soil P loss due to leaching in the absence of vegetative cover. The removal of biomass during harvesting further degrades the total P pool, as the P contained in removed biomass is lost (Elena Vanguelova et al., 2010). The total P in P pools vulnerable to loss during post-harvesting flux can exceed 50 % of the total P pool (Sohrt et al., 2017; Yanai, 1998). The net P loss in forest plantations will increase the prevalence of P deficiency and species suitability over time, which will impact species growth response and suitability. Atmospheric N deposition will compound P loss and further increase the area of forest where growth is limited by P accessibility (Yuan & Chen, 2015). Species suitability models suggest that nutrient poor soils are not suitable for establishment of most commercial conifer species and planting is limited to Scots pine (*Pinus sylvestris*), lodgepole pine (*Pinus contorta* Dougl. ex Loud.) and in some cases Sitka spruce (*Picea sitchensis*). Despite climatic suitability for Sitka spruce planting increasing across upland and northern areas of the U.K. (Figure 4-2), future viable planting area must also consider soil nutrient decline.

The application of inorganic mineral derived P fertilisers, rich in trace and heavy metals, including Cd, results in sequestration of these metals in plant tissue (Moffat, 1991). This may limit soil P loss during harvesting if rapidly applied (Callery et al., 2015). Research has suggested that trace and heavy metal stress can reduce frost hardiness, due to the alteration of cell membrane properties (Taulavuori et al., 2005). Sufficient trace and heavy metal uptake and sequestration to influence frost hardiness is only likely in newly planted seedlings treated with high dose inorganic mineral derived P fertilisers. As continentality (reflecting the length of the growing season) increases, according to climate projections, late frosts will become more frequent. It can be suggested that certain fertilisation interventions on nutrient limited sites may become unsuitable during establishment, necessitating novel alternatives to conventional fertilisation methods to overcome establishment limitations and risks.

Abiotic environmental plant stress factors can be classified into “short-term” stress, where seedlings can overcome the stress by acclimation and repair mechanisms, and “long-term” stress that require adaption and result in substantial irreversible damage (Kranner et al., 2010). Drought stress in a U.K. forest context can be broadly described as a “short-term” stress and soil nutrient related stresses can be described as a “long-term” stress. Stress factors considerably limit the tolerance of seedlings to additional stress factors and reduce seedling resilience. Seedlings affected by “long-term stress” factors have lower tolerance of “short-term” stress factors. Seedling under a stress response to “long-term” nutrient related stress factors are more vulnerable to acute damage caused by “short-term” abiotic stress (such as drought).

Seedling stress response can be divided into four phases (Mosa et al., 2017):

i. **Alarm phase**- the stress response reaction in the beginning of stress which limits physiological functions (including growth and photosynthetic performance). Only seedlings with low stress tolerance or under additional stress factors suffer acute damage.
ii. **Resistance phase**- the stage of resistance during continuing stress, where physiological function increases, and plant hardens. Growth remains low and cells are repaired. Restitution occurs if stress factor is alleviated during the alarm phase.
iii. **Exhaustion phase**- the stage of exhaustion as physiological stress responses fail due to high intensity or long-term stress, severe damage or seedling death occurs.
iv. **Regeneration phase**- the partial or full regeneration of seedling physiological function, following the removal of stress factors and contingent on limited seedling damage. Physiological functions are restored, generally at a lower level. Some damage will be permanent and seedling stress tolerance will decrease.

Physiology, ecophysiology, and phytopathology seedling stress responses can be directly evaluated by assessing the plant response in terms of growth, foliar analysis, and chlorophyll fluorescence results. Chlorophyll fluorescence measurements (F_v/F_m) can be used to measure photosystem II efficiency, which in turn indicates plant stress and is generally decreased during each stress response phase (Lichtenthaler & Rinderle, 1988; Mosa et al., 2017; Roháček et al., 2008). F_v/F_m is an adjusted measurement ratio that represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centres were open (dark adapted). This measurement is typically used to assess plant stress which limits photosystem function, PSII is altered in most plant stress responses, particularly when seedling distress is acute. Chlorophyll fluorescence cannot detect all plant stress and is specifically limited to measuring responses which alter PSII; it is also not a direct measure of photosynthesis. Care must be taken to reduce environmental variation during sampling which can influence measurements (Murchie & Lawson, 2013). The PSII operating efficiency (ϕPSII) is a measurement of the proportion of absorbed light that is actually used in PSII photochemistry and can therefore be used to estimate the rate of electron transport through PSII with knowledge of light absorptance by the leaf and photosystems (Murata, 1992; Murchie & Lawson, 2013).

Seedling growth can be used to assess the extent of long term or high intensity stress factors (Dobbertin, 2005). However, initial plant stress responses occur prior to observable reduced growth symptoms emerging, therefore growth measurements are not effective in detecting early stages of plant stress response (Rötzer et al., 2012). Seedling stress response also impacts growth, as plant resources are reallocated to maintain metabolism function. Supressed growth rates can be an indicator of long-term stress responses during the resistance phase and alarm phase, or degraded physiological functions in the regeneration phase following high intensity short-term stress (Brzostek et al., 2014). Seedling mortality can also indicate stress intensity or severity if stress is sufficient to exhaust the physiological seedling stress response. The phonology of the growth and dormancy of seedlings can have a considerable impact on seedling stress tolerance (Cumming & Burton, 1996). Phenological difference between seedlings particularly influence environmental stress factors which occur in autumn or spring, when seedlings move between growth and dormancy stages (Haase, 2011).

Limitations on forest fertilisation application and soil improvement in restock establishment to mitigate abiotic stress factors requires novel technologies (Schoene & Bernier, 2012). Site limitations, risk of diffuse pollution and operational constraints prevents blanket fertiliser applications (cost and application practicality). Specified biochar application as a novel fertiliser and soil improver has the potential to directly address the challenges to establishment caused by soil nutrient decline and climate related environmental stress factors. By targeting biochar application to the rhizosphere during planting, biochar nutrient delivery and rhizosphere nutrient uptake efficacy can be improved (Gao et al., 2019; Prendergast-Miller et al., 2014). The application of targeted biochar can improve seedling resilience to nutrient and water stress factors altering local soil conditions and stimulating root development (Razzaghi et al., 2020; Schmidt et al., 2021). Biochar is a source of available nutrients, particularly P, and can improve the wider soil available nutrient pools. Root, and associated mycorrhizae, structure can be enhanced by biochar treatment which can improve the ability of seedlings to access soil nutrients and water (Hammer et al., 2014). The highly porous, low density, structure of biochar can also benefit localised soil water properties and water availability (Edeh et al., 2020). The properties of biochar can be optimised to address the desired soil function improvements by altering feedstock and pyrolysis conditions, creating a specified biochar (Zhao et al., 2013). Biochar application, and subsequent impact upon mycorrhizal development, has been found to ameliorate drought stress in agricultural crops, however this has not been explored in a forestry context (Hashem et al., 2019). Targeted specified biochar has the potential to improve seedling responses to environmental stress factors by mitigating abiotic stress factors and improving seedling resilience to stress factors.

The purpose of this study was to assess the potential of biochar application in improving establishment success of Scots pine and Sitka spruce seedlings on nutrient limited planting sites. This study investigated: *i*) the impact of biochar and conventional fertiliser on seedling photochemistry to indicate resilience to stress factors, *ii*) the effect of biochar on seedling establishment (growth and survival) on a nutrient poor planting site and *iii*) the importance of seedling nutrition during the early establishment phase in a field environment, building on the findings of the experiment detailed in Chapter 3.

4.3 Methods

4.3.1 Site Selection

Forest and Land Scotland hosted the experimental trial and provided an initial list of potential sites due for restock planting. All sites were second or third rotation harvesting sites, which had been left fallow for >1 year following harvesting. These sites were assessed for species suitability and nutrient status using Ecological Site Classification (ESC) modelling (Wilson et al., 2001), Scottish Soils Knowledge and Information Base (SSKIB) (Lilly et al., 2004b) and Scottish soil finder. The SSKIB and Scottish Soils Database (Brown et al., 1987) were used to initially assess the soil nutrient status of the soils found at each site in order to ensure that the nutrient regime was poor. As the aim of the field experiment was to assess the potential of biochar as an alternative to conventional forest fertilisation on phosphorus limited sites, shallow peaty podzol or humus-iron podzol soil series were selected. These soils are typically low in available phosphorus and copper, freely drained and with a low water holding capacity. In the ESC model the properties of the selected soil series are comparable to VP3 (very poor 3) forest soils.

Sites which matched the selected soil type and soil nutrient regime criteria described were then assessed using the ESC model to identify the environmental variable which is likely to limit growth (and establishment success). The environmental parameters, excluding soil nutrient regime, of the ESC model showed that the selected species were suitable or very suitable for the sites. Soil nutrient regime was the limiting environmental factor according to ESC scoring for each species. Two potential sites were shortlisted, meeting the required soil and environmental characteristics. The sites were identified as suitable (Scots pine), marginal (Sitka spruce) and unsuitable (Douglas fir), depending on the nutrient demand of the species. These sites were located at Loch Ard Forest (56° 09′ 31″ N 4° 29′ 48″ W) and Trinloist Forest (57° 15′ 26″ N 4° 27′ 28″ W).

The two shortlisted sites were visited and surveyed to ensure that modelled soil parameters were accurate. Vegetation was surveyed to determine site soil nutrient regime (Wilson et al., 2001), ensuring that soils were very poor (and therefore tree growth limited by SNR). Soil was also sampled in the centre of the potential plot area to confirm soil type and series. The Site located in Loch Ard Forest was deemed as unsuitable for the field trial due to three principal factors. Vegetation surveys found the presence of species which indicate medium to rich soil nutrient status (*Digitalis purpurea* L. & *Rubus fruticosus* L. agg.) in localised pockets of higher nutrient status soils. The topography of the site was also challenging, with a steep slope present across the site, with multiple cardinal aspects. Pockets of deep peat (>50 cm O horizon) were found on the upper slopes. The heterogeneity of the soil on the site, coupled with the variable topography prevented the layout of an experimental design where inter-plot variability was controlled. It is likely that any effects of treatment on growth would be difficult to isolate from environmental variation. The Trinloist forest site was therefore selected for the field experiment. Finally, ESC modelling was reassessed using the results of the site survey to ensure that the predetermined site considerations were met (Table 4-1).

Table 4-1 – ESC model results for selected species at the Trinloist experimental site. Soil nutrient regime (SNR) is the limiting factor to yield class for each species. Colours show suitability according to ESC score; dark green (very suitable (0.75-1.00), yellow (suitable/marginal (0.50-0.74)) and red (marginal/unsuitable (0.30-0.49)).

	Species	
ESC Score	Scots pine	Sitka spruce
Ecological Suitability	0.52	0.35
Timber Suitability	0.5	0.3
YC	7	9
Limiting factor	**SNR**	**SNR**
Accumulated Temperature (AT)	0.96	0.86
Continentality (CT)	1	1
Exposure (DAMS)	1	1
Moisture Deficit (MD)	1	1
Soil Moisture Regime (SMR)	1	0.73
Soil Nutrient Regime (SNR)	0.52	0.35

4.3.2 Experimental Site

The experimental site was located within Trinloist Forest (57° 15′ 26″ N, 4° 27′ 28″ W), Scotland, U.K. (Figure 4-3). The forest is managed by Forest and Land Scotland. The site is dominated by peaty podzol and humus-iron podzol soils (with no iron pan present). Charr series and Countesswell series soils are present on the site. Countesswell humus-iron podzol soils were dominant, covering ~80% of the site. It is a freely drained soil with a thin surface organic horizon (<10 cm) (O horizon), shallow elevated mineral horizon (10-20 cm) (E horizon) over a bright orange subsoil (<30 cm) (B_h/B_s horizon). Cultivated mound planting positions are predominantly composed of the E and B_h/B_s horizons which have a high sesquioxide content (limiting P availability). There was no iron-pan present. The underlying lithology is a granitic intrusion to Moine thrust (schist), and the soil has moderate available N. The soil has a shallow

O horizon and is very low in available P and has moderate K. P is the limiting soil nutrient to seedling growth. Key soil properties of cultivated mounds are summarised in Table 4-2.

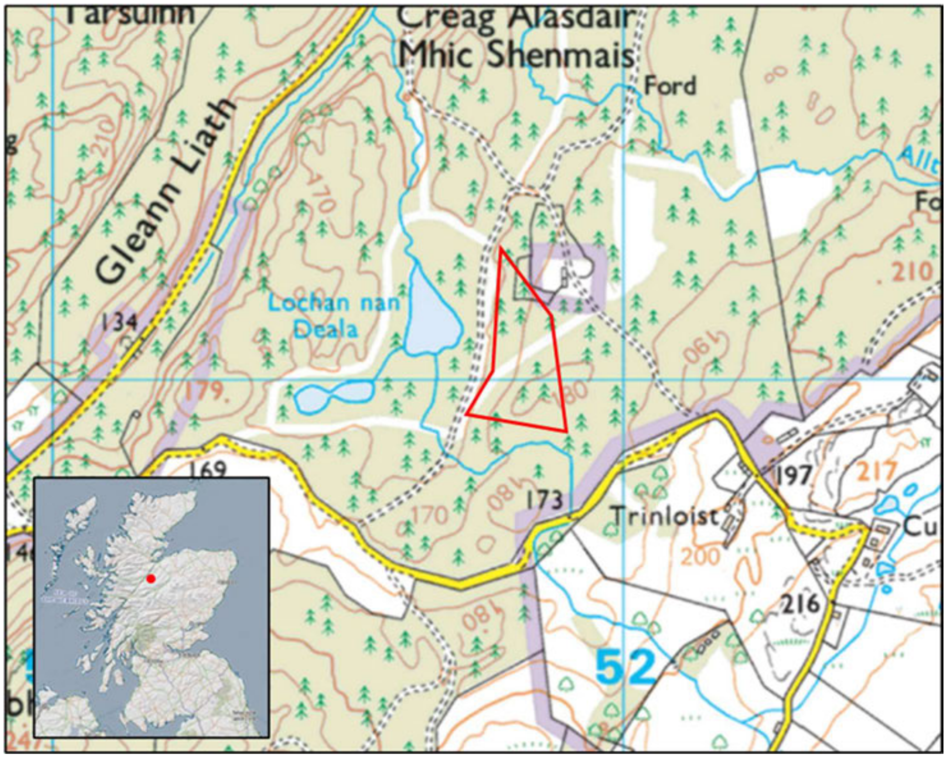

Figure 4-3 – Field experiment site location (red boundary). Centre of the map is; 57° 15′ 26″ N 4° 27′ 28″ W.

Table 4-2- Soil properties of the experiment site, sampled from mound planting positions.

Soil variables	Units	Mean	SD	Min	Max
Clay (%)	%	6.4	3.6	1.9	13.5
Sand (%)	%	74.6	6.4	61.4	84.1
Silt (%)	%	19.5	4.9	13.1	27.6
pH		4.2	0.4	3.8	4.9
Bulk density	kg m$^{3-1}$	1286	106	1201	1395
Water holding capacity	%	13.4	6.1	9.9	21.5
Exchangeable acidity	m$_{eq}$ 100 g^{-1}	14.5	5.6	8.4	21.4
CEC	m$_{eq}$ 100 g^{-1}	3.6	0.7	2.5	4.3
Base saturation (%)	%	3.4	2.9	2.2	4.6
Elemental C	%	4.3	2.6	2.1	5.9
Available P	mg P_2O_5 100 g^{-1}	45.1	26.5	16.1	84.4
Ca	m$_{eq}$ 100 g^{-1}	0.8	0.6	0.2	1.5
Mg	m$_{eq}$ 100 g-^{1}	0.17	0.15	0.03	0.38
K	m$_{eq}$ 100 g^{-1}	0.15	0.12	0.02	0.29
Total N	%	0.39	0.21	0.29	0.64

Site Preparation and Previous Management

Figure 4-4- Experiment site photographed during May 2018, following planting (March 2018). Site topography , brash and cultivation can be seem. The centre of the image is N.

The experiment site vegetation cover was acidic heath prior to planting (exact NVC type is unknown). Afforestation planting was during 1964, double mouldboard plough ground preparation was used where ground allowed. Significant P fertiliser was blanket applied, however dose rates and fertiliser type is unknown. A Sitka spruce/Scots pine mixed was used, planted at a 2:1 ratio. The Scots pine element was used as a nurse species and was allowed to self-thinned. Two light thinning operations were completed during 1990 and 2005. The site was harvested during November 2015 and left fallow for two years to limit *Hylobius abietis* damage. Ground preparation was completed during November 2017 by excavator mounding. Trench mounding was used in most of the site, except on steep ground where hinge mounding was used. Steep ground was not included in experimental plots. Mounds were evenly spaced at 2500 mounds ha^{-1}. Brash was cleared into rows and stumps were left in-situ. Mounds were left to settle for four months prior to experiment planting during March 2018. No adjacent blocks were harvested during the experiment period, avoiding potential *Hylobius abietis* ingress.

4.3.3 Experiment Set-up and Layout

The site was prepared prior to restock planting and experimental layout by trench mounding. Plots were placed between trenches. Each plot was ~15 m x 15 m, although exact dimensions were variable. Plot size was determined by the number of mounds in each plot, rather than spatial measurements. The dimensions of each plot ensured that there were at least 49 mounds per plot and care was taken to orientate plots into 7 x 7 mound squares. Wooden stakes were placed at the corners of each plot and serialised. Internal plots containing 5 x 5 mounds were marked with canes. These internal plots were used for data collection and the outer portion of the plot acted as a buffer zone. All mounds within plots were planted with seedlings and treatment applied.

A randomised block design was used, comprising of three blocks, each containing one plot of every treatment (six application treatments for two selected species). Each block contained 12 single species plots. Plots were assigned numbers after layout and treatments were randomly assigned to plots. The experiment consisted of 3 blocks, providing 25 sample seedlings per plot and 75 in total. Bare root seedling stock from seed was planted. Experimental layout was completed by Forest Research Technical Services Unit and planting was conducted by Forest and Land Scotland. Seedlings were planted at 2 m spacing (2500 trees ha^{-1}). Targeted fertilisation treatments (including biochar) were applied evenly around seedling roots during planting, directly into the planting hole.

Treatment Applications and Dose Rate

Three biochars were applied as separate soil additive treatments. These biochars are detailed in Chapter 2. The three biochars applied in this study were named and coded according to biochar feedstock. The biochar treatments used were softwood pellet biochar (SWP), vascular cambial zone biochar (VCZ) and softwood pellet biochar infused with additional P (SWP+P). The biochars were specified to provide different concentrations of P, with SWP low in P, VCZ relatively high in P and SWP+P P concentration similar to mineral TSP fertiliser (detailed in **Table *2-1***).These biochars are consistent with other chapters. The specified VCZ biochar, was developed to improve key seedling limiting soil functions on P limited establishment sites. The high porosity (interporosity and intraporosity), particle size and low bulk density (0.11 g cm$^{3-1}$) of VCZ biochar can improve soil water retention and hydraulic conductivity (Edeh & Mašek, 2022; Edeh et al., 2020).

All three biochars used were derived from woody biomass feedstock and produced by the UK Biochar Research Centre (UKBRC; University of Edinburgh, Edinburgh, UK). The equipment used was the Stage III system, a horizontal continuous feed pyrolysis kiln, manufactured by Ansac Pvt Ltd (Bunbury, Australia). The feedstock was pyrolyzed at a nominal highest heating temperature (HTT) of 550 ± 5°C with a heating rate of 78°C min^{-1}. Mean residence time in the kiln was 12 min, with 3.9 min at HTT. Details of the Stage III pyrolysis system can be found in Mašek et al. (2018). Details of the production process of SWP, SWP+P and VCZ biochar is described in Chapter 2.

SWP and VCZ biochar was applied to seedlings at a rate of 25 g seedling^{-1}. SWP+P was applied using a corrected mass dose of 25g biochar, plus the additional mass of infused P (and associated elements, mainly; Ca, Al and Cd). SWP+P mass dose was applied at 31.66 g (25 g biochar + 6.87 g TSP fertiliser). In results application dose rates of SWP+P biochar is stated as the biochar component dose rate (15 g, 25 g & 40 g). The selected application doses are the same as the intermediate doses in Chapter 3. Dose rate determination is detailed in Chapter 3. Biochar treatment chemical and physical properties are included in Chapter 3.

Figure 4-5- Planted Sitka spruce seedling treated with TSP, showing mineral soil mound, clear of vegetation.

A control treatment (CON), where seedlings had no application, was used in addition to two fertiliser treatments. Inorganic mineral derived P fertiliser application treatments were used to compare biochar treatments to conventional fertilisation practices (surface phosphate application (SPH)) and targeted triple superphosphate application (TSP). Triple superphosphate was selected as the targeted P fertiliser for use in this study. Surface phosphate (SPH) was also applied using triple superphosphate fertiliser that was consistent with targeted triple superphosphate treatments (TSP). Granular TSP (42 - 50% P_2O_5) was sourced from Yara Uk Ltd. TSP and SPH was applied at 7 g seedling^{-1} and 40g seedling^{-1} respectively. In the SPH treatment triple superphosphate fertiliser was evenly distributed on the surface on the mound over an area of 0.7 m^2 (30 cm diameter circle). Targeted triple superphosphate was evenly distributed around seedling roots following placement in the planting hole in the TSP treatment, prior to closing the planting hole Figure 4-5. The SWP + P, TSP and SPH dose rate was based on the recommended dose of P fertilization for conifer seedlings in P deficient soils (Taylor, 1991). The stated application rates deliver 60 kg available phosphorus ha^{-1}, which equates to 24 g available P per tree (requiring 39.87 g TSP per seedling). This rate is adjusted for targeted application and soil volume in cultivated mound, to maintain equivalent TSP:soil ratio. The adjusted dose rate for TSP (7 g seedling^{-1}) limits the detrimental impacts of high dose target fertiliser application and matches the available P dose of SWP+P biochar.

The six application treatments (SWP, SWP+P, VCZ, SPH, TSP and CON) were applied to; Scots pine (*Pinus sylvestris* L.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr). These species are two of the most important conifer species in U.K. forestry industry and were selected due to site suitability. Scots pine is a drought tolerant species, which is favoured on P deficient freely drained upland planting sites. Sitka spruce, which accounts for 50% of U.K plantation forest area, is considered a suitable species choice on poor planting sites. Sitka spruce is less drought tolerant compared to Scots pine and requires greater management input to establish on very poor nutrient regime sites (including forest fertilisation and nursing mixtures with pine spp.) (Savill et al., 1997).

Seedlings were planted during early March 2018, at which time biochar or fertiliser was applied. Seedlings were planted in accordance to best practice. Care was taken to prevent clustering of roots, in the planting hole. Bare root seedlings were used, sourced from Forest and Land Scotland Newton Nursery. Height grading was completed prior to planting, ensuring seedlings were 40-50 cm height.

Experiment Management

Following set-up, the experiment was managed by Forest and Land Scotland. The experiment was inspected bimonthly (six times per year) for browsing damage, weed growth and seedling health. Seedlings were inspected for pest and disease, particularly *Hylobius abietis* and powdery mildew. No extensive browsing, pest or disease damage was observed on seedlings. Due to the low soil nutrient regime, weed growth was minimal and no herbicide application was required. Natural regeneration in plots was removed during winter 2019/20. Any seedling mortality was not replaced in beat-up operations.

Temperature was recorded at the experimental site using a Gemini Tinytag TG 4017 data logger. Monthly mean daily high and low temperature was calculated. Precipitation was not recorded on site, and data from the closest Met Office monitoring station was used (9 km NE of the site at Drumnadrochit). Weather data for the site are displayed in Figure 4-6. Three drought (extended low rainfall) events were recorded during; April/May 2018, March/April/May 2020, April 2021, June 2021 and June/July 2022. No periods of extended extreme summer rainfall or temperatures were recorded.

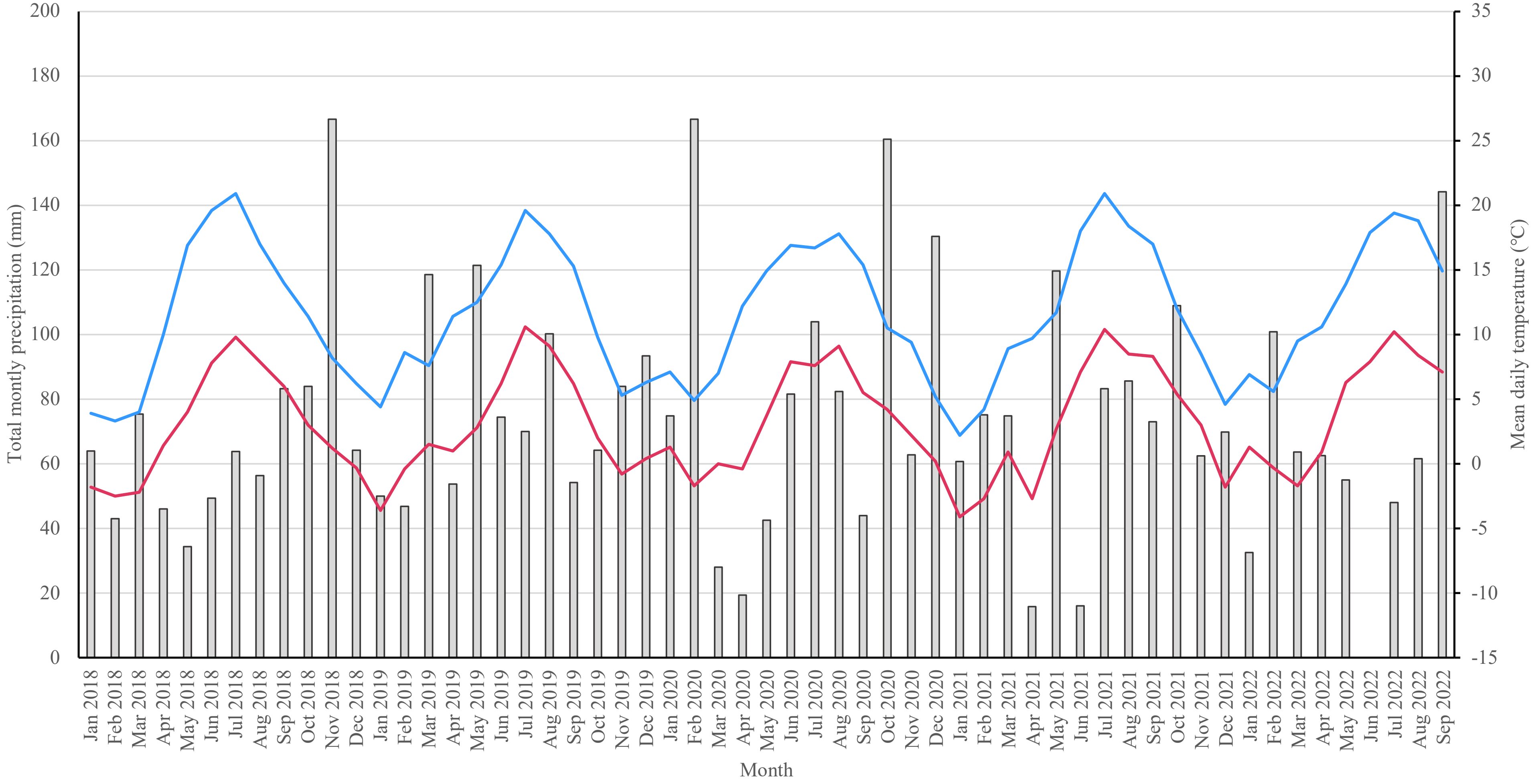

Figure 4-6- Climate data for Trinloist experiment site. Temperature displays monthly mean daily high temperature (°C) in red and monthly mean daily low temperature (°C) blue, measured on-site using Tinytag data logger. Precipitation data is sourced from the Met Office and was collected at the Drumnadrochit weather station (9 km NE of the experiment site).

4.3.4 Data Collection and Measurement

The site was sampled annually, collecting growth, survival, and chlorophyll fluorescence measurements. Data collection frequency was interrupted due to the COVID-19 pandemic, and efforts were made to mitigate data collection inconsistency. Three seedlings were randomly selected, using a random number generator, for detailed measurement and sampling. Where a seedling died, the closest surviving seedling was selected.

Seedling Establishment

End-of-season morphology was measured when seedlings were dormant (between October and March). Seedlings were measured during October 2018 (year 1), November 2019 (year 2), October 2020 (year 3). No measurements were taken during 2021 due to field work restrictions. Final growth measurements were completed in May 2022 (year 4). Seedling morphological parameters were collected. Root collar diameter, annual growth, seedling height and mean length of upper lateral branch whorl were measured. Seedling survival was also determined. Seedling survival and morphological measurements were collected for all seedlings in internal plots (25 seedlings plot^{-1}).

Soil and Foliar Sampling

Needle samples were collected, and chlorophyll fluorescence measurements were on three randomly selected sample seedlings in each plot in Year 2 and Year 4. The three selected sample seedlings were marked with tape and were consistent for the duration of the experiment. Soil samples were also collected (~5 g) from the mounds of sample seedlings, 15 cm away from the seedling root collar measured due north. Care was taken to ensure roots were not disturbed. As biochar was not found to alter bulk soil in the mound, due to targeted application, not all samples were analysed. Needles were sampled from the current shoot on the uppermost whorl of lateral branches (the leader was not used to minimise damage to seedlings) during July/August. Approximately 4 g (w/w) samples were collected. Only year 4 samples were analysed due to resource constraints.

Chlorophyll fluorescence of selected sample seedlings was measured during July/early August of each growing season (2018, 2019 & 2020). The timing of this was adjusted to ensure that weather variability was controlled. Sunny conditions, with a daily high temperature between 17 – 23 ℃, were selected and all measurements were completed within two days. As chlorophyll fluorescence is sensitive to environmental factors, inter-yearly analysis is indictive of chlorophyll fluoresce changes but there is a high degree of uncertainty in these results. Chlorophyll fluorescence was not measured during 2021 due to the COVID-19 restrictions. Seedling mortality was recorded.

Chlorophyll fluorescence parameters of photosystem 2 were measured using a pulse modulated Waltz Mini PAM II fluorometer (Heinz Walz GmbH, Effeltrich, Germany. The measured parameters are;

F_v/F_m – Maximum quantum efficiency of PSII photochemistry

ϕPSII – PSII operating efficiency: the quantum efficiency of PSII electron transport in the light.

Dark adapted measurements were taken using dark leaf clips (part no. 2030-B). Only needles on the uppermost lateral branch whorl were used. Care was taken to orientate needles to completely cover the clip aperture. For dark adaption to occur, the dark leaf clips were affixed to seedlings 30-40 minutes prior to measurements.

Chemical analysis methodology is described in Chapter 3.

4.3.5 Statistical Analysis

Data was analysed separately according to year and species. Inter year and interspecies analysis was also conducted, however there was high variability between variables and total yearly data was not normally distributed. Statistical analysis of growth data, chlorophyll fluorescence and survival data were completed separately for each species using Analysis of Variance (ANOVA). Each measured parameter was analysed with application treatment as fixed effect and block, replication and interaction terms treated as random effect. Data was tested according to treatment groups for equal variance using Levene’s test. This was used to assess whether data fulfilled the homogeneity of variance assumption required to accept ANOVA results. Where treatment groups showed homogeneity of variance ($p>0.05$) following Levene’s test for equal variance, one-way and two-way (with foliar chemical composition as a covariable) ANOVA statistical tests were used. Normality of residuals was tested using Shapiro-Wilk test. Where ANOVA obtained significant F-values, pairwise comparisons were tested using Tukey HSD test, adjusted for type I error. The variation between and within treatment groups was compared using the coefficient of variation (CV), which is a statistical measure of the relative dispersion of data points in a data series around the mean.

Growth and chlorophyll fluorescence trends across experiment years were tested using a simple linear regression analysis, due to limited temporal granularity. Residuals were tested to determine whether assumptions required for regression analysis have been met. Correlation between growth and chlorophyll fluorescence in selected sample trees was also assessed for each treatment independently using Pearson correlation coefficient.

Growth, survival, and chlorophyll fluorescence results are presented with treatments grouped by yearly results. Whilst this may seem counter-intuitive, it allows trends across the experiment period to be observed. As growth rate diverge (growth is correlated to seedling size) from year variation in growth rates increases and yearly comparisons between treatments is lost through natural variation.

4.4 Results

4.4.1 Seedling Growth and Survival

Seedling Survival

Very low seedling mortality was observed in year 4 (1 seedling total), therefore these data have not been included in the results. Seedling mortality was high in Year 1, exceeding 40% in some plots. There was higher variability in mortality per plot in Sitka spruce treatments, compared to Scots pine. There was no difference in seedling mortality rate or intraspecies variation across the experiment period for Scots pine treatments ($p>0.05$). Sitka spruce mortality was significantly lower ($p=0.043$) during Year 1 in the VCZ treatment than CON, SWP+P and TSP treatments. There was no difference in Year 2 and Year 3 mortality. Year 3 cumulative mortality rate was significantly lower ($p<0.001$) in the VCZ treatment compared to the TSP treatment in Sitka spruce.

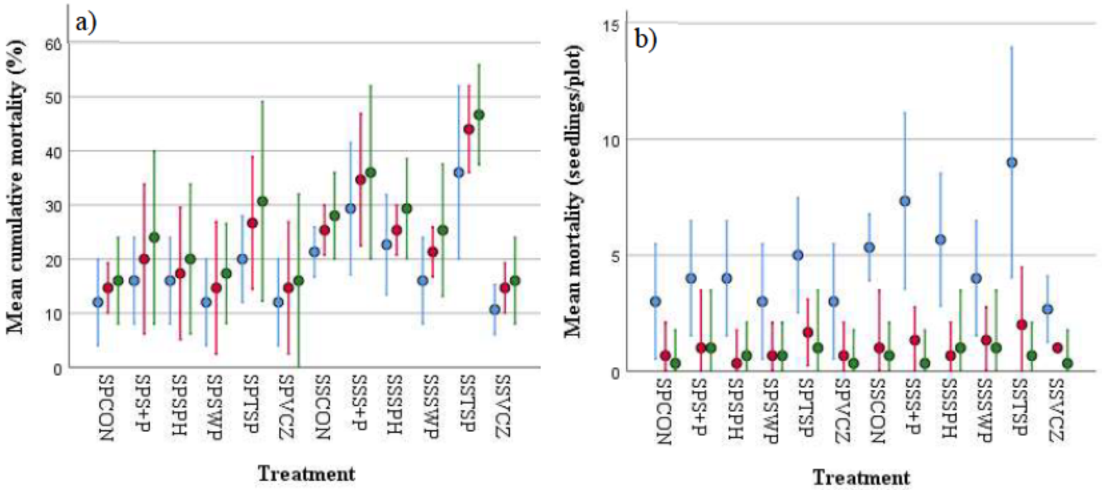

Figure 4-7 – Mean seedling mortality displayed as cumulative percent mortality (a) and annual mortality per plot (25 seedlings per plot) (b). Error bars display 95% confidence intervals (CI) in a) and standard deviation (SD) in b). Years 1, 2 and 3 are displayed in blue, red and green respectively. *n=3*.

Seedling Growth

In Year 1 there were no significant differences in height growth rate (cm yr^{-1}) across all treatments and species ($p>0.05$). Seedling diameter and lateral branch length showed no significant difference ($p>0.05$) between application treatments. Significant differences in growth rate between planting treatments and species emerged in Year 2 and was continued in Year 3 and Year 4 (Table 4-3). Sitka spruce seedlings treated with biochar had significantly higher growth rate (cm height increase yr^{-1}) ($p<0.001$) than the control treatment (CON) at Year 3. Surface phosphate (SPH) application resulted in no significant difference in growth rate, however targeted TSP application was found to improve growth comparable to biochar treatments in Year 3. In Year 2 there was a lesser observed effect of treatment on seedling growth, with a significant ($p=0.012$) difference in growth between VCZ biochar application compared to CON and targeted TSP treatments (Table 4-3). Height growth in Year 3 was low, corresponding with the longest period of low rainfall during the experiment. Growth recovers in Year 4, however Scots pine do not show this correlation between growth response and of low rainfall.

Year 4 growth followed similar growth patterns to Year 3. Growth between Year 3 and Year 4 was only significantly different ($p=0.38$) in Sitka spruce seedlings treated with SWP+P biochar.

Growth declined to Year 2 levels in this treatment. Mean growth in Sitka spruce seedlings treated with VCZ biochar application was significantly higher than all other treatments, and significantly exceeded all treatments (excluding SWP application) in Scots pine seedlings. Inorganic mineral derived P fertiliser application (TSP & SPH) did not significantly alter mean growth rates over the control treatment (CON). Biochar application improved Sitka spruce seedling growth in the tested field environment during the early establishment period, with VCZ biochar having the greatest positive impact on growth (Figure 4-8).

There was no difference in the annual growth of Scots pine seedling in Year 2 (p>0.05). In Year 3 growth was found to be significantly higher (p=0.013) in SWP biochar treated seedlings compared to CON and SPH treatments. SWP and VCZ biochar show a weak positive linear growth trend across growing seasons (R^2=0.27-0.56). The CON treatments showed a stable, low growth rate. Inorganic mineral derived P fertiliser (TSP and SPH) and SWP+P treatments in Scots pine, and TSP and SWP+P treatments in Sitka spruce, appears to improve growth to Year 3, followed by a slight decline in growth.

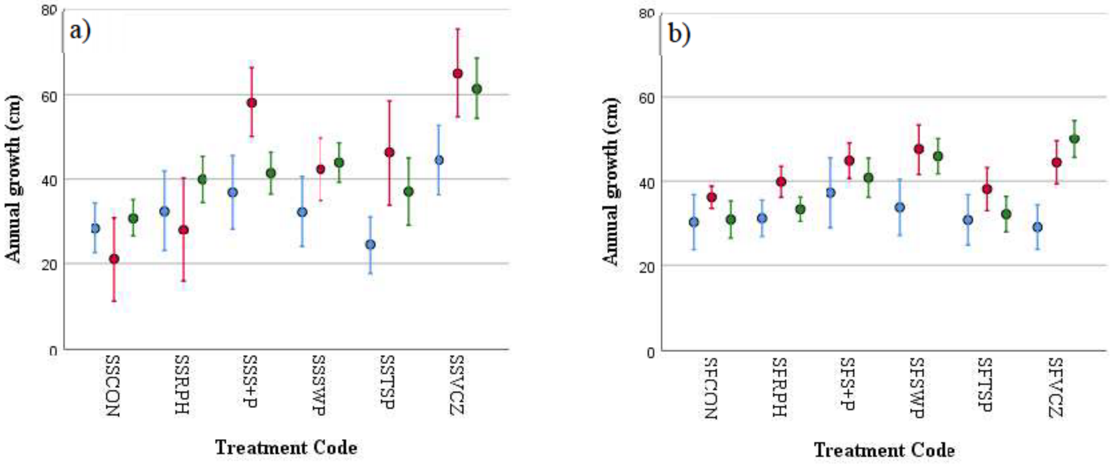

Figure 4-8 – Annual seedling growth rate (cm yr^{-1}) for year 2 (blue), year 3 (red) and year 4 (green) following planting for each application treatment. Each species is shown in separate charts, Sitka spruce (a) and Scots pine (b). SWP+P treatment has been abbreviated to S+P and treatment codes include the species code (SP, Scots pine and SS, Sitka spruce). RPH treatment code is used for surface applied phosphate (SPH), *n=75*.

Interspecies comparisons showed no difference in the year 2 growth rates for Scots pine and Sitka spruce seedlings. All treatments had similar growth across species in year 2, except for VCZ treatments. Sitka spruce seedlings showed significantly higher year 2 growth (p<0.001) than Scots pine in seedlings treated with VCZ biochar. Year 3 growth showed significant variation across species and treatments (p<0.001). Scots pine seedlings had higher mean growth in CON, SPH and SWP treatments compared to Sitka spruce seedlings, however this was not significant (p>0.05). Despite the site and expected yield class assessment as less suitable for Sitka spruces, according to ESC modelling, seedlings treated with VCZ biochar showed significantly higher year 3 growth than Scots pine (p=0.011). All other treatments (TSP and SWP+P) had higher mean year 3 growth in Sitka spruce seedlings, however the high variation in Sitka spruce growth resulted in high variance and no significance (p>0.05).

Table 4-3 – Annual seedling height growth (cm yr^{-1}) for the field experiment at year 2 and year 3 following planting. ACOVA results and post-hoc Tukey groups are displayed, along with standard error in parenthesis, for application treatments. Variance between blocks is not reported (p>0.05).

	Annual height growth (cm)					
	Sitka spruce			Scots pine		
Treatment	Year 2	Year 3	Year 4	Year 2	Year 3	Year 4
Control (CON)	28.5 (2.70)^{a}	21.08 (4.50)^{a}	30.83 (1.98)^{a}	30.39 (2.79)	36.22 (1.13)^{a}	31.00 (1.89)^{a}
Surface phosphate (SPH)	32.46 (4.30)^{ab}	28.08 (5.53)^{ab}	39.92 (2.45)^{ab}	31.29 (1.94)	39.83 (1.66)^{a}	33.42 (1.29)^{ab}
Triple superphosphate (TSP)	24.42 (3.07)^{a}	46.21 (5.59)^{bcd}	37.08 (3.57)^{ab}	30.92 (2.68)	38.13 (2.29)^{ab}	32.25 (1.89)^{a}
Biochar (SWP)	32.29 (3.78)^{ab}	42.29 (3.31)^{bc}	43.83 (2.09)^{b}	33.83 (2.99)	47.5 (2.71)^{b}	45.83 (188)^{bc}
Biochar (VCZ)	44.42 (3.68)^{b}	64.92 (4.72)^{d}	61.33 (3.27)^{c}	29.21 (2.37)	44.38 (2.30)^{ab}	50.00 (2.03)^{c}
Biochar (SWP+P)	36.89 (4.07)^{ab}	58.11 (3.88)^{cd}	41.39 (2.32)^{ab}	37.28 (3.57)	44.78 (1.80)^{ab}	40.78 (2.00)^{ab}
F	3.178	13.253	13.845	1.061	4.082	18.906
p	0.012	<0.001	<0.001	0.391	0.003	<0.001

Superscripted lowercase letters indicate significant differences (p<0.05) and groups from ANOVA with post-hoc Tukey test.

Values in parenthesis are standard errors of mean, *n=75*.

4.4.2 Seedling Nutrient Deficiency and Chlorophyll Fluorescence

There was no significant differences in mean foliar nutrients, trace metals or heavy metals (p>0.05). All Sitka spruce treatments had needle P concentration which were below optimal concentration (<1.8% P, oven dry weight), except VCZ and TSP treatments. Mean foliar needle P concentration was below the deficient concentration (<1.2% P, oven dry weigh) in the control treatment (CON). Scots pine seedlings in the CON treatment had marginal needle P concentration (1.2% P, oven dry weight), suggesting potential P deficiency. SWP+P, VCZ and TSP seedling had optimal mean needle P content (>1.4% P, oven dry weight). SWP and SPH treated seedlings had less than optimal foliar P concentration (<1.4 % P, oven dry weight), which is not considered as deficient. Needle K or N concentration was not below deficiency thresholds in any treatment. Foliar nutrient concentration is considered in more detail in Chapter 3.

Sitka spruce seedlings had significantly higher ϕPSII (PSII operating efficiency) and F_v/F_m (maximum quantum efficiency of PSII) recorded in seedlings treated with VCZ biochar than CON, SPH and TSP treatments in Year 2 (p<0.001) and Year 3 (p<0.001) (Table 4-4). SWP+P treated seedlings had elevated Fv/F_m compared to the CON during Year 1 (p=0.019) and Year 3 (p=0.006), and higher ϕPSII over the experimental period. ϕPSII and F_v/F_m were increased over the CON in SWP treated trees during Year 2 and Year 3 (p<0.05). TSP and SPH fertiliser treatments did not result in different chlorophyll fluorescence measurements compared to the CON treatments, suggesting no difference in seedling stress response. There was no difference (p>0.05) within treatments for chlorophyll fluorescence measurements between years, however some trends in F_v/F_m emerged. F_v/F_m in VCZ biochar treated trees gradually increases over the study period, following planting. CON, SWP and SPH treatments exhibit a decline in F_v/F_m over time, which is most pronounced in the CON treatment, indicating an potential stress response. Inorganic mineral derived P fertilisers application resulted in a close to significant (p=0.053) decline in F_v/F_m during Year 2, followed by a recovery in year 3 suggesting a more complicated seedling response, however high variation, and limited temporal data, result in low confidence in this trend. The Sitka spruce results are displayed in Figure 4-9 & Table 4-4.

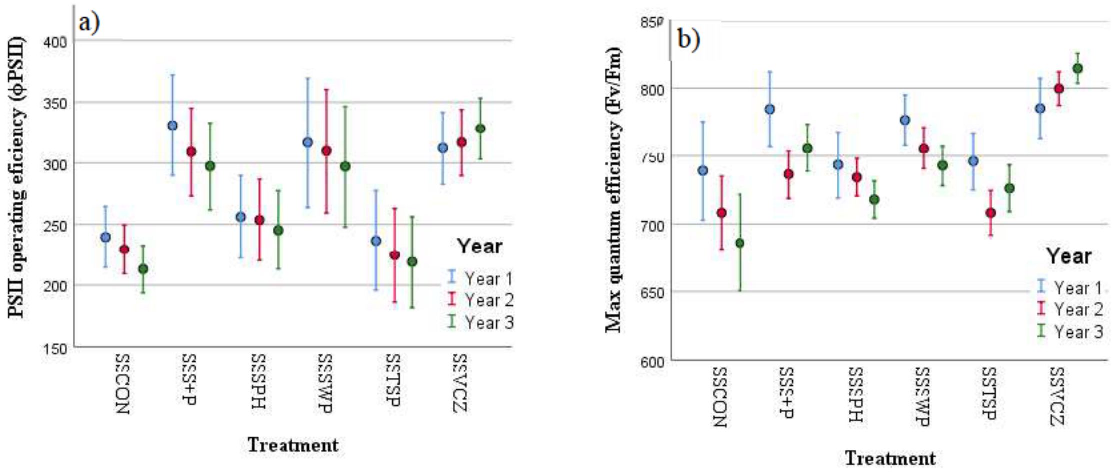

Figure 4-9- Sitka spruce needle chlorophyll fluorescence measurements for years 1, 2 & 3. Years 1, 2 and 3 are displayed in blue, red and green respectively. Graphs show a) ϕPSII - PSII operating efficiency: the quantum efficiency of PSII electron transport in the light and b) F_v/F_m - Maximum quantum efficiency of PSII photochemistry, $n=9$.

In Scots pine seedlings there was no difference in ϕPSII across treatments ($p<0.05$), however F_v/F_m was significantly different ($p<0.001$) (Table 4-4). VCZ biochar treated seedlings had elevated F_v/F_m compared to CON, SPH and TSP treatments in Year 2 ($p<0.001$) and Year 3 ($p<0.001$), and these seedlings could be considered as having a reduced stress response. VCZ biochar also exceeded the CON in Year 1 ($p=0.022$). Other biochar types (SWP and SWP+P) have significant ($p<0.05$) modest F_v/F_m improvement over the CON (SWP+P, Year 3) and TSP (SWP+P, Year 2 & Year 3 and SWP Year 3) treatments. F_v/F_m variation within treatments was low in VCZ biochar, compared to the CON (all years) and TSP, SWP+P and TSP (Year 1). This suggests a consistent effect on seedling stress response in VCZ treated trees and variable seedling stress response in the other treatments.

Scots pine and Sitka spruce exhibit similar trends in F_v/F_m change across, suggesting similar response mechanisms across species. The lower error in Scots pine increases confidence in the stress response trend following inorganic mineral derived P fertiliser application, where F_v/F_m declines during year 2 (showing increased seedling stress response mechanisms), before an improvement in Year 3. This is likely a combination of water and nutrient related abiotic stress factors.

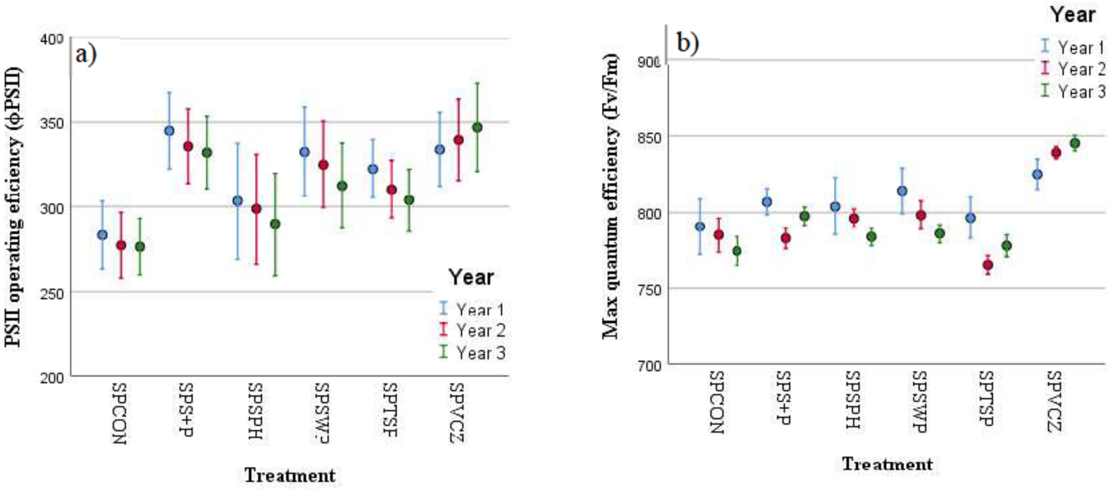

Figure 4-10 – Scots pine needle chlorophyll fluorescence measurements for experiment period. Years 1, 2 and 3 are displayed in blue, red and green respectively. Graphs show a) ϕPSII - PSII operating efficiency: the quantum efficiency of PSII electron transport in the light and b) F_v/F_m - Maximum quantum efficiency of PSII photochemistry, $n=9$.

Table 4-4- Yearly chlorophyll fluorescence results showing; max quantum efficiency (Fv/Fm) (*top*) & PSII operating efficiency (ϕPSII) (*bottom*). Mean with standard error and ANOVA Tukey groupings are displayed for each treatment.

	Max quantum efficiency (F_v/F_m)					
	Sitka spruce			Scots pine		
Treatment	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Control (CON)	739.1 (18.1)^{a}	708.3 (13.4)^{a}	686.2 (17.8)^{a}	790.6 (9.3)^{a}	784.8 (5.7)^{b}	774.3 (4.7)^{a}
Surface phosphate (SPH)	743.4 (12.2)^{ab}	734.3 (6.9)^{ab}	717.9 (6.8)^{ab}	803.9 (9.4)^{ab}	796.2 (3.1)^{b}	783.6 (3.0)^{ab}
Triple superphosphate (TSP)	746.0 (11.0)^{ab}	708.3 (8.1)^{a}	726.2 (8.6)^{ab}	796.5 (6.9)^{ab}	765.2 (3.0)^{a}	777.7 (3.6)^{a}
Biochar (SWP)	776.8 (9.2)^{ab}	755.9 (7.6)^{b}	742.9 (7.4)^{b}	814.1 (7.5)^{ab}	798.3 (4.7)^{b}	785.8 (3.1)^{ab}
Biochar (VCZ)	758.3 (11.0)^{ab}	800.0 (6.1)^{c}	814.4 (5.5)^{c}	824.8 (5.0)^{b}	838.9 (2.0)^{c}	845.2 (2.5)^{c}
Biochar (SWP+P)	784.7 (13.6)^{b}	736.5 (8.9)^{ab}	756.2 (8.7)^{b}	807.0 (4.2)ab	782.7 (3.4)^{b}	797.7 (3.0)^{b}
F	0.019	15.010	18.842	2.829	41.501	60.019
p	2.939	<0.001	<0.001	0.022	<0.001	<0.001

	PSII operating efficiency (ϕPSII)					
	Sitka spruce			Scots pine		
Treatment	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Control (CON)	239.6 (12.4)^{ab}	229.4 (10.1)^{a}	213.1 (9.8)^{a}	412.9 (65.4)	410.7 (66.9)	405.8 (68.3)
Surface phosphate (SPH)	256.1 (16.9)^{abc}	253.6 (16.7)^{ab}	245.2 (16.1)^{ab}	303.3 (17.2)	298.7 (16.2)	289.7 (15.1)
Triple superphosphate (TSP)	236.7 (20.4)^{a}	224.6 (19.2)^{a}	218.9 (18.3)^{a}	322.5 (8.7)	310.4 (8.6)	303.7 (9.2)
Biochar (SWP)	316.6 (26.5)^{bc}	309.9 (25.2)^{b}	297.0 (24.6)^{bc}	332.5 (13.2)	325.0 (12.9)	312.4 (12.6)
Biochar (VCZ)	312.2 (14.8)^{abc}	316.7 (13.5)^{b}	328.2 (12.5)^{c}	333.9 (10.7)	339.7 (12.0)	347.0 (12.9)
Biochar (SWP+P)	331.1 (20.6)^{c}	309.1 (18.0)^{b}	297.4 (17.8)^{bc}	344.9 (11.2)	335.8 (10.9)	332.1 (15.1)
F	4.926	5.828	7.587	1.675	1.777	1.914
p	0.001	<0.001	<0.001	0.153	0.130	0.104

Superscripted lowercase letters indicate significant differences ($p<0.05$) and groups from ANOVA with post-hoc Tukey test.

Values in parenthesis are standard errors of mean, *n=9*.

4.5 Discussion

4.5.1 Seedling Response

The growth response of the tested species showed clear differences depending on forest fertilisation method. Scots pine, as a species, has a high tolerance to nutrient deficiency and often performs well on soils low in available P and Cu. Seedlings require low levels of these nutrients to achieve adequate growth, and are more readily limited by other environmental factors. The dose of available P applied, depending on treatment, had no impact on growth rates. Foliar analysis results suggest that Scots pine seedlings were not highly deficient in P. Conventional mineral fertiliser had little effect on seedling annual growth rate, suggesting that mineral fertiliser may be ineffective at mitigating seedling nutrient deficiency in the early establishment phase.

The observed increase in seedling mortality is consistent with other experimental work detailed in this thesis and the additional work conducted during this project (Chapter 3 and Appendix 2 (Lei et al., 2022)). The high mortality and low growth rate in Year 1 for all treatments may indicate seedling stress or planting shock, highlighting the seedling sensitivity to environmental factors immediately following planting. Mortality rate declined in all treatments as seedlings became established. During Year 1, seedlings treated with targeted high available P fertilisers (including infused P SWP+P) showed high rates of rapid seedling mortality, occurring in the initial 4 weeks following planting. The highly soluble form of P in the applied fertiliser products is highly mobile and can create localised extreme acidic conditions in the rhizosphere. This can cause root mortality, which in turn may result in seedling death. Overall, seedling mortality showed the same trend across treatments, although mortality was lower in the field experiment, compared to the controlled environment experiment (Chapter 3). The lower mortality rate is likely due to the small volume of growing media used in Chapter 3 concentrating the effects of high available P treatments, coupled with the higher buffering capacity in the mineral horizons of podzols (Shamrikova et al., 2005). Soil pH will stabilise, and new root growth can be observed. High available P concentrations, decreases root proliferation and creates small dense suppressed root systems (Rashid et al., 2022). Fertilisation with conventional P fertilisers provide a short-term nutrient source, which can supply the tree during the establishment phase. The detrimental impact of such fertilisers on root development and mycorrhizal associations may impact future growth, soil nutrient cycling and crop resilience to drought.

4.5.2 Photochemistry and Seedling Stress

Biochar application was shown to improve seedling photochemistry, which may indicate increased drought tolerance, in Sitka spruce,. VCZ biochar application resulted in the most pronounced seedling improvements. The consistent and high annual growth rate and low seedling mortality, indicating minimal seedling stress responses, observed in VCZ treatments contrasts to non-biochar treatments. Seedling growth rate in non-biochar treatments was observer to sharply decline during Year 3, likely in response to increased drought stress. Despite no clear morphological evidence of drought stress in Scots pine, variance in seedling drought stress response across treatments was clearly observed in seedling photosynthetic response as measured by the chlorophyll fluorescence. The structure of VCZ biochar will improve water retention and hydraulic conductivity in the rhizosphere (Edeh & Mašek, 2022; Edeh et al., 2020). This will create localised areas in the soil which will improve water retention, when available water in surrounding bulk soil declines during drought. Seedlings will then be able to access water held within internal spaces of biochar particles (see Chapter 5 for access strategies). Targeted application will ensure that water is preferentially allocated to seedlings, rather than competitive herbaceous plants.

VCZ biochar has been found to improve root development, proliferation and mycorrhizal abundance (described in detail in Chapter 3, Chapter 4 and Appendix 2 ((Lei et al., 2022)). This enhancement of the rhizosphere due to application of specified VCZ biochar may improve the accessibility of water in the bulk soil, by increasing the surface are of roots and mycorrhizal fungi compared to aboveground biomass. Increased root and mycorrhizal surface area can improve water uptake efficiency (Peterson et al., 1999). The water transfer pathway between mycorrhizal fungi and conifer seedlings is strengthened with increased mycorrhizal association and abundance (Plamboeck et al., 2007). Mycorrhizal fungi are also recognised as an important contributor to hydraulic redistribution in a field environment, where moisture is transferred to deficient areas (such as a planting mound) (Warren et al., 2008). This mechanism may however also redistribute higher water concentration held within biochar particles, through mycorrhizal fungi, however this has not yet been established. Biochar also stimulates root hair development, which enhances the efficacy of root water and nutrient uptake around biochar particles (Persson, 1980). The dense structure and low available P content of SWP biochar likely limits the effect on root development compared to VCZ biochar and the high available P content and low pH of SWP+P biochar suppress root and mycorrhizal growth (Chapter 5 and Appendix 2). Whist some studies suggest that high pH can decrease plant water uptake, the liming effect of

VCZ biochar is not sufficient (Tang et al., 1993). These mechanisms increase the resilience of seedlings to drought related abiotic stress factors and beneficially improve the seedling physiological response during each subsequent drought period.

Results suggest that seedling stress response to nutrient deficient P conditions can be limited through the application of available P to soil by some biochar and inorganic mineral derived fertiliser. It is difficult to separate the reduced stress response within Sitka spruce seedlings treated with VCZ and SWP biochar between water and nutrient related abiotic stress factors Seedling growth and foliar nutrition was expected to improve from TSP and RPH application to the low P soil of the study site, compared to the CON. The was not observed in the results of this study, likely due to the impact of these fertilisers on rhizosphere development and nutrient transfer. Inorganic mineral derived P fertiliser has been found to supress root development (with a root avoidance response) and cause mycorrhizal decline (Chapter 3 and Appendix 2 (Lei et al., 2022)) (Treseder, 2004). This limits the ability of seedlings to access available P in the soil and amended fertiliser. Results suggest that both surface and targeted inorganic mineral derived P fertiliser application has little effect on seedling establishment success or stress mitigation and can therefore be considered as an ineffective forest management intervention on comparable sites. The direct available P delivery from biochar particles, increase in soil available nutrients from improved nutrient cycling (from increased mineralisation of organic nutrients and desorption of inorganic nutrients), coupled with increased nutrient accessibility from enhanced root and mycorrhizal development may contribute to a reduction in nutrient related abiotic stress factors. VCZ biochar application resulted in comparable growth, survival and F_v/F_m between both tested species, supporting and expanding on findings by Lyu et al. (2016). This suggests that drought and nutrient stress may have been limited in Sitka spruce seedlings, resulting in establishment success equal to Scots pine, therefore increases the projected suitability of Sitka spruce. The decrease in mortality in Sitka spruce to the same rate as Scots pine, with VCZ treatment, further confirms the potential improvement in suitability.

The phenological differences between species and within Scots pine seedlings (where seed orchard planting stock was used) may have contributed to the observed differences between species. Phenological differences were not assessed in this study and can influenced seedling survival and growth in early establishment (Nielsen & Roulund, 1996). Site heterogeneity, in terms of soil properties, topography and exposure can cause phonological differences between seedlings. Early bud burst and active growth may increase seedling susceptibility to frost damage and increase the severity of spring drought stress (Haase, 2011). This may have contributed been a factor in the high year 1 mortality. The effect of biochar on seedling phenology was not assessed but may influence the observed growth and photochemistry differences between treatments. Further research is required to investigate the effect of biochar on seedling phenology on nutrient limited planting sites upland establishment sites.

The seedling stress response to nutrient and drought stress factors cannot be seen as independent. The synergistic negative impact of abiotic stress factors on seedling growth, survival and seedling health is greater than the sum of individual stress responses. When considering the viability of soil nutrient stress mitigation interventions, such as fertiliser application, the indirect and direct impact to seedling sensitivity to other stress factors must also be assessed to determine overall efficacy on seedling establishment success. The multiple direct and indirect effects of biochar on the rhizosphere and wider soil environment (Schmidt et al., 2021) may contribute to the mitigation of water and nutrient stress factors, which in turn may limit the required seedling stress response and improve establishment success. The seedling response to VCZ treatments (which gradually increases growth rate and reduces seedling stress response over the early establishment period) is likely to stabilise over time, resulting in a stable high growth rate. Conversely, CON treatments showed a negative trend (indicating an increase to seedling stress response). This will likely stabilise at a low growth rate, however stress factors may cause a collapse in growth rate and nutrient induced check. SWP+P and TSP treatments are expected to become less resilient to water and nutrient stress over time, due to rhizosphere function and development suppression, limiting the ability of seedlings to uptake water and nutrients. Specified VCZ biochar has been shown to viably improve early-growth in the establishment phase and seedling resilience to abiotic stress on freely drained nutrient limited conifer planting sites. As water related stress becomes more acute and frequent due to climate change, and as soil nutrient pools decline, specified VCZ biochar may become an important tool in maintaining establishment productivity and improving diverse species suitability.

4.5.3 Experimental Limitations

Chlorophyll fluorescence is used to assess photosynthetic efficiency caused by not only abiotic and biotic stress factors but also by natural heterogeneity in several physiological processes which influence the response of seedlings to environmental factors. High natural heterogeneity is expected. This can be seen in PSII operating efficiency (ϕPSII) results, which has high intra-treatment variability. Stress responses assessed through PSII chlorophyll fluorescence may not identify stress in another part of the seedling (e.g., below-ground), which will not influence seedling photosystems until later staged of stress response or unless stress factors cause acute damage.

Whilst the results of this field experiment show a clear trend that VCZ biochar out-performs conventional management interventions (supported by Chapter 3), care must be taken when extrapolating results to other sites. Only one field experiment site was used due to resource considerations. Response mechanisms to biochar is highly dependent on soil type. Results should only be extrapolated to similar freely drained nutrient poor soils and species. Non-woody biochar was also not tested and broadleaf species were not included. Surface biochar application was also not included, and would likely have reduced effect, as there would be less interaction with the rhizosphere. Data was collected for the first four years following planting, growth rate improvements should not be assumed to continue as seedling move towards canopy closure. It is expected that growth rates will homogenise across treatments as seedlings mature to saplings.

4.5.4 Biochar and Forest Resilience to Climate Change

The sensitivity of seedlings to drought stress is exacerbated if other stress factors, such a nutrient deficiency, are also applied. It is therefore critical that both soil water and nutrient accessibility and availability are considered synergistically when implementing measures to adapt forests to climate change. Biochar may alleviate drought stress and ameliorate soil nutrient deficiency during early establishment. Seedlings treated with VCZ were shown to have limited photochemical response to drought stress. Biochar can improve soil water holding capacity and plant available water (Edeh et al., 2020). The physical properties of VCZ biochar may locally improve soil water properties in the rhizosphere. The improvement to soil nutrient availability from biochar addition can also alleviate contributary nutrient related stress factors, allowing effective seedling resource allocation to drought responses, such as osmotic adjustment and regulation (Fang & Xiong, 2015). The improvement to root traits following biochar application (Xiang et al., 2017) could contribute to further root development as a response to increased drought frequency and severity sue to climate change. Biochar may enhance the drought avoidance, drought tolerance, drought escape, and drought recovery responses of seedlings, however there is limited evidence of the direct impact of biochar on seedling drought response. Further research into the potential integration of biochar with other measure to adapt forests to climate change should be prioritised.

Seedling photosynthetic response as measured by chlorophyll fluorescence, suggests that biochar can effectively improve seedling health by ameliorating soil related abiotic stresses. As previously discussed, there is synergy between combined stress factors and seedling resilience. An increase in physiological robustness due to biochar application may improve seedling response to biotic stress factors, which are at an increasing risk to U.K. forests due to expanding population ranges from climate change. Research has also found that biochar can increase systemic resistance to plant pathogens, including *Phytophthora* spp. (Zwart & Kim, 2012). Zwart and Kim (2012) suggest that this may be due to an improvement to tree health, which indirectly increases resistance to the pathogen. This support the potential use of biochar as a climate change mitigation measure by improving seedling health and therefore resilience to climate change related stress factors.

4.5.5 Operational Use of Biochar in Forest Management

This field trial was designed to be consistent with existing forest management practices. Operational and site constraints on restock establishment sites prevent viable blanket application of fertiliser or alternative soil amelioration amendments, such as biochar in this study, which also risk certain negative environmental impacts (such as diffuse pollution). There are operational and environmental concerns with high dose blanket surface applied biochar onto cultivated bare ground risks entering water courses, which could contribute to diffuse pollution. Surface applied biochar onto bare ground also risks redistribution by wind on exposed sites. Low dose ha^{-1} rate, applied directly to roots during planting ensure that nutrients are preferentially allocated to seedlings rather than competitive herbaceous plants. This application method can also provide adequate biochar doses in soil exploitable by seedlings during the early establishment phase, without creating a significant operation burden (applying 62.5 kg ha^{-1} and 25 g seedling^{-1}, which is equivalent to a blanket application of 18 t when corrected for equivalent soil volume). No effect is expected once trees are established, and soil nutrient turnover/availability increases towards canopy closure. Biochar can rapidly be adopted by the forest industry as an effective management intervention during establishment using current practices and planting methods. Biochar supply and cost is likely a barrier to industry adoption. Inclusion of biochar in U.K. woodland creation or restock grant schemes, or addition to Forest Research/Scottish Forestry establishment guidance, would accelerate the acceptance of biochar by landowners and managers.

4.6 Conclusion

Seedlings established on nutrient poor soils are sensitive to environmental stress factors. Targeted specified biochar application has been found to increase seedling growth and survival in the early establishment phase, in turn ameliorating nutrient deficiency and the resultant seedling stress response. Specified biochar may also improve seedling resilience to drought stress, by enhancing the ability of seedlings to uptake water and improving local soil water properties. Biochar, when correctly used, may have the potential to help mitigate declining forest productivity caused by climate change and soil nutrient loss. The efficacy of biochar types are highly dependent on physical and chemical properties. Operational constraints can be overcome by considered application systems and biochar should be considered as a suitable technology for use in establishment by policy makers.

Inorganic mineral derived P fertiliser can locally mitigate temporary nutrient availability. However, negative impacts on rhizosphere development can reduce seedling resilience to drought stress and reduced rhizosphere area can limit nutrient accessibility. Silvicultural practices and biofertilisers, including biochar, which holistically improve root development and the wider soil environment should be considered over conventional forest fertilisation.

4.7 Contributions

Forest Research’s Technical Services Unit set-up the experimental blocks and plots, and work was coordinated by Alistair MacLeod. Dr Mike Perks, Dr Saran Sohi and Gerrard English kindly assisted with experiment mensuration. Kenny Hay and Forest and Land Scotland (formerly Forest Enterprise Scotland) managed the planting programme and experimental site inspections. Dr Gail Jackson kindly provided use of a MINI-PAM II Fluorometer for part of the experiment. Chemical analysis was completed by John Mormon, University of Edinburgh and Francois Bochereau, Forest Research. Prof. Ondrej Masek produced the biochar at the

UKBCE Stage III facility. This experiment was made possible by the support of Mike Perks and Kenny Hay, and their organisations, Forest Research and Forest and Land Scotland. Seedlings were provided by Forest and Land Scotland Newton Nursery.

Chapter 5

Rhizosphere and charosphere interaction: The influence of biochar properties on root and ectomycorrhizal colonisation and nutrient uptake strategies

5.1 Abstract

Ectomycorrhizal fungi form a critical nutrient pathway for host trees to access soil nutrients and increase the cycling of nutrients from organic pools. Whilst biochar has been found to improve arbuscular mycorrhizal abundance, through hyphae nutrient foraging and proliferation in nutrient replete soil patches, the interaction between biochar and ectomycorrhizal (ECM) fungi has received little research attention. This study investigates the nutrient foraging strategies of ECM fungi and host plants depending on biochar structure. Optical, fluorescence and SEM microscopy was used to quantify ECM fungi and qualify the effects of biochar structure on hyphae distribution and the morphology of the ectomycorrhizal extramatrical mycelium (EEM) when interacting with biochar particles. This study provided insights into the accessibility of biochar nutrients to the host plant and explore the potential impacts of increased ECM hyphae distribution on wider soil nutrient cycling in the context of nutrient transfer pathways. Specified vascular cambial zone derived (VCZ) biochar increased ECM abundance in surrounding growing media, compared to softwood pellet biochars, indicating hyphal nutrient foraging for P and subsequent EEM proliferation. High available P concentration treatments (inorganic mineral derived P fertiliser and P infused biochar) profoundly reduced ECM abundance, show a clear avoidance and suppression effect. Biochar physical microstructure was found to be deterministic to EEM morphology and hyphal colonisation of biochar particles. Pelletised feedstock restricted internal hyphal growth to fissures between pellet aggregates, limiting nutrient accessibility. Surface growth followed depressions and fissures in the surface forming undifferentiated strands. High available P was also shown to reduce ECM abundance. ECM fungi were able to utilise the cellular structure of unprocessed wood derived biochar to colonise internal spaces entering biochar through large diameter axial tracheids and resin canals to exploit nutrient replete areas within biochar particles. Hyphae were found to proliferate throughout the internal structure of unprocessed biochar, however surface entry points were limited to pores <20µm in diameter. The EEM formed a dense

labyrinthine morphology on unprocessed biochar until surface enter points were found. Results show that biochar structure is critical to the exploitation of internal nutrients by ECM fungi. The interaction between the rhizosphere and charosphere determines the nutrient accessibility to the host tree, which influences the efficacy of biochar for forest nutrition.

5.2 Introduction

Rhizosphere nutrient pathways are dependent on root structure and mycorrhizal associations. The morphology, colonisation and total surface area of roots and mycelium will contribute to the acquisition efficacy of the rhizosphere to uptake soil nutrients and water. The development of the rhizosphere in conifer seedlings is highly dependent on biotic and abiotic factors, including soil type, nutrient addition and ecosystem community composition (Fogel, 1983; Lei et al., 2022; Rasheed et al., 2017). The symbiosis of roots and mycorrhizal fungi is essential in soil nutrient cycling, which can increase the available nutrient pools (Li et al., 2021). The extent and morphology of mycorrhizal associations in plants under nutrient or water stress has been found to directly alter the nutrient and water acquisition capacity of these plants, and in turn are determinantal to the stress response (Yang et al., 2022).

Mycorrhizal fungi, including arbuscular mycorrhizal (AM), ectomycorrhizal (ECM) and ericoid mycorrhizal fungi, are widely recognised as having vital roles in terrestrial ecosystems (Wurzburger et al., 2017). Mycorrhizal fungi alter soil nutrient cycling and can create a nutrient transfer pathway from soil to plant (Phillips et al., 2013). In a forest context ectomycorrhizal symbiosis provides a route for soil phosphorus (P) to enter tree roots, and the interaction of ECM fungi with host plant and soil is an integral part of soil nutrient cycles. Many forest trees in the U.K., including commonly planted conifer species, readily form mutualistic ECM symbiosis with basidiomycete and ascomycete fungi, playing a vital role in forest establishment (Baird & Pope, 2022). ECM fungi can colonise the fine roots of trees, forming a nutrient, water and carbon transfer interface through the mantel layer from which the Hartig net extends between epidermal and cortical root cells to enable transfer between the ECM fungi and the host tree (Agerer, 2006). Extraradical hyphae grow from the mantle layer and form a ectomycorrhizal extramatrical mycelium (EEM) which forages for nutrients in the surrounding soil (Genney et al., 2006). The EEM produces a large surface which can uptake soil nutrients (particularly N and P) and water which can then be allocated to the host tree. Hyphae, which form part of the EEM, can proliferate in nutrient replete areas or colonise other fine roots, forming new mantle layers which strengthen the nutrient transfer pathway between soil, ECM fungi and tree .

The importance of ECM fungi in soil N cycling and tree N acquisition has been widely studied. ECM fungi have been found to increase the soil NH_4^+ pool through increased mineralisation. ECM colonisation of fine roots and EEM development can improve the accessibility (a combination of uptake efficiency and rhizosphere size) of the NH_4^+. In P limited soils, ECM have also been shown to create a P uptake pathway. Organic P mineralisation is increased by ECM activity, enhancing the soil available P pool (Dong et al., 2018). ECM fungi have been consistently found to improve tree P acquisition by increasing soil available P and the accessibility of soil P pools (Lambers et al., 2010). Mycorrhizal associations have been found to strengthen when the host plant is deficient in P, and mycorrhizal development may be an indirect stress response to deficient soil P. There is also considerable evidence to suggest that mycorrhizal fungi can increase drought tolerance of host plants (Augé, 2004; Gehring et al., 2017; Kipfer et al., 2012). Improved water uptake by ECM can mitigate drought stress factors through increased root hydraulic conductivity and improved contact with soil particles from hyphal binding which enables water to be extracted (Gosling et al., 2006). In addition, water stress accentuates the role of mycorrhizal fungi in P acquisition in nutrient limited soils, showing a synergistic response to multiple abiotic stresses (Püschel et al., 2021). Soil management interventions should consider the impact on ECM fungi, which are vital to the resilience of host plants to abiotic stress factors.

Inorganic mineral derived N and P fertilisers and high nutrient additions to soil have been shown to decrease rhizosphere development and mycorrhizal abundance through suppression and avoidance effects (Gosling et al., 2006; Wallander & Nylund, 1992). This may limit the benefits of mycorrhizal fungi on nutrient cycling and transfer, reducing tree nutrient uptake and water accessibility. Biochar, a carbon rich product of biomass pyrolysis, provides nutrient addition to the soil whilst also enhancing the rhizosphere, including mycorrhizal fungi (Prendergast-Miller et al., 2014). Mycorrhizal fungi have been found to use biochar as a physical growth matrix and directly uptake nutrients held within biochar particles (Hammer et al., 2014). When plants are treated with both biochar and mycorrhizal fungi plant nutrition and yield increases, compared to biochar and mycorrhizal fungi only (Hammer et al., 2015). Most research of the interaction between biochar and mycorrhizal fungi has focussed on AM and there has been little direct research of ECM interaction with biochar particles. AM and ECM fungi employ different nutrient acquisition strategies, which make extrapolation of AM-biochar interaction nutrient transfer studies challenging (Phillips et al., 2011). AM fungi scavenge nutrients, including P, which are released by saprotrophic microbes. ECM fungi can mineralise nutrients held within organic pools and can access nutrients which are not available to plant roots, therefore the ECM response to biochar critical for nutrient accessibility and availability (Dong et al., 2018).

Biochar has been found to improve seedling growth in nutrient poor soils and increase rhizosphere development (Lei et al., 2022; Robertson et al., 2012), however nutrient transfer pathways are poorly understood and ECM interaction with the charosphere across a range of biochar structures has not been assessed. The complex microstructure of biochar particles is highly variable according to feedstock, processing and pyrolysis temperature (Hassan et al., 2020; Shafie et al., 2012). Biochar available P is generally held physically in biochar internal spaces, and the cellular structure can limit available P egress (Ippolito et al., 2015). Understanding rhizosphere interaction with the charosphere and internal biochar spaces is key to assessing the accessibility of available nutrients held and physically immobilised inside biochar particles. This is likely a principal factor in the efficacy on certain biochars in ameliorating soil nutrient deficiency (Joseph et al., 2018). Understanding ECM interaction with biochar particles is also required to investigate the accessibility of water held within biochar particles. Low hydraulic conductivity in some biochar structures make direct water exploitation by roots ineffective (Edeh et al., 2020). ECM hyphae may act as a water uptake pathway, by binding to internal structures and extracting water from micropores.

This study aims to *i*) investigate the fungal abundance in soil adjacent to biochar and fertiliser particles applied to the rhizosphere of Scots pine seedlings and *ii*) observe how biochar structure influences hyphae morphology and distribution in biochar produced from unprocessed and processed wood feedstocks.

5.3 Methods

5.3.1 Sample Collection

Samples were collected from the controlled environment experiment during the end of experiment sampling (detailed in Chapter 3) for analysis using Scanning Electron Microscopy (SEM) and Optical Microscopy (OM). Samples were collected from all end of experiment sample trees which were treated with biochar only (SWP, SWP+P and VCZ treatments) for all species and doses (see Chapter 3). Biochar samples were collected during root extraction and particles with fungi visually present were assessed to improve the probability that visual fungi were ectomycorrhizal. The fungi present on biochar particles was visually traced along the mycelium to seedling roots. Roots were inspected using an optical dissection microscope for the presence of a Hartig net and mycorrhizal associations which was linked through the hyphae to the biochar particle. Where no visual fungi were present and no biochar particles were linked to roots, biochar was randomly sampled from within the root zone (adjacent to seedling roots). Identification of ectomycorrhizal fungi for samples was attempted, however there was low confidence in the accuracy of identification by microscopy in the absence of identification by DNA extraction and the potential presence of nursery species. To reduce sample numbers for analysis by SEM, due to resource constraints, the intermediate biochar treatment dose of 25 g seedling (for SWP, SWP+P and VCZ biochar) was selected for SEM analysis (which was the biochar dose used in Chapter 4). Examples of biochar-root-mycorrhizae interaction were also collected during root sampling. These samples were photographed and retained for further OM/SEM analysis.

Fluorescence Microscopy samples were collected from the experiment detailed in Appendix 2. The treatments were consistent (dose adjusted) with Chapter 3 and Chapter 4, however only Scots pine (*Pinus sylestris* L.) seedlings were used.

5.3.2 Fluorescence Microscopy

Growing media samples were collected from selected sample trees grown as part of the experiment detailed in Appendix 2, during the end of experiment sampling (Lei et al., 2022). Full experimental methods are detailed in Appendix 2. Seedlings were firstly removed from rhizoboxes, including all root biomass. Care was taken to limit disturbance to the growing media. Growing media samples (variable mass weight) were collected from each rhizobox. Two samples were individually collected from each rhizobox Figure 5-1. The two sample areas (SA) were 90° truncated sectors, with a 20 mm straight edge from the boundary of the treatment area (TA). The central axis of sample area 1 (SA1) was orientated at 0° to the TA and the central axis of sample area 2 (SA2) was orientated at 90°. Using a guide plate, all growing media within the sample areas was collected using a micro-spatula. The volume of soil removed was variable depending on treatment (as dose was mass based, the radius of the TA varied across treatments and therefore the SA area varied). Material was passed through a 2 mm aperture soil sieve, to remove any residual root biomass. Growing media samples were dried for 48 hours at 40 °C and ground using a ball grinder to homogenise samples. Based on existing methods for the detection of fungi in tissues using calcofluor white and fluorescence microscopy, (Monheit et al., 1984; Rasconi et al., 2009), all the samples were oven dried for 48 hours, ground (using a ball grinder) and further sieved using a 65µm mesh sieve (this was to remove sand, which would interfere with slides and microscope equipment).

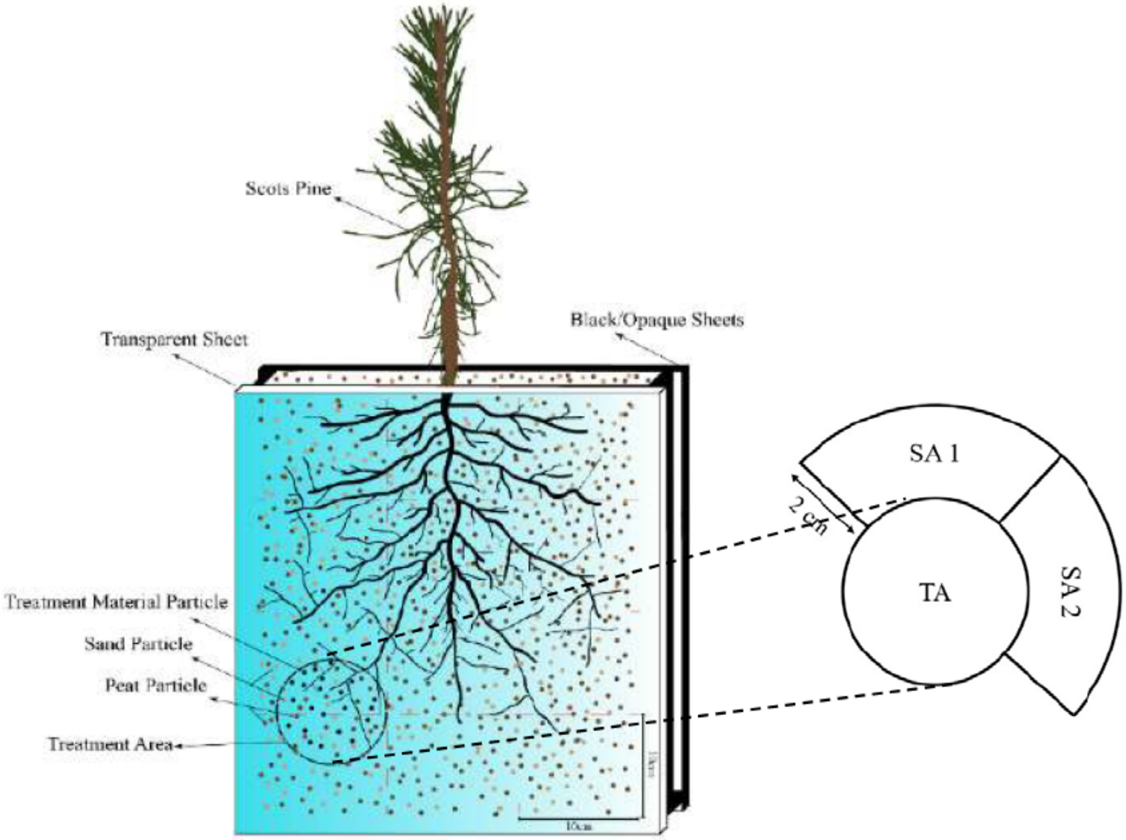

Figure 5-1- Rhizobox design, showing seedling, roots and treatment area (TA). The TA is in the lower left of the rhizobox, and this is the section where treatment/growing media mix was applied. Fluorescence microscopy samples were collected from the adjacent area (2 cm) of the rhizobox. Two samples were collected, independently collecting all material from SA 1 & SA 2. See Appendix 2 for more detail.

Slides were prepared using 5 mg of each growing media sample, which was evenly applied to each sample slide within a 0.25 cm^2 area using a micro-spatula. Two samples were separately prepared on each slide. Calcofluor-white Stain (SIAL, Sigma-Aldrich was applied to the slide, using a Thermo Scientific Finnpipette adjustable pipette at a rate of 0.025 ml slide $^{-1}$. Following application of the stain, 0.025 ml of 10% Potassium Hydroxide solution was added to each slide. Pipette tips were changed between chemical applications. Coverslips were carefully placed over the specimen and slides were left for 1 min prior to examination. Reflective index 1.515 oil was used.

Fluorescence microscopy (FM) was conducted using a Zeiss Axio Imager 1. All samples were analysed under UV light (λEx = 355 nm) at 500 x magnification and reflective shutter exposure was set at 50.0178 ms (decimal places are due automatic rounding). Each slide was imaged using a Zeiss Axiocam. Four images were taken per sample. Images were at each cardinal point, 1 mm from the centre of the sample (coverslip).

5.3.3 Optical Microscopy

Biochar/root/mycorrhizae sample collection is described in Section 5.3.1 and Chapter 3. Samples were kept at 5°C prior to optical microscope (OM) assessment. To maintain root, biochar and mycorrhizal hyphae structure samples were not prepared for high magnification OM. Samples were imaged using a variable magnification stereo microscope. Graph paper (1mm) was used to provide scale. Images were taken using a digital microscope camera. All imaging was completed with 14 days of sample collection. Surface interaction between biochar particles and mycorrhizal and root structures were firstly visually assessed and documented photographically. Percentage cover of mycorrhizal hyphae was recorded for each biochar particle (which was ~1 cm^3), along with the total root attachment (number of roots). Samples were then cut to observe internal structures using single edge backed razor blades. Cylindrical SWP and SWP+P biochar particles were cut along the polar axis in the mid-point of the longitudinal axis. VCZ biochar particles (which has variable shapes) were cut to expose inter radial, longitudinal and tangential wood planes.

5.3.4 Scanning Electron Microscopy

Biochar sample collection is described in Section and Chapter 3. Samples were firstly cut to <1 cm^3, with a max dimension of 10 mm to allow mounting on SEM stubs. As with OM sample preparation, samples were then cut to observe internal structures using single edge backed razor blades. SWP and SWP+P biochar particles (which are cylindrical) were cut along the polar axis in the mid-point of the longitudinal axis. VCZ biochar particles (which has variable shapes) were cut to expose inter radial, longitudinal and tangential wood planes. The observable wood planes were dependent on the feedstock wood structure and not all planes were observable on each sample biochar particle. The samples were then air-dried for 72 hours. Biochar particles were prepared and loaded onto SEM sample stubs using carbon tape, with the imaging surface orientated upwards. Mounted samples were then gold coated (100 nm) and imaged in high vacuum at 2 keV using a Carl Zeiss SIGMA HD VP Field Emission SEM. This method provided best resolution of surface details Three biochar samples were retrieved from the roots of each species (Sitka spruce, Scots pine and Douglas fir) and biochar type (SWP+P, SWP & VCZ) for preparation and analysis. Imaged VCZ biochar contained one sample for each species and at least one imaged surface for the following: outer surface, internal tangential, internal radial and internal longitudinal. SWP+P and SWP surface faces (base and curved face) and internal structure was imaged. Multiple images were taken for each sample. A total of 31 samples were examined, and a total of 228 images were recorded. Energy Dispersive X-Ray Analysis (EDX) was not undertaken in this instant. Softwood derived VCZ biochar particle faces are described in adherence to wood description convention (transverse face, tangential face and radial face).

This work was completed using the Scanning Electron Microscope (SEM) facility at the School of GeoSciences, University of Edinburgh.

5.3.5 Statistical Analysis

Fluorescence microscopy images were analysed using an adapted established methodology utilising ImageJ software (Bankhead, 2014; Hartig, 2013). Fluorescence intensity was used to compare fungal hyphae abundance between treatments. The fluorescence intensity reflects the total mycelium mass in each sample. Fluorescence intensity is recorded in arbitrary units (a.u.), suited only in comparisons of images acquired using consistent fluorescence microscope set-up.

The underlying design and sample preparation damaged hyphal structures but maintained total mycorrhizal mass (which was assessed using fluorescence intensity). Macro-data for pixel fluorescence intensity was created for each sample by creating a mean pixel fluorescence intensity distribution from the four images produced for each sample. Analysis provides a fluorescence intensity result for each pixel, from which the distribution curve is created. The mean fluorescence intensity curve for each treatment (including 95% C.I.) were created distribution curves. Intensity >4000 a.u. was recorded to indicate the presence fungi fluorescence. Analysis was conducted using the number of pixels for each sample fluorescence exceeded this threshold. The data were tested for normality, and compared using one-way Analysis of Variance (ANOVA) with post-hoc-Tukey test. Data was normally distributed and there was equal variance across treatments. The use of Tukey's HSD multiple range test protect against type I error.

Optical microscopy data for the particle surface was further tested using one-way ANOVA with post-hoc-Tukey test. Images produced by SEM was not quantitatively analysed and root and mycorrhizal interaction with biochar particles was described qualitatively based on visual observations.

5.4 Results and Discussion

5.4.1 Mycorrhizal Abundance

Fungal structures were clearly observed during fluorescence microscopy within experimental treatments. High intensity fluorescence generally had poor form, partly due to the method of sample preparation (sample areas where homogenised to mitigate variation within each sample slide). Clear ECM structures were observed in VCZ, SWP and CON treatments. In some samples hyphal structure was intact with a visible network of prosenchyma of hyphae visible. Ectomycorrhiza were readily identifiable based on their septate cell structure.

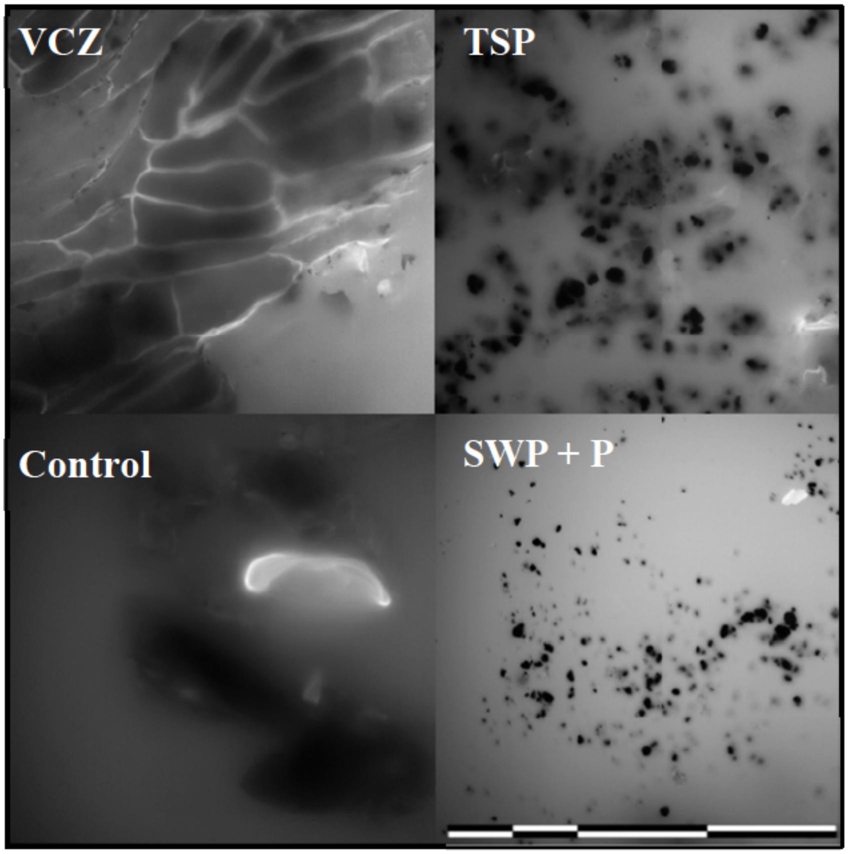

Figure 5-2- Example images produced using fluorescence microscopy for subjectively representative selected experimental treatments applied to Scots pine. No clear fluorescence can be seen in TSP and SWP+P, however soil particles are visible. Some fluorescence can be observed in the control (CON) treatment. An ectomycorrhizal septate strand can be seen in the VCZ treatment. Scale bar is 150 µm.

Mean pixel count, for fluorescence intensity >4000 a.u., differed significantly across treatments (F=211.24, $p<0.001$). There was no difference between SWP and CON treatments (Figure 5-3). VCZ biochar resulted in a significantly higher ($p<0.001$) result than SWP and CON treatments. TSP and SWP+P had no pixels with a fluorescence intensity >4000 a.u. and were therefore excluded. The clear spike in fluorescence intensity >4000 a.u. in the VCZ treatment, suggest that there is a higher abundance of ECM fungi in the proximity of VCZ biochar particles. This could be due to a developed nutrient transfer pathway from biochar to colonised seedling roots through an extraradical hyphal network. The absence of any clear fungal structures, or increased fluorescence due to staining, in SWP+P and TSP suggest that the chemical properties of these treatments suppress growth or stimulate an avoidance response of ECM fungi and other fungi in the soil. This potential fungal response to SWP+P and TSP would be consistent with the root response observed in Appendix 2 and support finding by Treseder (2004) who found that mineral P fertiliser application decreases mycorrhizal abundance.

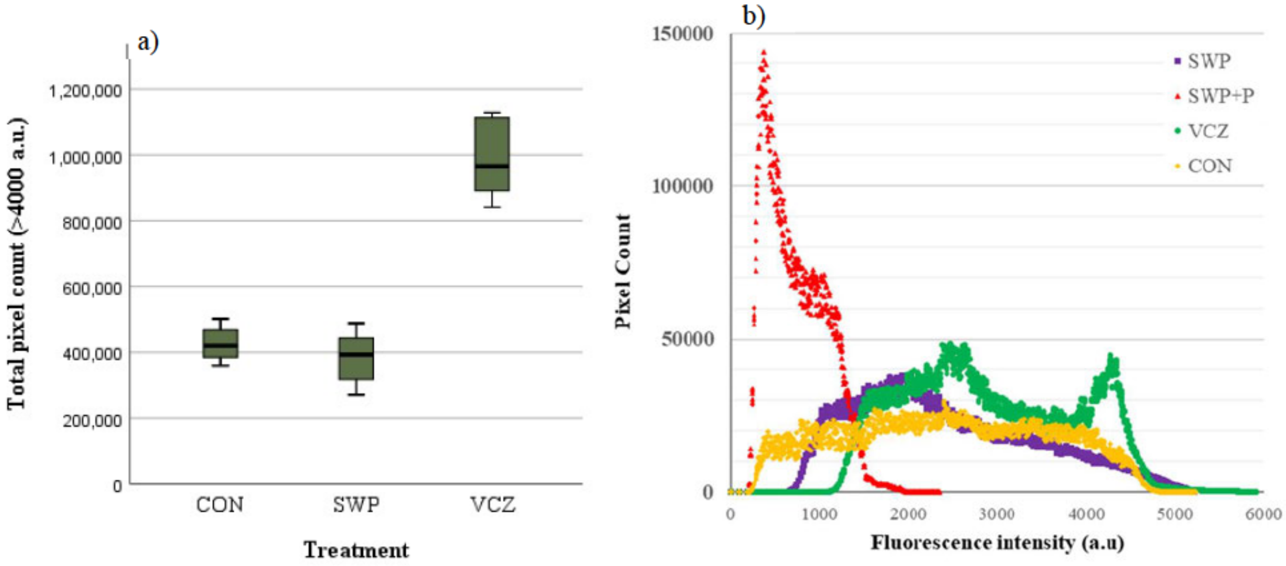

Figure 5-3- Fluorescence microscopy results showing; a) box plot of total mean pixel count >4000 a.u. (arbitrary unit), which indicated fluorescence from fungal cells reacting to the stain, for CON, SWP and VCZ treatments. SWP+P and TSP had no fluorescence recorded >4000 a.u. The TSP treatment had the same distribution as SWP+P and was excluded for data visualisation clarity. *n=12*. b) total pixel fluorescence distribution curves for SWP, VCZ, SWP+P and CON treatments, showing the data for all samples is shown. Clear spike in VCZ biochar at high intensity, suggesting presence of fungal matter. The seedling species tested was Scots pine.

Whilst the fluorescence microscopy image analysis results suggest that VCZ has higher abundance of fungal biomass and therefore mycorrhizal abundance, potential artefacts of the fluorescence method must be considered. Calcofluor-white stain is commonly used to detect fungal biomass. The stain binds to 1-3 β and 1-4 β polysaccharides of chitin and cellulose, including the chitin in the fungal cell wall. In addition to ectomycorrhizal extramatrical mycelium (EEM), calcofluor-white stain will also bind to other fungi in the growing media and some components of residual plant matter. The results can be used to state which treatment exhibited higher fluorescence intensity due to the stain binding to chitin and cellulose. This indicated differences in fungal abundance. Visual observations further indicate higher mycorrhizal abundance on VCZ biochar particles (Section 9.1.3) Fungal abundance was not directly measured, nor was ectomycorrhizal fungi differentiated from other fungi in the growing media. These results should be considered together with other data presented in

Appendix 2, Section 9.1.3 and Section 5.4.2. As previously mentioned, the results depend on consistent microscope configuration, although this did not change in the course of imaging reported here.

5.4.2 Biochar colonisation and nutrient foraging strategies

Biochar root colonisation by conifer seedling roots and ectomycorrhizal fungi was highly dependent of biochar structure and chemical composition. The outer surfaces of biochar particles were typically colonised, with undifferentiated strand hyphae or single filamentous hyphae extending into the internal spaces of biochar. Patterns of colonisation for internal biochar spaces varied according to biochar structure.

External Colonisation

There is distinction in apparent strategies for biochar surface colonisation by roots and ECM fungi, providing insights into the interactions between rhizosphere and charosphere. These strategies of colonisation were *i*) direct fine root growth on and within biochar particles, *ii*) root growth on biochar surface with fungal mantle and extraradical hyphae penetrating the internal spaces of the biochar and *iii*) ectomycorrhizal extramatrical mycelium (EEM) contact with biochar particles (with the absence of direct root contact) and filamentous hyphae penetrating the internal spaces of the biochar. The extent of biochar colonisation within these categories appeared highly dependent on biochar structure and may be influenced by available P concentration.

i) direct colonisation by fine roots

External colonisation of biochar particles was observed by fine roots, extraradical hyphae and the wider EEM. Conifer roots have been shown to actively forage towards biochar particles, through an acquisitive root growth strategy (Appendix 2). Roots in direct contact with biochar particles retrieved in this study have been observed growing and proliferating within biochar macro-structure. Unprocessed wood derived biochar maintains the structure of original feedstock and is prone to splitting with along the grain due to mass loss, volume loss and steam egress. This creates longitudinal fissures, which were directly exploited by the fine roots of seedlings (Figure 5-4).

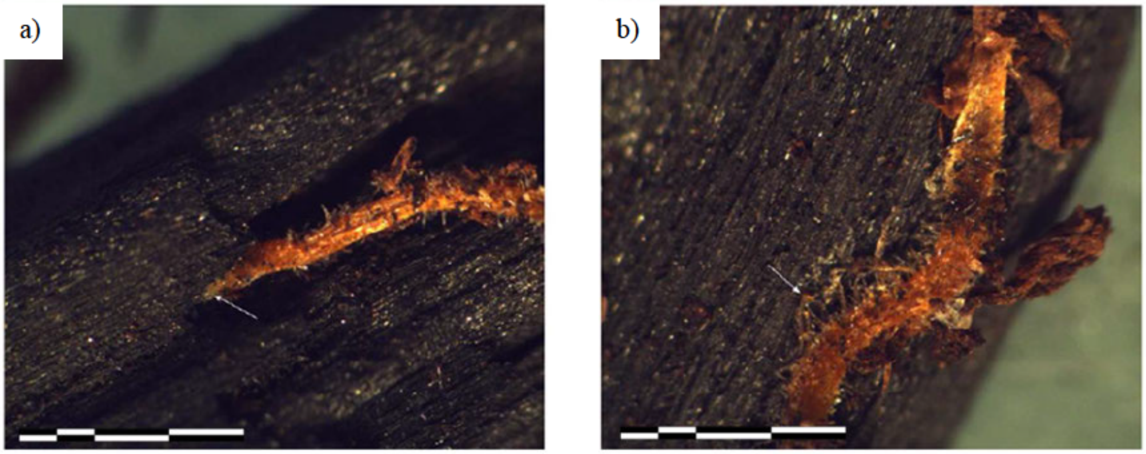

Figure 5-4 – Example of visualised root colonisation for VCZ biochar. a) observation of root penetration into internal macro-structure through longitudinal fissure (white arrow) with no mycorrhizal structures present. b) preferential growth of fine root along VCZ biochar surface (with clear ectomycorrhizal mantle layer and extraradical hyphae), root hairs can be seen entering biochar through micro-fissure. Scale bar is 6 mm.

Wood pellet biochar appeared more difficult for direct colonisation by roots. During the production process of pelletised woody feedstock, wood is re-structured under heat and pressure. Lignin is amorphous and will soften upon heating. This results in the liquefaction of cell walls, allowing restructuring and densification of hemicelluloses and cellulose upon pressure. The lignin hardens following cooling, creating a reformed wood pellet. During pyrolysis longitudinal fissuring does not occur. Fine roots were observed exploiting surface weaknesses in biochar pellets, between individual restructured pin-chip aggregates (which are the feedstock for pellet production) Figure 5-5. Pellet aggregates have a smooth dense microstructure and rough macro-structure which may make it difficult for root attachment. Lateral growth of roots on the surface of biochar particles was less extensive compared to VCZ biochar. Direct fine root contact and lateral growth along biochar surface is infrequent on SWP+P and SWP particles, compared to VCZ biochar. This may be attributable to the avoidance effect caused by high available mineral derived P concentration in SWP+P biochar (Lei et al., 2022). The low available P concentration of SWP biochar may be insufficient to allow the preferential foraging and proliferation of conifer roots observed around P-replete biochar particles (Hammer et al., 2014). The dense structure of pelletised biochar can physically trap P in the internal structure and may limit P signalling. Where fine roots do enter biochar particles through fissures or voids, secondary and primary growth rapidly split biochar particles.

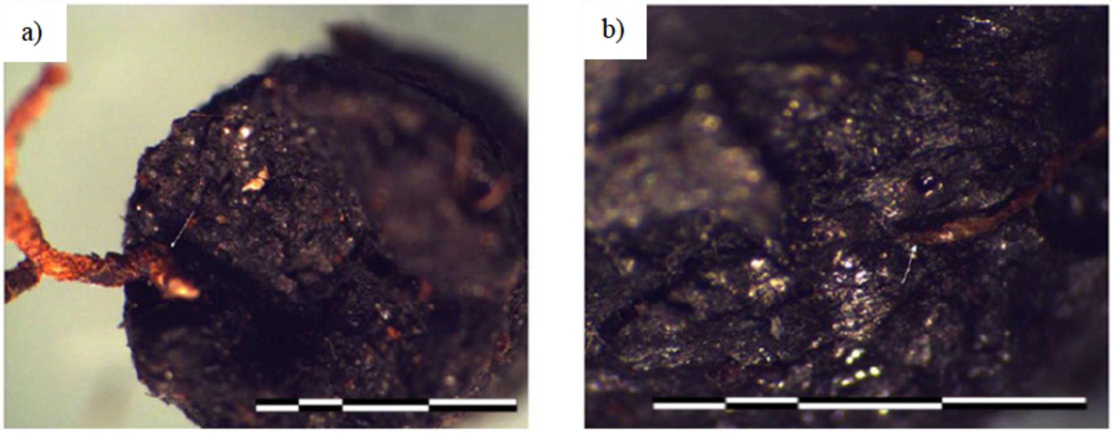

Figure 5-5- Examples of fine root penetration of biochar from pelletised feedstock, with no observable mycorrhizal structures. a) shows fine a root exploiting the weakness in pellet structure between aggregates to access internal spaces within SWP biochar. b) surface exploitation of weakness between aggregates is being exploited by a fine root, allowing lateral growth along SWP+P biochar surface. The root entered the biochar particle once a sufficiently sized void is found. Scale bar 6 mm (a) and 3 mm (b).

ii) root colonisation with mantle layer and EEM

As previously stated, conifer roots have been shown to actively forage for biochar particles (due to the presence of available P) and proliferate fine root growth around biochar surfaces. Fine roots in contact with biochar particles can then be colonised by ECM fungi. A mantle layer can form on the root surface, directly in contact with the biochar surface, anchoring the root to the biochar particle. The subsequent development of ECM extraradical hyphae (Figure 5-6) is highly dependent on biochar structure. In VCZ biochar an extensive EEM forms across the biochar surface, with individual filamentous hyphae entering the biochar where possible. It was observed that fine roots in contact with SWP biochar were frequently colonised by ECM fungi and a mantle layer. Individual filamentous hyphae generally do not form as extensively on and within SWP biochar particles, when compared to VCZ biochar. Hyphae morphology appears to favour undifferentiated strand formation on the surface of SWP biochar. These strands spread across the biochar surface and enter through voids into the external microstructure Figure 5-6. Moderate P biochar has been found to increase root colonisation by ECM fungi, which may increase ECM abundance on the surface of VCZ biochar (Robertson et al., 2012).

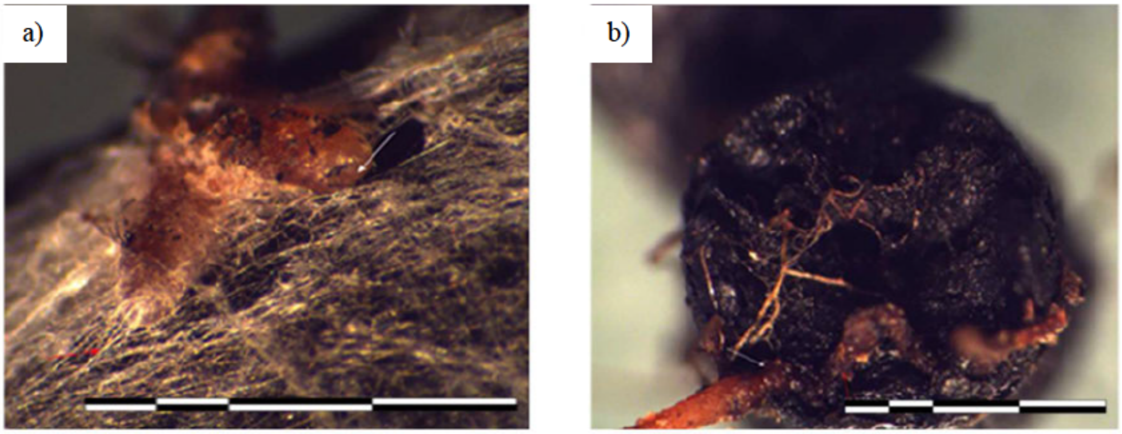

Figure 5-6- Example surface root contact by fine root with fungal mantle layer and extraradical hyphae. a) extensive EEM structure on the surface of VCZ biochar emanating from ECM mantle layer on the root tip. b) fine root with ECM mantle layer, root growth has split SWP particle and undifferentiated strand hyphae are visible. Scale is 3 mm (a) and 6 mm (b).

The variation in EEM morphology and hyphal density is likely a combination of both biochar structure and nutrient content. The observed increase in surface colonisation in VCZ biochar compared to SWP biochar, supports findings by (Hammer et al., 2014) which show that increased biochar nutrient content increases mycorrhizal colonisation of biochar particles. The limited root and ECM colonisation of biochar in SWP+P biochar is likely due to three principal factors: cadmium (Cd) toxicity, low pH and high inorganic mineral derived P. ECM fungi in pine has been shown to develop poorly in highly acidic conditions (Marx & Zak, 1965). The surface pH of SWP+P biochar is sufficient to limit hyphal development until the pH is buffered. The high cadmium content of biochar may contribute to the reduced ECM colonisation. Cd has been found to result in variable hyphal development, although further research is needed to determine the response to cadmium toxicity by EMC fungi (Wang et al., 2020). Getman-Pickering et al. (2021) found that inorganic mineral derived fertiliser can limit mycorrhizal development and the associated benefits to the host plant. ECM root tip colonisation is negatively correlated to available P at root tip (Kluber et al., 2012), which may limit root tip colonisation by ECM fungi near SWP+P biochar surfaces. EEM hyphae distribution clearly exploits biochar surfaces using different strategies according to biochar structure. Hyphae growing on the surface of SWP particles form undifferentiated strands, which are linear aggregations of hyphae composed of only one type of single layer hypha (Ingleby, 1990). These undifferentiated strands follow weaknesses between aggregates of the biochar particle. The EEM forms a labyrinthine distribution of single hyphae, which evenly spreads across the biochar surface (Figure 5-7).

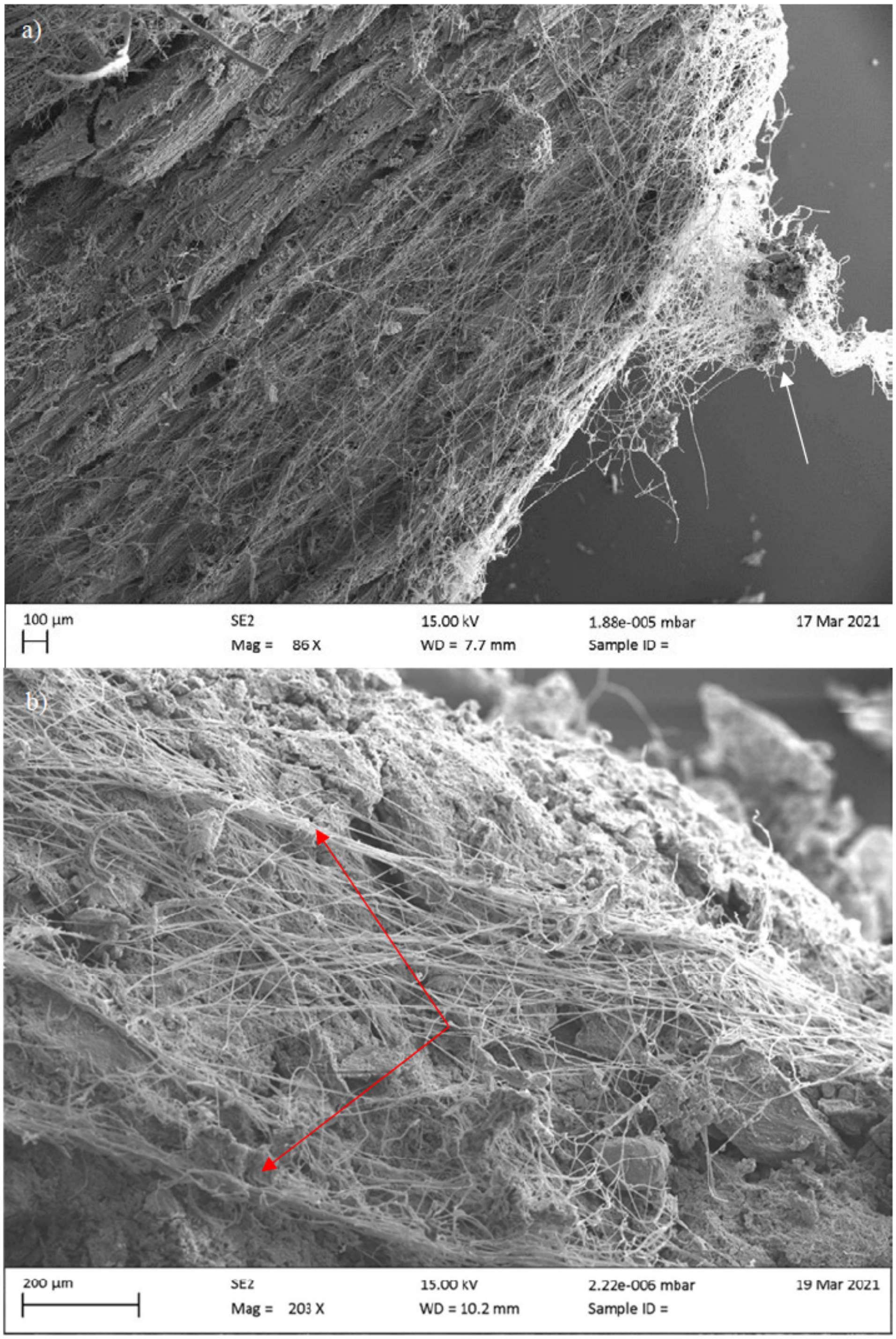

Figure 5-7 – Example of external colonisation of biochar by ECM hyphae. EEM extends across biochar surface as extraradical emanating hypha are produced from the root mantle layer. a) show a VCZ biochar particle colonised by a fine root visibly attaching to biochar (white arrow), the EEM produces a labyrinthine structure of hyphal elements. b) the EEM exhibits a less dense and distinct morphology in SWP biochar, creating undifferentiated hyphal strands (red arrows) from which single filamentous hyphae extend across the biochar surface.

iii) direct colonisation by EEM

Direct colonisation of biochar particles by EEM was observed in both VCZ and SWP biochar. This is where ECM hyphae were found on the surface and within biochar particles without direct contact by fine roots with a mantle layer. Direct colonisation of SWP+P biochar, in the absence of mantle contact, was not observed (likely due to extreme P concentration, Cd toxicity and/or extreme pH, which are described in the preceding pages). The extent of direct colonisation and hyphal development varied between VCZ and SWP biochar. When EEM came into contact with VCZ particles there was dense hyphal proliferation on the biochar surface (Figure 5-8). Early hyphal colonisation was observed to enter biochar internal spaces prior to surface growth. This could be related to nutrient signalling, identifying P-replete regions within biochar particles and stimulating surface growth due to higher nutrient concentration, which creates a strengthened nutrient pathway between mycorrhizal fungi and the host tree (Daguerre et al., 2016; Harrison, 2005).

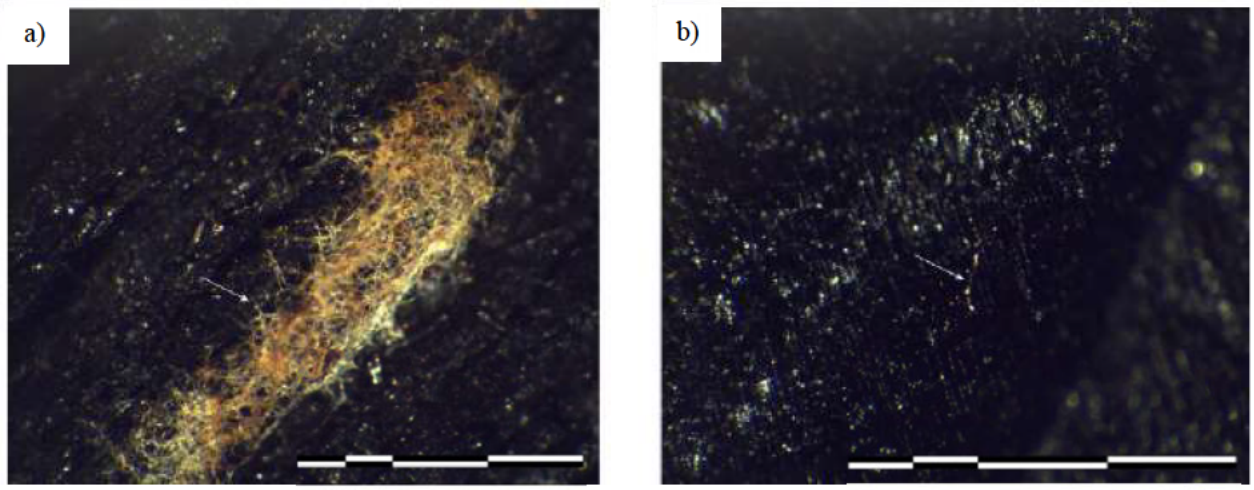

Figure 5-8- Direct EEM colonisation of VCZ particles, showing dense hyphal proliferation on VCZ biochar surface (a) and early colonisation by single filamentous hyphae (b) in the absence of mantle contact with biochar. Scale bars are 6 mm (a) and 3 mm (b).

EEM does not create dense hyphal masses on the biochar surface in pelletised SWP biochar particles. Hyphae appear to attach to weaknesses between pellet aggregates and grow through voids into the internal structure. Hyphae on the biochar surface did not frequently branch and there was no evidence of proliferation, with sparse single hypha on the biochar surface (Figure 5-9). This again highlights the variable ECM response to biochar properties, which results in clear morphological and developmental differences.

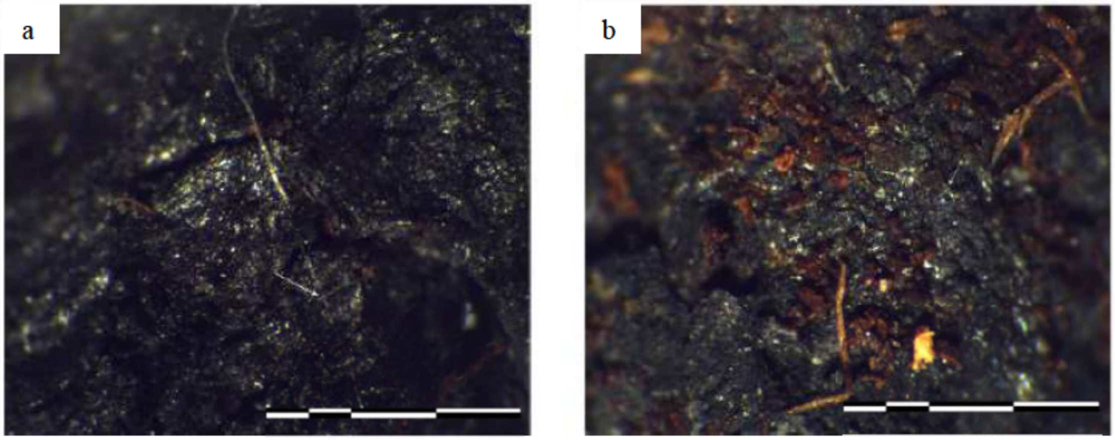

Figure 5-9- Direct EEM colonisation of SWP particles, showing dense hyphal proliferation on VCZ biochar surface (a) and early colonisation by single filamentous hyphae (b) in the absence of mantle contact with biochar. Scale bars are 6 mm.

The SEM study evidence that EEM used different biochar colonisation strategies (in the absence of mantle root contact) (Figure 5-10). The EEM forms a dense labyrinthine mass of hyphae across the biochar surface, with limited strand development. The cellular structure of wood (used as feedstock in VCZ biochar) appears to prevent hyphae entering the internal spaces in radial and tangential orientations (except through sparsely distributed resin canal structures). Maximum pore size in the cell structure on these surfaces is 0.5 µm to 1.5 µm (axial tracheid pits), these pores appear to be unexploitable by most filamentous ECM hyphae. Surface exploration and proliferation decreases when the transverse face is reached, and growth preferentially favours internal exploration. Pelletised biochar surfaces do not form complex hyphal masses, and growth is limited to single filamentous hypha. It appears that once weaknesses in the pellet structure are found by single hyphae, further hyphae develop toward the internal opening, forming undifferentiated strands. ECM fungi seem to require mantle contact to biochar particles to extensively colonise the outer surface of biochar particles. This may be due to the low nutrient content of SWP biochar, limiting proliferation, and the rough structure preventing hyphae extensively spreading across the surface (contrasted with the relatively flat surface structure in VCZ biochar).

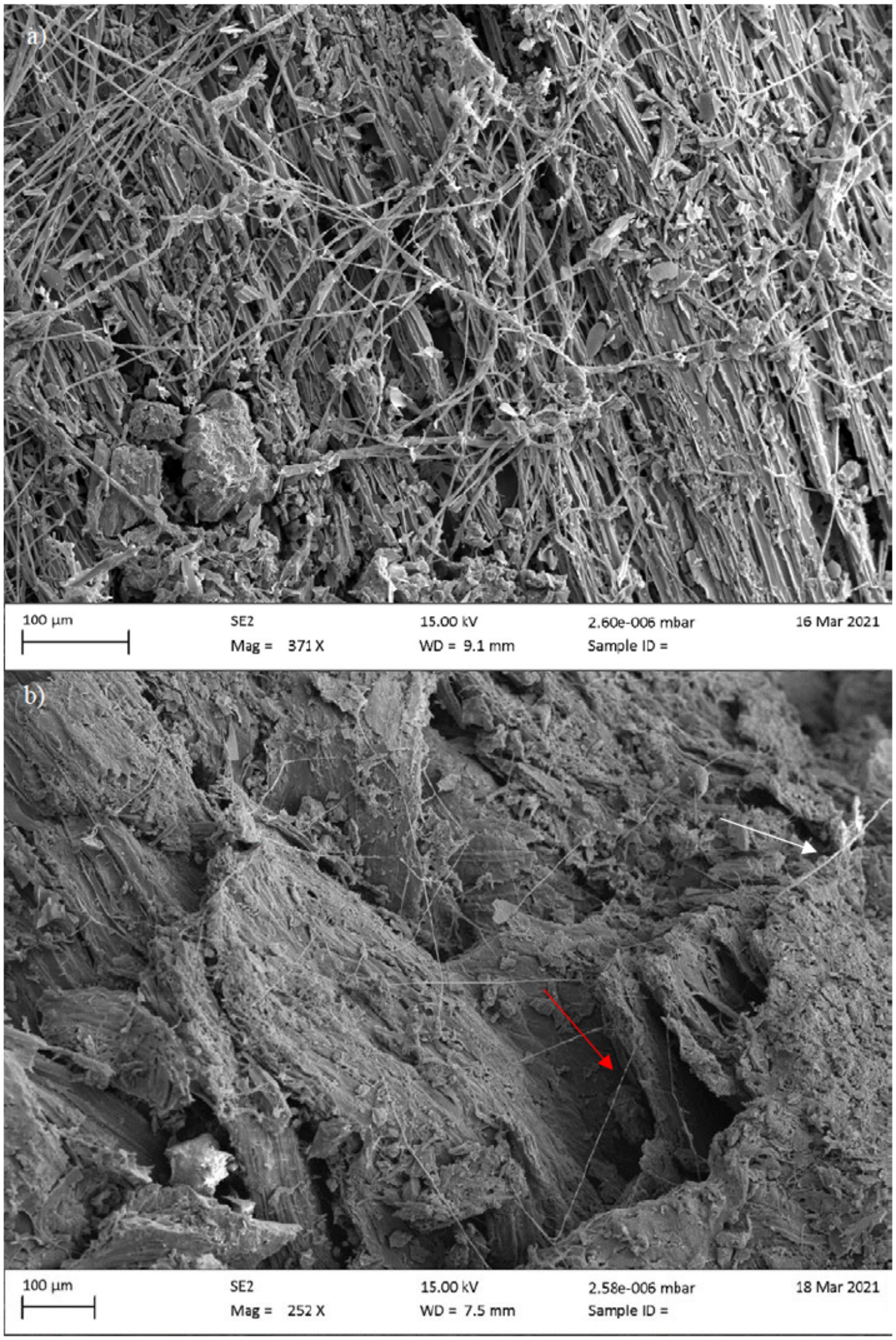

Figure 5-10- Example of surface colonisation of biochar by EEM. a) Dense EEM covers the tangential face (wood orientation) of VCZ biochar particle, hyphae. Axial tracheids are visible, hyphae appear not to enter the internal structure through. b) sparse filamentous hyphae extend across SWP biochar (red arrow), stands form when weaknesses into biochar internal structure are found and hyphae preferentially grow into the internal structure (white arrow).

Internal colonisation

Internal colonisation was highly dependent on cellular structure. No direct colonisation of internal biochar cellular microstructure was observed by fine roots. Some particles were colonised by fine roots exploiting longitudinal fissures (VCZ) or inter-aggregate weakness/voids (SWP+P & SWP). The microstructure of VCZ biochar, which is determined by the anatomy of wood, appeared to be difficult for fine roots to penetrate as no fine roots were observed within the internal cellular structure of biochar. Axial tracheid diameter in VCZ biochar was 20 µm to 40 µm, which were not penetrated by fine roots. These cells have greatest diameter close to the vascular cambium, where new sapwood has thin cell walls. Whilst resin canals in VCZ biochar are of sufficient diameter for some fine roots to access (<100 µm), ash and carbon deposits within the resin canal effectively blocks ingress by roots. For seedlings to access nutrients or water held in internal biochar microstructure, EEM hyphae are required, thus contributing to nutrient accessibility.

Biochar internal colonisation strategies were found to vary according to biochar structure (Figure 5-11). In pelletised biochar the preferential growth of the EEM along surface fissures and weaknesses allows hyphae to "discover" voids in the aggregate matrix which enable hyphae to enter the internal spaces of biochar. Hyphae proliferates into internal spaces of SWP biochar, likely to access nutrients (particularly P) which are held within the biochar particle. The undifferentiated hyphal strands found on SWP biochar surfaces appear to branch into single hyphae once internal spaces have been reached. This may suggest that hyphal strands develop along the path of least resistance on the biochar surface. The morphology of the EEM then changes to favour nutrient uptake in internal spaces (with branching hyphae). The EEM colonises the surface of VCZ biochar expanding in all directions, as a dense labyrinthine hyphal mass, across all surfaces until adequately sized pores in the biochar structure are found to allow hyphal growth into the biochar particle. This usually occurs on the transverse face of biochar (wood orientation), where hyphae can directly exploit larger diameter axial tracheid cells, found in earlywood, and resin canal structures. Infrequent lateral resin canals can occur on other biochar faces. Other wood cellular structures are not exploited by ECM hyphae probably due to insufficient diameter and lack of linear structure (e.g., some tracheid cells and ray cells). Once internal access points are found by hyphae, extensive EEM surface development does not occur, and hyphal growth preferentially favours internal structures. This suggests active nutrient foraging by ECM fungi when biochar particles are colonised. SWP+P internal biochar colonisation is constrained to limited extraradical hyphae around the mantle. The outer bark component of VCZ particles appear to be more difficult for the EEM to exploit due to the lack of vascular structures (Figure 5-12).

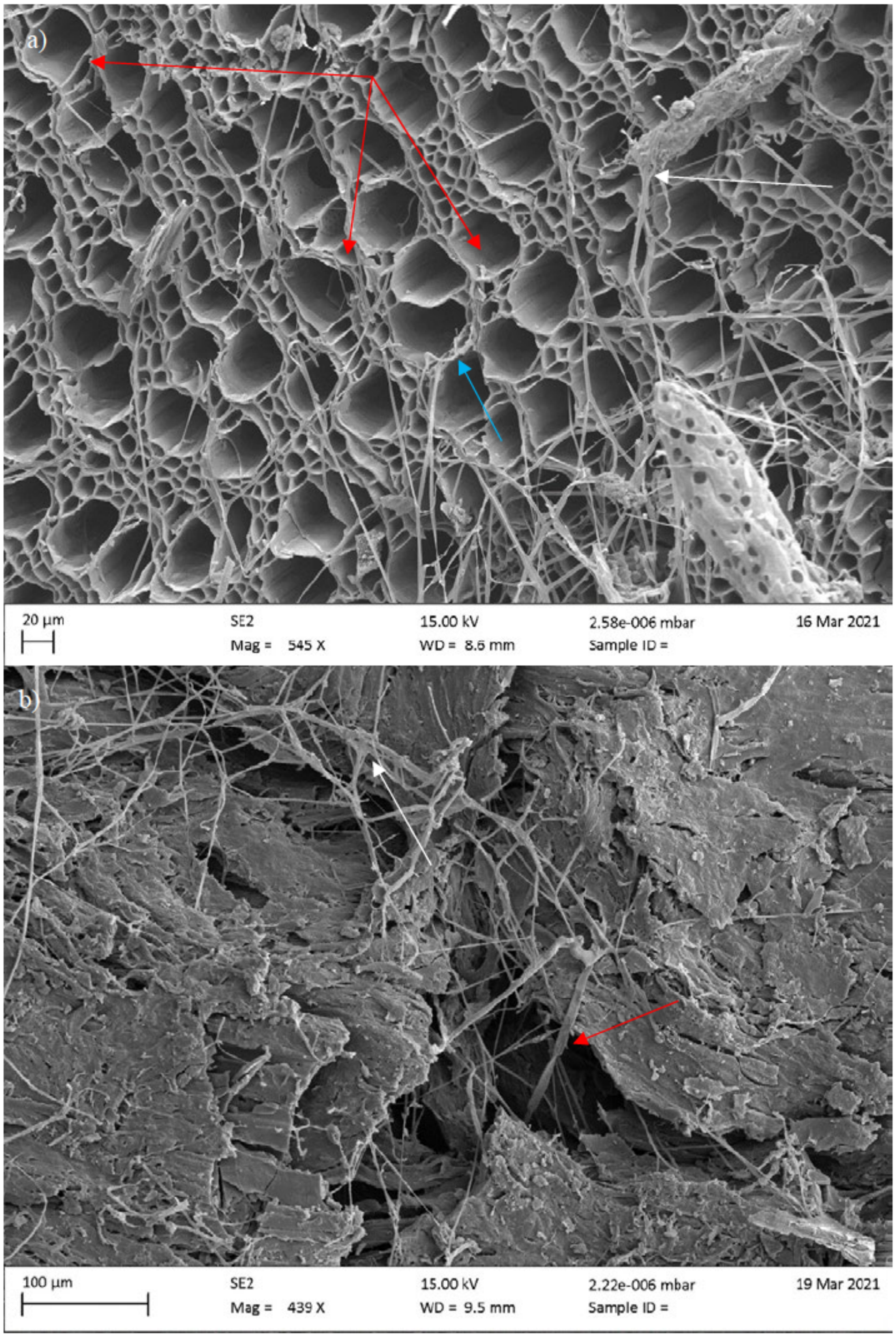

Figure 5-11- EEM development on biochar surfaces with hyphae entering biochar internal structure. a) extraradical hyphae can be seen extending from root mantle layer (white arrow) across the transverse face (wood orientation) of VCZ biochar. Hyphae enter internal biochar structure through large axial tracheids which are found in new sapwood near the vascular cambium (red arrows) and further expand during pyrolysis, tortuous hyphae growth near the opening to axial tracheids suggests preferential growth towards opening (blue arrow). b) Branching undifferentiated strand hyphae following weakness between aggregates in an SWP biochar particle (white arrow). Hyphae enter internal structure through void between aggregates (red arrow).

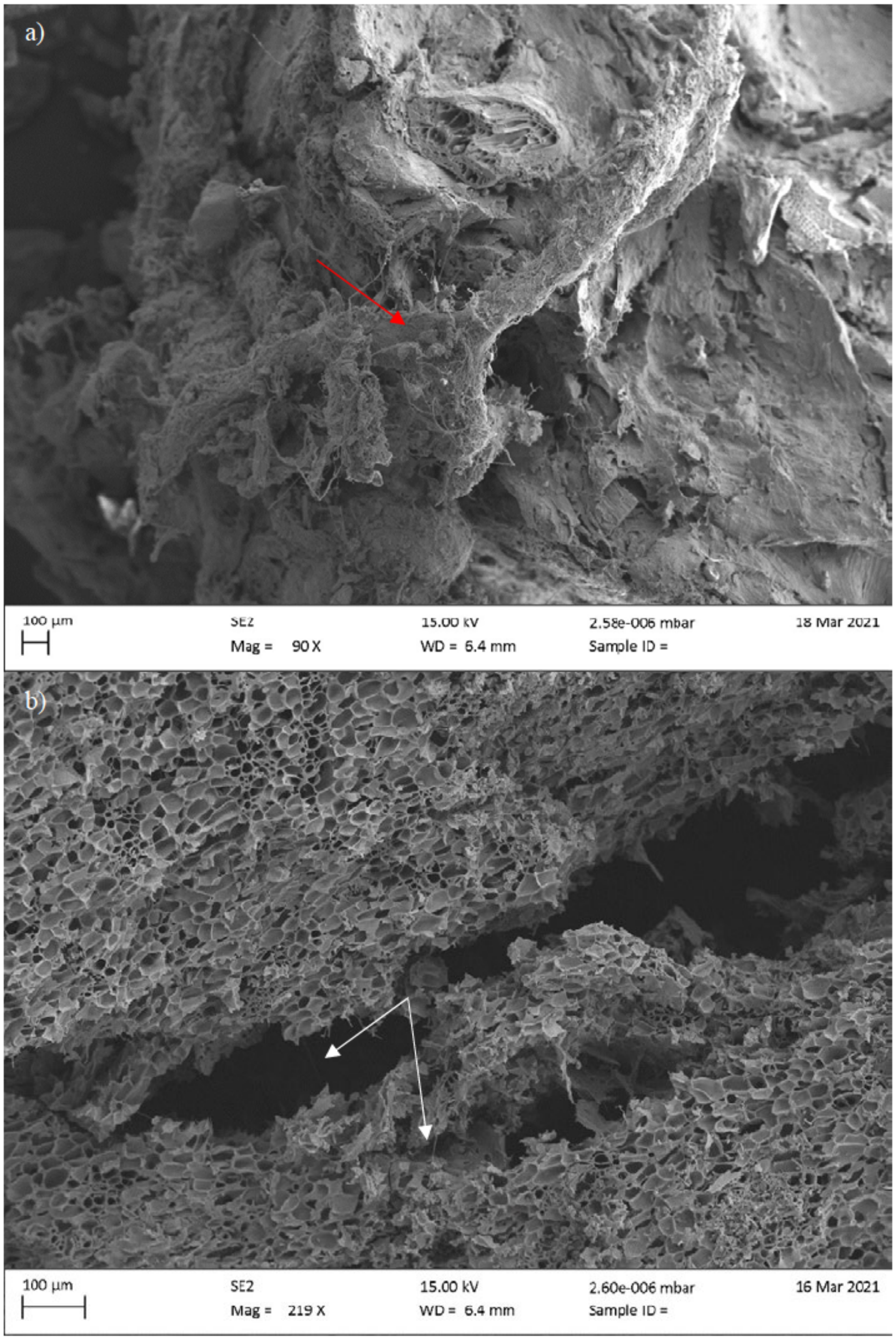

Figure 5-12- Example of limited internal colonisation of SWP+P and bark component of VCZ biochar. a) internal colonisation of SWP+P biochar is constrained to close proximity to the mantle layer, with some extraradical hyphae development. Fine root and fungal mantle layer is visible in the image centre (red arrow). b) VCZ particle comprising of exclusively pyrolysed bark. The lack of vascular structure and pores between cells prevents extensive colonisation. Sparse filamentous hyphae can be seen in voids and fissures (white arrows).

Following the initial colonisation of the internal space in biochar particles by ECM, the EEM was able to exploit the internal structure of biochar to proliferate within biochar particles. The extent of hyphal development and the growth strategies was highly dependent on the physical structure of biochar and previous studies have shown that mycorrhizal distribution is affected by the chemical properties of biochar (Hammer et al., 2014; Warnock et al., 2007; Warnock et al., 2010). As previously mentioned, ECM distribution in SWP+P biochar was limited. Where fine roots, which were colonised by ECM fungi, developed within SWP+P biochar particles some extraradical hyphae were observed. The environmental conditions within SWP+P biochar particles appear to have limited hyphae development, this could have been through ECM suppression or avoidance (because of low pH, Cd toxicity and high available P concentration, discussed in a previous section). There was limited direct rhizosphere interaction with biochar particles in SWP+P biochar, potentially decreasing the accessibility of nutrients physically immobilised within the SWP+P pellet aggregate matrix through the EEM. The moderate chemical properties of SWP biochar suggest that hyphal development in the internal structure of SWP biochar is not chemically suppressed. The low P concentration likely results in a decreased P foraging and hyphal proliferation effect (Hammer et al., 2014). The physical structure of SWP biochar likely results in the observed low hyphal abundance and distribution in the internal spaces of SWP biochar particles. High density and lack of vascular structures limits EEM development with biochar particles to voids and fissures between pellet aggregates Figure 5-13. These structures are generally discontinuous and high pore size is likely to limit P retention in biochar particles. Hyphal growth through voids was found in some cases to result in the biochar particle to shear along the line of structural weakness. This may release nutrients which are physically immobilised in the pellet structure, as it breaks down and the aggregates are released.

ECM hyphae growing within VCZ biochar particles have been observed “exploring” linear vascular structures (large diameter axial tracheids and resin canals) (Figure 5-13). Filamentous singular hyphae appear to exploit these structures extending until nutrient replete areas are found. In resin canals, hyphae were observed creating multiple dichotomous branches to exploit ashy deposits on the resin canal wall Figure 5-14. Hyphae were also observed exploiting large diameter axial tracheid structures to colonise adjacent spaces in VCZ biochar particles. Hyphae were able to traverse degraded cell walls and middle lamella, between axial tracheid cells, which create a longitudinally exploitable pathway between cells. Hyphae were found to move through axial tracheid pits into adjacent small diameter tracheid cells which could not be transversely accessed, penetrating pit diameters which were <10 μm. This shows that maintaining the vascular cellular structure, which provides internal biochar pore connectivity, of biochar feedstock material is vital to ensuring that the internal spaces of biochar can be effectively colonised by EEM hyphae. In turn, this will improve nutrient and water accessibility, furthering interaction between the rhizosphere and charosphere.

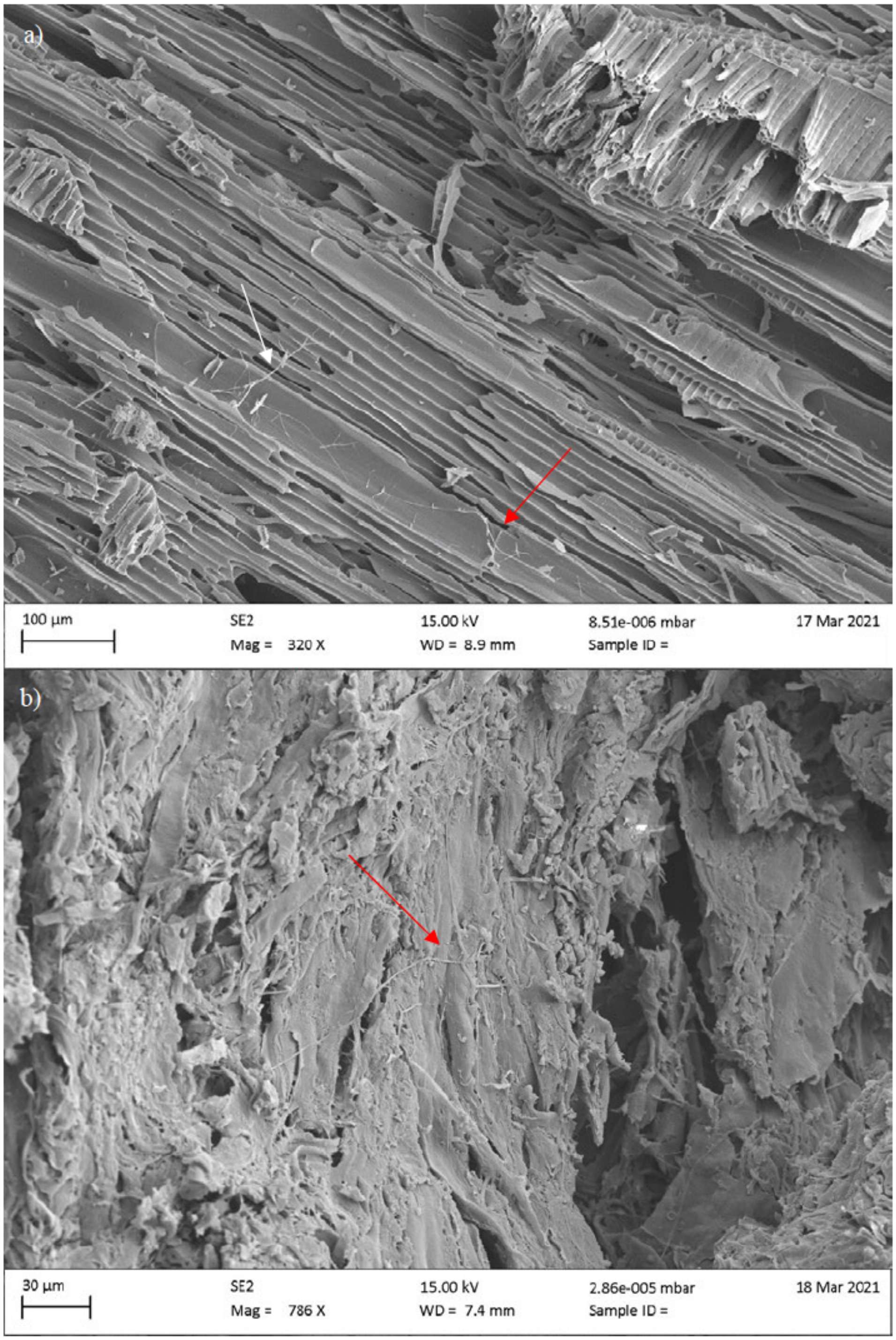

Figure 5-13- Example of hyphae which were observed actively explore internal biochar structure, exploiting weakness in pelletised particles and the cellular structure of VCZ biochar. a) hypha can be growing within the internal structure of VCZ biochar, through large diameter axial tracheids and passing through degraded cell walls between tracheid cells (red arrow). Branching hypha can be seen laterally growing into adjacent cells through a pit (white arrow). b) Sparse filamentous hyphae grow within SWP biochar, utilising weaknesses between aggregates (red arrow). Dense structure prevents extensive distribution.

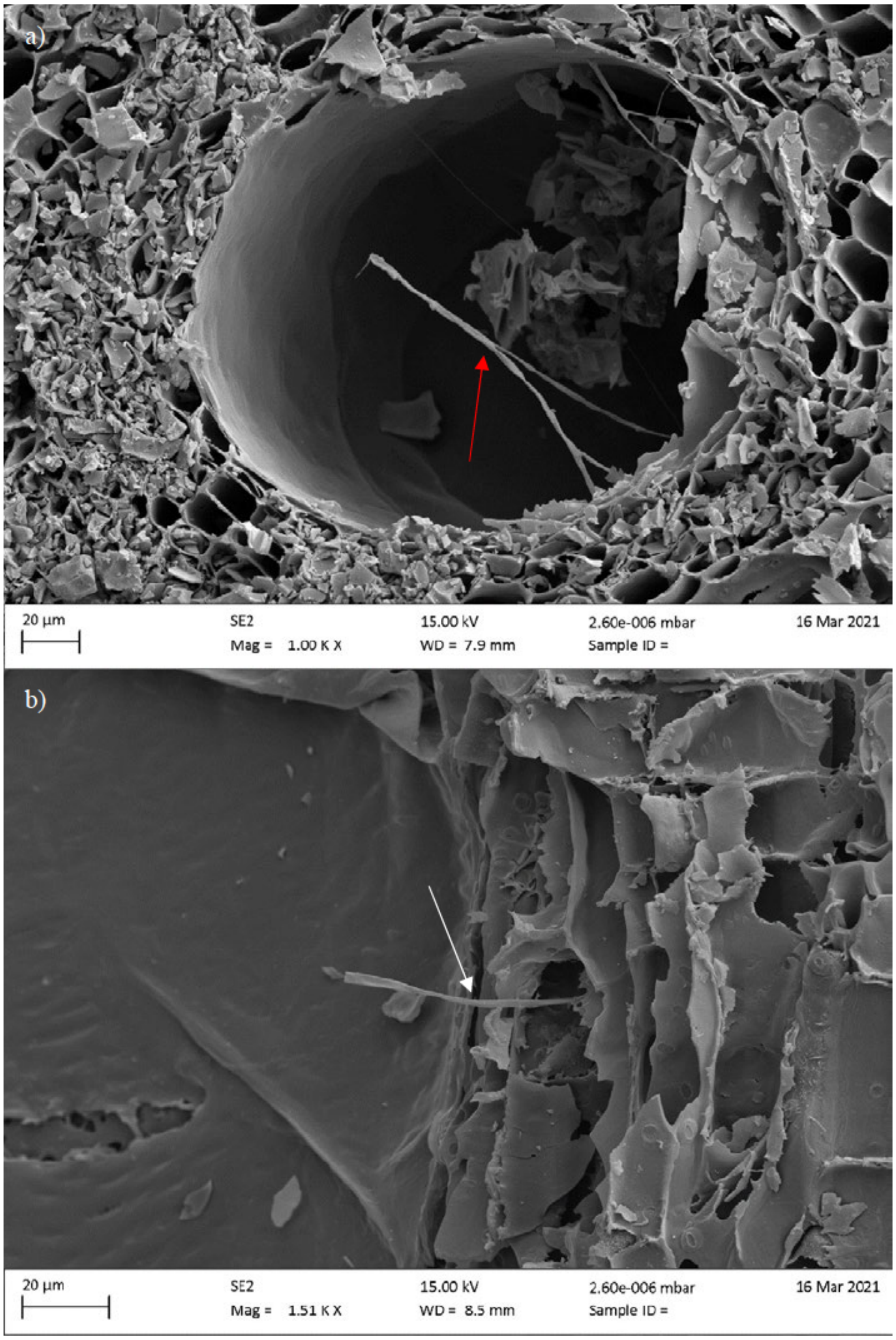

Figure 5-14- Example of hyphae exploiting resin canal structures in VCZ biochar particles. These structures have a diameter up to 20x larger than axial tracheids and have no internal barriers to hyphae exploration. a) cut section of resin canal, showing dichotomous branching hypha exploiting the structure (red arrow). b) part of hypha (which extended through resin canal prior to cutting) extending laterally into surrounding cellular structure of biochar particle through horizontal resin canal (white arrow).

5.4.3 Nutrient Transfer Pathways

Biochar has been found to influence mycorrhizal abundance and function and mycorrhizal interaction with biochar particles is considered as a vital aspect of the nutrient transfer pathway between biochar particles and the plant (Hammer et al., 2014; Jaafar, 2014). Hyphal contact with nutrient replete biochar surfaces is known to increase nutrient uptake to the associated plant (Hammer et al., 2014). Clear evidence of hyphal proliferation on nutrient replete surfaces was observed within VCZ particles (Figure 5-15). The highest P concentration within VCZ biochar particles is found on the surface of the vascular cambium and in the living secondary phloem. Hyphae were found to branch upon contact with the vascular cambium within, or on the surface of, biochar particles. The palmate branching distribution was only observed in these P replete areas of VCZ biochar, showing that the chemical properties of biochar influence ECM morphology. These hyphal structures increase hyphal contact with the P rich surfaces, increasing exploitation. It is likely that this enhanced colonisation on P rich surfaces will result in increased P transfer to the host plant and increase the accessibility of P within biochar particles. Hyphal branching was also observed to exploit ash deposits within vascular structures and resin canals.

Sclerotia were extensive on the outer surface of VCZ biochar. These fungal structures were only found on the transverse face of biochar (wood orientated), where hyphae entered the internal structure through large diameter axial tracheid cells and resin canals. Sclerotia act as energy stores for hyphae and can buffer fluctuations in carbohydrate supply from the host tree, enabling consistent hyphal growth. The presence of these structures at internal access points suggests resource allocation for preferential growth within biochar particles.

It is expected that some of the nutrients are physically occluded in biochar particles, particularly P, and these nutrients will gradually diffuse into the surrounding soil. Such diffusion would facilitate captured by surface EEM, where there has been extensive hyphal development, as observed for VCZ biochar. This highlights the potential importance of ECM fungi in improving biochar P capture and efficiency, preventing P loss, and helping to close the soil P loop.

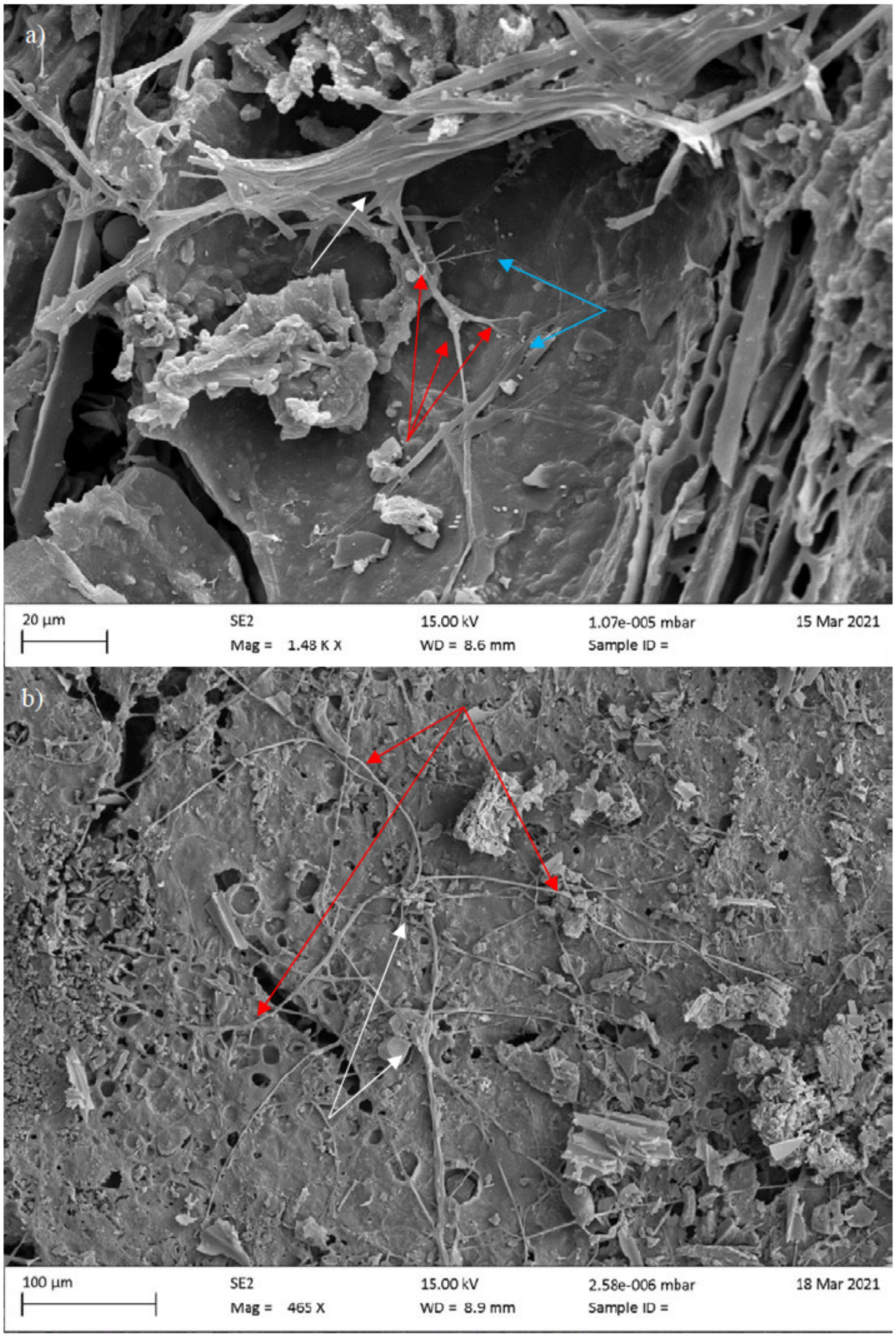

Figure 5-15- Example of hyphae branching when nutrient replete zones within the internal spaces of biochar are found. EEM morphology is distinctive in these areas. Extensive multiple branching of single hypha was not observed outside internal nutrient replete spaces of VCZ biochar. a) lateral hypha branch emanates from undifferentiated strand towards section of vascular cambium (white arrow) within a VCZ biochar particle. The hypha dichotomously branches multiple times (red arrows) and branching terminate on the vascular cambium surface (blue arrows). b) Single hypha branches multiple times from two single points (white arrows). Extensive hyphae then expand across the nutrient replete vascular cambium (red arrows) within a VCZ biochar particle, showing distinctive morphology.

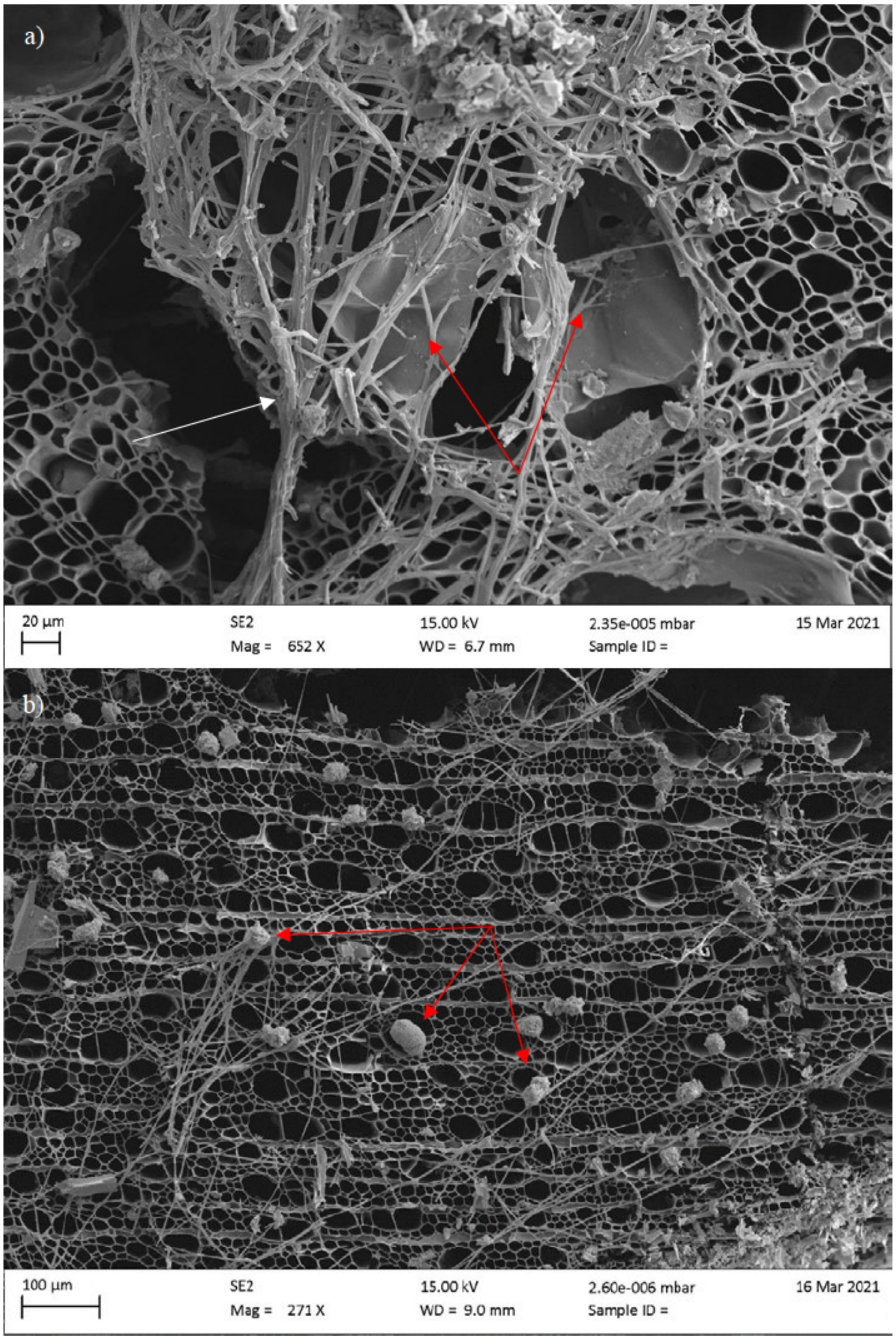

Figure 5-16- Examples showing that EEM exploiting nutrient rich deposits near VCZ biochar surface, indicated by hyphal proliferation a) branching undifferentiated strand at probable ash deposit on VCZ biochar surface (white arrow) can be observed. Dichotomous branching of individual hyphae can as be observed, which then terminate on nutrient rich ash surface (red arrows). b) sclerotia can be seen (white arrows) on the transverse face of VCZ biochar. These spherical fungal masses provide fungal energy reserves.

5.4.4 Biochar Refugia and Nutrient Cycling

The provision of refugia by biochar for fungal grazers could have negative effects on ECM fungi (Warnock et al., 2007). There has been little research on the abundance and impact of fungal grazers on nutrient cycling within biochar particles. Fungal grazers (including mites and nematodes) were found in internal spaces within biochar particles. The low colonisation rate and ECM abundance in SWP+P biochar, resulted in no observed microorganisms within the SWP+P biochar. This is probably due to scarcity of amenable substrate, although the low pH may contribute. Microfauna was found extensively within and on the surface of VCZ biochar particles. Soil mites and eggs were observed exploiting the refuge provided by longitudinal fissures in VCZ particles, which were produced during pyrolysis (Figure 5-17). The dense pellet structure of SWP biochar did not enable micro-fauna to access biochar particles, except where aggregates had fragmented through weathering.

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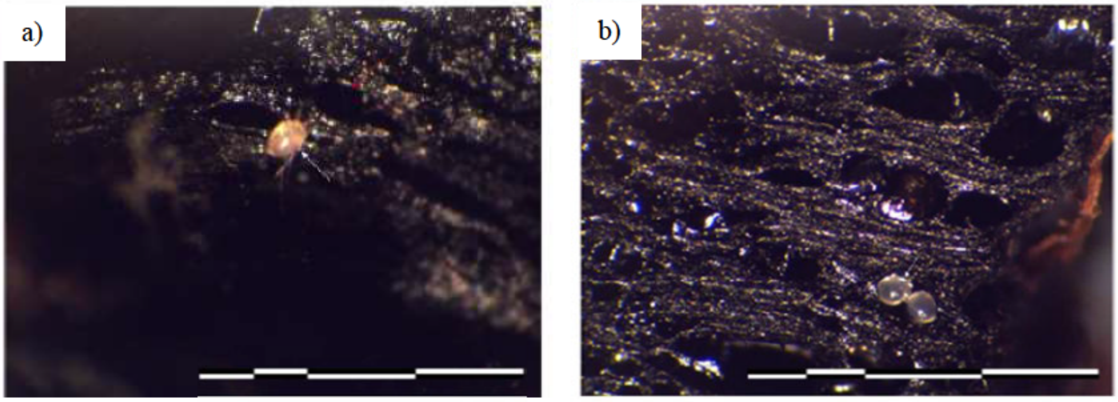

Figure 5-17- Examples of VCZ biochar particles that act as refugia for soil invertebrates, which influence nutrient cycling and transport nutrients back into biochar particles. a) a soil mite (0.5 mm length) can observed within cut biochar particles. Mites cannot access cellular structure on biochar and is confined to longitudinal fissures in biochar particles. b) invertebrate eggs within longitudinal fissures in VCZ biochar particles. Scale bars is 3 mm.

Nematodes were observed grazing hyphae on the external biochar surfaces of VCZ biochar and were also found grazing within longitudinal fissures (Figure 5-18). The relatively low abundance of hyphae on SWP biochar, coupled with distinct EEM morphology likely contributed to the infrequency of observed association of nematode grazers on SWP biochar. The formation of undifferentiated hyphal strand structures found on SWP biochar are likely more difficult for nematodes to graze compared to the singular filamentous structure of the EEM on VCZ biochar particles. Grazers within biochar particles may promote mineralisation of degraded biochar particles and this could be accelerated by passive ingestion of biochar particles. The general abundance of microfauna in biochar particles was low, when compared with the bulk soil, and is unlikely to limit ECM distribution or function. These organisms may however have an important role in nutrient cycling within biochar particles.

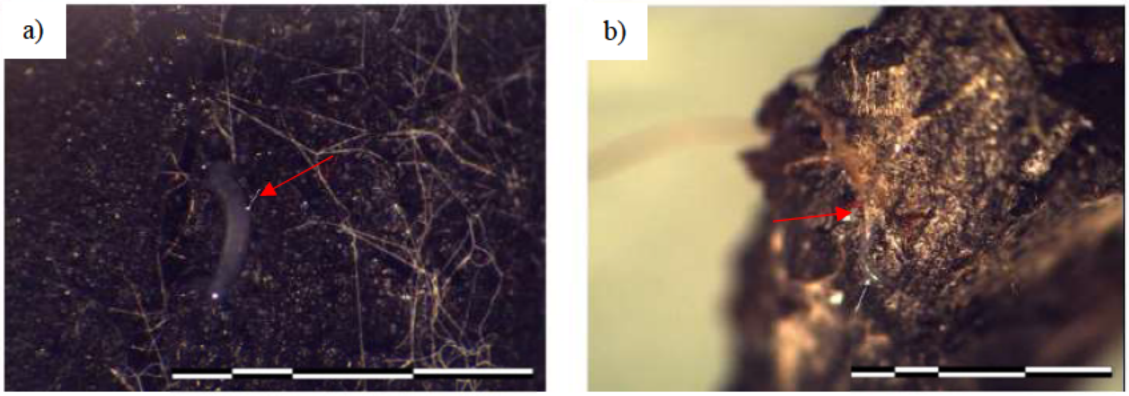

Figure 5-18 – Examples of Nematodes which were observed extensively within the internal spaces, and external surfaces, of VCZ biochar particles. Nematodes were observed grazing on ECM hyphae (red arrow in a) & b)). Scale bars are 3mm.

The nutrient balance within biochar particles is unlikely to be diminished by the presence of fungal grazers and/or microfauna using biochar as refugia. Rather, invertebrates migrating into biochar particles from surrounding soil could import nutrients in organic matter. Nutrients in microbial biomass should eventually become available to ECM hyphae colonising biochar. Such interactions were visualised within a VCZ biochar particle (Figure 5-19). A mycorrhizal hypha was observed entering a deceased mite, presumably to access resources replete inside of the organisms. This suggests that ECM hyphae actively forage opportunistically for new nutrient sources and acquire non-native nutrients within biochar. The proliferation of ECM fungi around biochar particles suggests some potential for biochar to act as a nutrient sink for nutrients held within ECM hyphae. Hyphae which decompose within biochar, potentially through grazing, would be cycled back into living hyphal structures. Biochar particles could effectively maintain a closed P cycling loop, except for nutrient transfer via ECM fungi, where the physical structure increases EEM proliferation and limits lateral P loss.

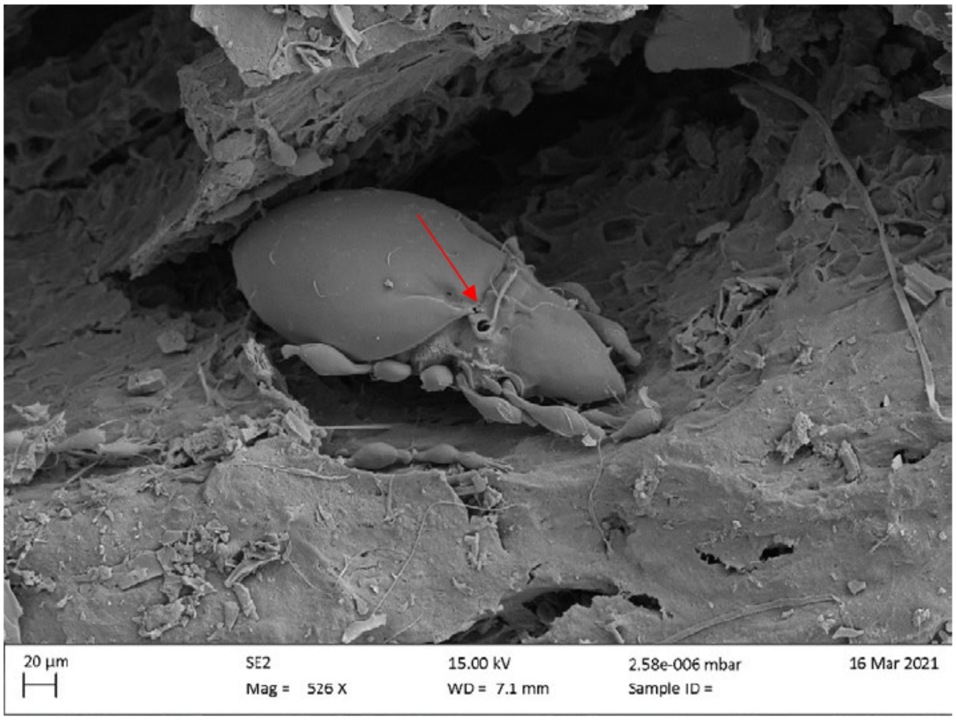

Figure 5-19- Example of a biochar particle providing apparent refuge for a microscopic dust mite (*Acari* spp.). The deceased mite appears to offer a nutrient source for ECM. A hypha enters the internals of the mite through a pore (red arrow). The precision of this hyphal development may indicate preferential foraging behaviour of hyphae within biochar particles.

5.4.5 Biochar Structure and Nutrient Accessibility

Roots did not colonise the microstructure of biochar particles and internal penetration was limited to external, macroscopic fissures. Internal colonisation of biochar was visually evidenced by the extent of surface EEM. There was extensive expansion of EEM across biochar surfaces, but this varied greatly with surface texture. Highly irregular or rough surfaces confined ECM colonisation and growth to fissures. This resulted in lower EEM coverage of biochar pellets and greater coverage in the planar structures of biochar from unprocessed wood, such as VCZ. Mycorrhizal hyphae have been shown to directly access immobilised P in biochar, in the absence of root contact with particles (Hammer et al., 2014). The high hyphal growth observed on VCZ biochar particles may increase P accessibility to the host tree, however the increase in fungal biomass may influence the allocation of other nutrients. Extensive ECM growth rates result in hyphae retaining a considerable amount of captured nitrogen for their own growth, thus reducing the amount transported to the host plant (Colaert et al., 1992). A decreased N transport to the host seedling may lead to increased N deficiency in some soils. It is not thought that ECM fungi allocate elevated P levels at high growth rates (Treseder, 2004).

It appears that EEM require large diameter tracheid cells to colonise cellular microstructure of biochar, or resin canals to exploit the cellular structure of woody biochar. Filamentous hyphae have a diameter less than that of tracheids, but most tracheid cells were not exploited. There was clear preference for biochar external pores >20 µm. The mechanisms that result in the absence of observed hyphae entering tracheid cells that have a diameter <20 µm is not known, but could be due to hydraulic conductivity, or poor hyphal signalling. Large diameter tracheid cells are generally found near the vascular cambium, where new sapwood has thin cell walls which thicken as cells mature, and in earlywood which are lower density for xylem transfer (with latewood higher density for structural support). It is likely the ECM colonisation of woodchip biochar produced from heartwood or maturing sapwood would be difficult due to the thickening of cell walls and resulting in decreased in biochar micropore size in the tracheids. This will likely be highly species dependent, with lower density wood more likely to be effectively colonised by ECM fungi. Alternative, hardwood feedstock may provide a conducive structure for EEM colonisation, as the presence of vessel elements will enable hyphae to enter the biochar microstructure regardless of tracheid diameter. High temperature pyrolysis, which results in greater mass loss, may open the cellular structure allowing hyphae to colonise. Hyphae were effective at traversing the internal structures of VCZ biochar once surface colonisation located the transverse face of biochar particles. It is likely that EEM would render most of the P held in the VCZ cellular matrix available to the host tree root.

5.5 Conclusion

The physical structure of biochar, determined by the feedstock properties, effected the distribution and colonisation of mycorrhizal fungi. Unprocessed woody biochar, such as VCZ biochar, retains the cellular structure of wood. Hyphae appear to exploit the tracheid cells of wood to proliferate within internal spaces, potentially creating a nutrient transfer pathway. Processed, pelletised, wood derived biochar has a dense structure with irregular pore structure which limits hyphal colonisation of internal spaces. Pelletised biochar also creates inaccessible spaces within aggregate particles, holding nutrients which roots, or ECM fungi cannot acquire. These nutrients may remain inaccessible until weathering sufficiently degrades the physical structure of biochar aggregates. The internal hyphae density on a mass basis will be constrained in biochar particles which lack a cellular structure or equivalent pore distribution and connectivity. The chemical properties of biochar and mineral fertiliser was found to influence fungal abundance around biochar and fertiliser particles. This study found evidence that suggests hyphae foraging and proliferation close to VCZ particles. Mineral fertiliser was shown to suppress fungi colonisation around fertiliser patches The difference between fungi abundance between VCZ biochar and mineral TSP fertiliser suggests that mycorrhizal accessibility of nutrients in soil applications may be limited by the chemical properties of the amendments and this may influence the efficacy and accessibility of fertilisers and biochars. The different interaction between the rhizosphere and charosphere between distinct biochars likely has implications on seedling resilience and growth in the early establishment phase.

5.6 Contributions

Dr David Kelly and other staff at Centre Optical Instrument Laboratory (COIL), the University of Edinburgh, provided training and assistance during fluorescence microscopy. Dr Nicola Cayzer, SEM Facility Manager at the School GeoSciences SEM Facility, completed SEM imaging due to COVID-19 restrictions. All sample preparation was completed by the author, including assistance during SEM imaging. Kaiyu Lei managed the experiment detailed in Appendix 2, which provided samples for use in fluorescence microscopy. He also assisted in sample collection for Chapter 3, which provided samples for SEM analysis and assisted with optical microscopy. The experimental work detail in Appendix 2 was a close collaboration between Kaiyu Lei and the author (who oversaw the MSc research project). Experiment concept and principal design was design was conceived by the author to supplement this PhD thesis. Sincere thanks must be given to all the people who contributed to this work, which was completed during COVID-19 restrictions. They went above and beyond expectations to make this chapter possible in a very limited working environment. Particular thanks to Kaiyu Lei for working so diligently over this difficult period and surviving months isolated in the lab with the author.

Chapter 6

Establishment soil carbon loss and the mitigation potential of biochar in the context of NetZero: Insights from two empirical soil carbon models

6.1 Abstract

Woodland creation and biochar are both accepted as land-based carbon removal and carbon storage mechanisms; however the additionality of these technologies has not been assessed. The net greenhouse gas removal and carbon balance of woodland creation over the first rotation is directly dependent on; initial soil organic carbon (SOC) stocks, soil carbon losses associated with cultivation disturbance, biochar application and the balance between tree carbon sequestration and respiration. The comparison of two independent empirical models for soil C change in organo-mineral soils following afforestation show consistent total change at the end of first rotation carbon balance for all modelled scenarios. The results further suggest that woodland creation on organo-mineral soils should not be conducted under the assumption of short-term net carbon sequestration. Reducing cultivation disturbance on all O horizon depths in organo-mineral soils can improve end of rotation total net carbon balance and considerably advance the year to attain positive carbon balance by limiting SOC mineralisation during establishment. Model variation in the rate of soil carbon loss (comparing linear and modified Boltzmann sigmoidal models for soil C loss) highlights the importance of soil carbon loss dynamics over the establishment period when assessing the year of positive carbon balance for woodland creation, at which point afforestation improves the carbon stock of land and contributes to greenhouse gas removal (GGR). The potential improvements during growth in the establishment phase due to the modelled benefits of targeted biochar application provides early indication of the potential of biochar to reduce the time to reach positive carbon balance post establishment and compensate for soil carbon loss from mounding in nutrient limited organo mineral soils. Blanket biochar application during woodland creation can be used to ensure that the carbon balance of woodland creation remains positive, and the required dose rate can be calculated from the developed soil carbon models.

6.2 Introduction

Governments throughout the world have made commitments to reduce net greenhouse gas (GHG) emissions in order to mitigate the effects of anthropogenic climate change over the next century to limit global warming to below 1.5˚C (IPCC, 2022). The U.K. and Scottish Governments have committed to reach net zero GHG emissions by 2050 and 2045 respectively (Climate Change Act 2008 [U.K.] & The Climate Change Act 2019 [Scotland]). Net GHG reduction commitments are to be met through reducing the direct emissions of greenhouse gases and sequestering atmospheric CO_2 using greenhouse gas removal (GGR) technologies (BEIS, 2021) of which Land Use, Land-Use Change and Forestry (LULUCF) is a major potential contributor (Matthews et al., 2009; Romppanen, 2020; Smith et al., 2019). The conservation and enhancement of semi-natural land based carbon sinks can be effective at long term carbon sequestration and storage (Alcalde et al., 2018; Smith et al., 2016). For the UK government's commitment to achieving net zero GHG emissions by 2050, the Climate Change Committee (CCC) recommends afforestation of 30,000 hectares per year (ha yr^{-1}) by 2025, and 50,000 ha yr^{-1} by 2035 (CCC, 2020).

Woodland creation is widely accepted as a cost effective GHG removal method (Morison, 2012). Plant biomass carbon stock can be rapidly increased over decadal timescales, and the contribution of afforestation to delivery of NetZero can be maximised with the planting of productive species on low carbon soils (Forster et al., 2021; Matthews et al., 2020). However, the rate of net carbon sequestration and the contribution of woodland creation to NetZero targets is likely to be over-estimated without adequate consideration of below ground carbon stock change, especially in respect to woodlands established on organo-mineral soils (Friggens et al., 2020). Organo-mineral soils, which represent a significant portion of available planting area in the U.K., are carbon rich and vulnerable to degradation of carbon stocks through land use change and cultivation disturbance (Wuaden et al., 2020). The soil carbon pool of soils can be degraded following afforestation on grassland and ericaceous heathland, with losses particularly pronounced in organic carbon rich soils (Guo & Gifford, 2002; Hartley et al., 2012). Mitigation of soil carbon loss is critical for the greenhouse gas removal (GGR) contribution of woodland creation and forest establishment in the context of NetZero targets.

Biochar is a land based GGR technology which increases the stable carbon pool of soils (Smith et al., 2019). Labile carbon, stored in biomass, is thermochemically transformed by pyrolysis.

During "carbonisation", dehydrogenation, hydrogen transfer and isomerisation processes result in the formation of rings of C atoms which condensate into larger sheets and stacks (Wang et al., 2016). Carbon aromaticity increases, creating a recalcitrant form of carbon in biochar. Aromaticity and degree of aromatic condensation are two indices to evaluate the stability of biochar materials and is principally determined by pyrolysis temperature and residency time (Fang et al., 2015). Higher temperature pyrolysis >600°C produced increased carbon stability and carbon storage longevity (Leng & Huang, 2018). Biochar is recognised as an effective land based GGR technology, which can rapidly increase soil recalcitrant carbon. Biochar can also alter soil properties which impacts crop productivity and rhizosphere development. Biochar is not an inert product and interacts with carbon cycling processes, influencing net soil carbon storage (Cross & Sohi, 2011).

Rates of soil carbon loss during woodland creation are affected by soil microbial and mycorrhizal community dynamics (Bailey et al., 2019; Zhao et al., 2018). Soil microbial and mycorrhizal communities are altered due to soil environment and ecosystem changes through management practices (Mojeremane et al., 2012) and vegetation change (Wardle David et al., 2004). Direct soil disturbance from ground preparation and drainage prior to planting aerates soil and stimulates decomposition of plant matter and soil organic carbon (SOC) resulting in rapid CO_2 release (Leiber-Sauheitl et al., 2014) and degradation of the soil nitrogen (N) pool (Egnell et al., 2015). Reduction in carbon stocks from the bulk soil increases as trees establish and plant communities shift, with the rate of carbon loss from undisturbed soil (between mounds or furrows) stabilising at canopy closure (Hargreaves et al., 2003). Afforestation typically reduces soil moisture and can increase soil summer temperature, in moist soils, which increases soil respiration and carbon decomposition and turnover (Friggens et al., 2020; Hermans et al., 2022). Following the stabilisation of soil C turnover, SOC stocks have been found to recover after canopy closure, particularly in conifer dominated woodlands (Bárcena et al., 2014; Hernandez-Ramirez et al., 2021). The gradual soil carbon accumulations expected following canopy closure are due to high organic matter input through leaf litter (Reidy & Bolger, 2013). This accumulated SOC stock is vulnerable to disturbance during forest management operations (Yamulki et al., 2021) and changes to soil decomposition processes following tree root death following harvesting or wind damage (Subke et al., 2011).

Effective woodland establishment practices often require some form of cultivation (Mason & Quine, 1995) which provides benefits including; reduced sward competition, elevated root temperature, soil mineralisation providing nutrients and localised drainage. The level of soil disturbance is predicated on cultivation method and depth of soil disturbance. U.K forestry policy categorises cultivation methos according to level of disturbance (none, low or high) (Scottish Forestry, 2021). High disturbance afforestation cultivation, such as shallow ploughing, increasing the rate of soil carbon loss during afforestation. Low disturbance cultivation practices, include excavator mounding, mitigates a proportion of SOC loss during the first rotation of afforestation due to a reduced volume of disturbed soil (Vanguelova et al., 2018) . Labile soil carbon stocks in semi-natural organo-mineral soils have low resilience to cultivation disturbance during afforestation (Mojeremane et al., 2012). However, there is potential to mitigate the loss of labile carbon through the negative carbon priming effect from biochar application, which has been reported as -18.9% (Wang et al., 2016; Zimmerman et al., 2011). Soil carbon losses resulting from woodland creation on organo-mineral, and historically on organic soils, are dependent on the thickness of the organic horizon, which accounts for the majority of SOC stock in these soils (Grüneberg et al., 2010; Vanguelova et al., 2018). Productive woodland creation on organo-mineral soils with an O horizon >20 cm degrades the labile soil carbon pool during the first rotation, which then subsequently increases during second rotation to reach similar carbon stocks to unplanted grassland (Benham & Vanguelova, 2016; Vanguelova et al., 2019; Zerva & Mencuccini, 2005). Subsequent felling cycles result in further periods of soil carbon decline, followed by recovery as restock establishment reaches canopy closure (Paul et al., 2002; Thiffault et al., 2011). Biochar has the potential to mitigate these soil carbon fluctuations and by increasing the recalcitrant carbon pool, which is resistant to soil carbon loss during harvesting/restock cycles and protects soil carbon stocks from loss during cultivation (Li et al., 2018; Lu et al., 2014).

Studies (Ball et al., 2011; Brown, 2020; Friggens et al., 2020; Matthews et al., 2020) have found that the soil carbon loss resulting from afforestation is greater than the increases in plant biomass stored carbon under certain scenarios and timescales following afforestation.. These scenarios are limited to low yield, high total SOC and/or high disturbance afforestation. The relative GHG removal benefit of woodland creation options as a GGR contributor to NetZero commitments must account for changes in below ground carbon stocks following planting and variable rates of biomass accumulation (Matthews et al., 2020). Targeted specified biochar application has been found to improve growth in the establishment phase in the early establishment phase, potentially reducing the time to canopy closure (Chapter 3 and Chapter 4). Whilst biochar induced yield improvements during establishment cannot be extrapolated to increased growth following canopy closure, the increase in establishment speed and success could have profound carbon balance implications. Modest increases to growth rates following specified biochar addition, coupled with the direct carbon storage of biochar, may offset the soil carbon loss from disturbance during establishment in certain scenarios. Soil carbon loss dynamics and variable total tree biomass accumulation rates should be considered when assessing the contribution of individual woodland creation proposals and the potential additional GGR technologies, especially with respect of NetZero commitments where decadal timescales for recovery of establishment losses and achieving net GGR through land use change are crucial. Temporal carbon changes should be used to calculate the delay before schemes deliver increases in the net carbon stock of land.

Two independent soil carbon empirical models were used in this study to assess the impact of difference intensity establishment cultivation techniques, soil O horizon depth and forest yield (productivity) on afforestation site carbon stock changes. These models were compared to assess the influence of modelled soil carbon loss dynamics on total carbon balance to the end of the first rotation (40 years) for selected woodland creation scenarios on organo-mineral soils. The impact of targeted specified biochar application on carbon balance was also assessed for both models. Model outputs were compared to appraise the relative efficacy of afforestation scenarios in the context of GGR and NetZero. Model outputs of the tested scenarios were used to identify establishment scenarios where low-dose application of specified biochar could mitigate early establishment carbon loss and to create a predictive model for calculating the required biochar application rate to mitigate negative carbon balances in low yield planting. The validation of model outputs was outside the scope of this study. This study provides indicative analysis on carbon stock changes in woodland establishment and does not provide validated predictions. The purpose of this study was to *i*) develop an independent model for soil carbon loss during afforestation for use in U.K. forest policy, *ii*) assess the modelled impact of cultivation disturbance and yield on the net carbon balance of woodland creation to the end of the first rotation in the context of "Net Zero" policies and *iii*) identify potential of biochar in mitigating net soil carbon loss through modelling.

6.3 Methods

6.3.1 Soil Carbon Stock

The soil carbon stock was separately calculated for peaty gley organo-mineral soils managed under undrained unimproved grassland using data from the Scottish Soils Knowledge and Information Base (SSKIB) (Lilly et al., 2004a) and BioSoil soil network (Vanguelova et al., 2013). Soil carbon calculations based on SSKIB data calculated for the organic (O) and mineral (E/B) horizons (kg C m^{-3}) separately for a range of O horizon depths . These values were then totalled and converted to give soil carbon stock (kg C m^{-3}). Total soil C stock (t C ha^{-1}) was calculated to a total soil depth of 1 m for organic horizon depths of 5 cm, 10 cm, 20 cm 30 cm & 40 cm. Soil organic carbon (SOC) (%) and bulk density (BD) (kg m^{-3}) varies across peaty gley soils, general peaty gley SOC and BD was calculated from mean values of SOC and BD in spatially extensive peaty gley soils series in Scotland (Lilly et al., 2004b). Soil carbon calculated from BioSoil data used mean soil carbon stock at fixed peat (O) horizon depths.

Equation 6-1 – Equation for soil horizon carbon content (kg C m^{-3})

$$Soil\ horizon\ C\ content\ (kg\ C\ m^{-3}) = BD\ x\ \frac{SOC\ (\%)}{100}$$

Table 6-1 – Calculated soil carbon stocks (to 1 m depth) for peaty gley soils under unimproved grassland management dependent on O horizon depth prior to land use change through afforestation, calculated from Hutton Institute soil data and comparative Forest Research data for deep peat soils.

O Horizon Depth (cm)	Soil Carbon Stock (t C ha^{-1}) SSKIB	Soil Carbon Stock (t C ha^{-1}) BioSoil
5	33	31
10	60	64
20	106	131
30	152	199
40	198	266

6.3.2 Creber Model of Soil Carbon Loss

The Creber soil model used in this study was extracted from a wider woodland establishment life-cycle analysis (LCA), with only the soil carbon sub-model component retained for model comparisons. The underpinning soil data for the empirical model was derived from Scottish Soils Knowledge and Information Base (SSKIB) dataset (Lilly et al., 2004b), to produce an independent soil carbon change model from the ECOSSE model which also uses some data from SSKIB (Smith et al., 2007). A modified Boltzmann distribution, a generalized 4-parameter logistic function, often referred to as Boltzmann sigmoidal equation (Kent et al., 1972), was used to model the cumulative rate of soil carbon loss from the bulk soil (Equation 6-2). The rate of bulk soil C loss was adjusted for drained and undrained scenarios by reducing $T_{half,}$ (the time at which half of bulk soil carbon loss occurred) where additional drainage was included (undrained scenarios were used in this study). The T_{half} and slope used in the Boltzmann distribution equation used to calculate bulk soil C was adjusted dependent on cultivation method. The T_{half} for soil C loss from the bulk soil was lower in high disturbance linear cultivation and higher in low disturbance cultivation methods, with additional drainage reducing the half value further. T_{half} for raised linear disturbance, where disturbed soil creates elevated planting positions and linear troughs are produced, was reduced as soil carbon loss from soil occurs at a faster rate under these conditions. The half value occurred before canopy closure for all cultivation methods. The year of canopy closure following planting was calculated by yield class curves. Total soil carbon change at the end of rotation was validated against afforested soil data from SSKIB and Forestry Commission planting and yield data for peaty gley soils.

Equation 6-2 – Modified Boltzmann sigmoidal equation used to calculate yearly cumulative soil carbon loss from the bulk soil, *t* (time from planting) and T_{half} (the time from planting at which half of bulk soil carbon loss occurred).

$$Cumulative\ soil\ C\ loss = total\ soil\ C\ loss\ \times (\frac{1}{1+\exp\frac{(t-T_{hal}\ )}{slope}})$$

Carbon loss from directly disturbed soil was calculated as percentage loss of total SOC in the volume of directly disturbed soil. The volume of soil directly physically disturbed during ground preparation (cultivation) prior to planting was calculated for shallow plough (high disturbance), hinge mounding (low disturbance) and direct planting (no disturbance) cultivation methods according to area, depth, and shape of disturbed soil. Physical soil disturbance during cultivation was calculated at a rate of 720 m^3 ha^{-1} (shallow plough), 150 m^3 ha^{-1} (hinge mound) and 0 m^3 ha^{-1} (direct plant). Soil carbon directly released from physically disturbance was applied using a linear rate of soil carbon loss over a 10-year period from year of cultivation. The rate of soil carbon loss was dependent on the volume of soil disturbed, with 50% of the total disturbed soil carbon loss occurring at year 5 and 100% at year 10. Soil carbon accumulation from litter was included from canopy closure at a fixed 0.6 t C ha^{-1} yr^{-1}.

The net soil carbon change model, using adjusted T_{half} and slope in the fitted Boltzmann sigmoidal equation was validated using 20 cm O peaty gley soil and YC 16 SS scenarios for high and low disturbance cultivation scenarios (Figure 6-1). Mean soil carbon stock from 5 selected sites for each data point was used to validate model fit. Soil data collected as part of SSKIB sampling were used to provide mean soil carbon stock for Sitka spruce compartments with a known age and cultivation method. SSKIB sample location was cross referenced with Forest and Land Scotland Compartment Database to identify data points which met the required soil type (peaty gley), O horizon depth 20 cm (+/- 1 SD), tree species (Sitka spruce), cultivation method (mound or shallow plough), yield class (YC14 to 18) and time from planting (+/- 3 years). Five suitable data points (which matched required parameters) at year 10, 20 and 35 were identified for both cultivation methods. There was insufficient data to calculate mean soil carbon stock for year 35 mounding. Soil carbon change was calculated from baseline unplanted peaty gley carbon stock measurements and standard error was calculated. Model fit against measured soil carbon data is displayed in Figure 6-1. The modelled soil carbon change had a strong fit to measured soil carbon data (R^2>0.7).

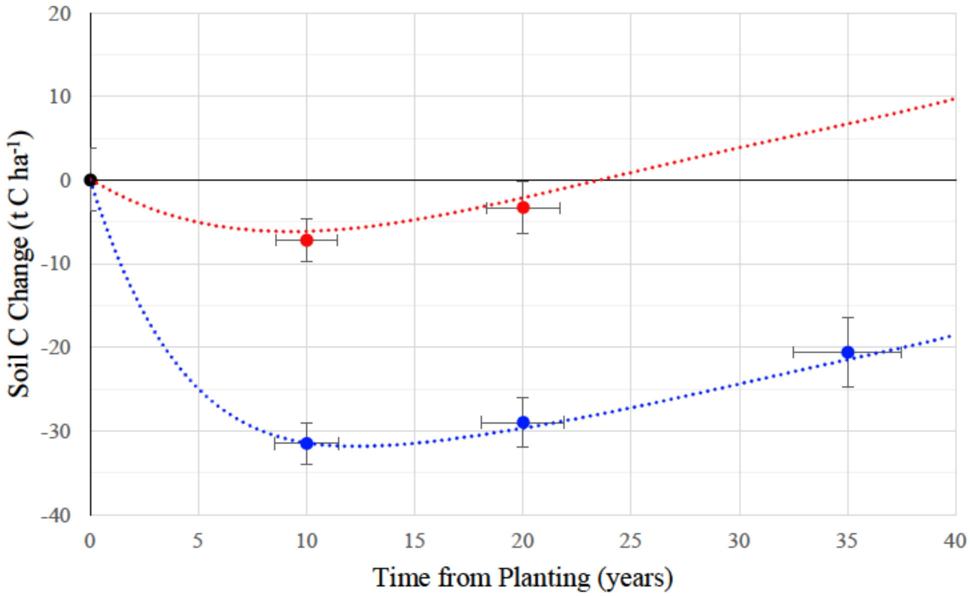

Figure 6-1 - Modelled cumulative net soil carbon change in ~20 cm O horizon peaty gley soils over one YC14-18 Sitka spruce rotation (40 years) using modified Boltzmann sigmoidal equation fitted to SSKIB data. Soil carbon change from baseline soil carbon stock is displayed for hinge mounding (red) and shallow plough cultivation scenarios. Mean soil carbon stock from SSKIB data is displayed for both cultivation practices and standard error is displayed (n=5). Net soil carbon change curves were modelled for 20 cm O horizon peaty gley soils for hinge mounding (red and shallow plough (blue).

6.3.3 Vanguelova model

This model is based on empirical relationships drawn from total soil carbon stocks prior to cultivation disturbance and total carbon stock in the soil profile after disturbances. The model accounts for different periods of subsequent forest growth (e.g., 20 or 40 years of first rotation), so total carbon change and annual rate of C change could be modelled. Calculations are based on measured C stocks in organo-mineral soils sampled from the BioSoil soil network (Vanguelova et al., 2013) and calculated % loss of soil C due to cultivation practices. Cultivation disturbance was calculated as low physical soil disturbance (from 60 m^3 ha^{-1} to 380 m^3 ha^{-1} soil volume disturbed, soil carbon loss from 1% to 10% of total stock), medium physical disturbance (from 560 m^3 ha^{-1} to 840 m^3 ha^{-1} soil volume disturbed, soil carbon loss from 10% to 20% of total stock) and high physical disturbance (from 1000 m^3 ha^{-1} to 2500 m^3 ha^{-1} soil volume disturbed, soil carbon loss from 30% to 50% of total stock). The modelled estimates of carbon loss are dependent on soil volume disturbance and were based on the limited available scientific evidence of direct measurements of soil C loss due to disturbances in peaty gley soils (Vanguelova et al., 2019; Zerva & Mencuccini, 2005). The main assumptions made for this model are: *i*) rate of soil carbon change is constant between years over the first rotation, resulting in a liner change in total soil carbon stock, *ii*) litter accumulation rate is also constant and is at 0.6 t C ha^{-1} y^{-1}, as used in previous assessments (Hannam et al., 2017; Lilly & Chapman, 2015; Vanguelova et al., 2019) ; *iii*) the % loss of soil carbon is limited to the depth of disturbance caused by the individual cultivation technique and assumes that this depth is an organic layer.

6.3.4 Modelling Comparison

To limit the number of afforestation scenarios modelled, management variables were selected to represent common U.K. woodland creation practices on organo-mineral soils. Peaty gley soils were used in all scenarios as these soils account for the majority share of organo-mineral soils afforested with Sitka spruce (*P. sitchensis* (Bong.) Carr) in the U.K. (Brown et al., 2014). Modelled values generated for peaty-gley soils are similar to other organo-mineral soil types, notably peaty podzols, with <5 % variation in expected soil carbon change across organo-mineral soil types (Lilly et al., 2020). We compared multiple afforestation scenarios focussed on typical yields for commercial conifer and native broadleaf woodland creation on nutrient poor soils. Conifer biomass accumulation was modelled for Sitka spruce, which is the principal planting component of grant funded conifer woodland creation schemes in the U.K. Native broadleaf woodland creation on organo-mineral soils was represented by downy birch (*Betula pubescens* (Ehrh.)) and sessile oak (*Quercus petraea* (Matt.) Liebl.) mixed planting at a ratio of 2:1. Scenarios modelled soil carbon change for three soil cultivation regimes; high disturbance (shallow plough (SP)), low disturbance (excavator mound (MO)) and no disturbance (direct plant (DP)) at a range of O horizon depths. Model outputs were compared at year 20 and 40, non-linear polynomial regression models were fitted to assess cumulative net carbon balance change over the first rotation.

Incorporating a full Life Cycle Assessment (LCA) approach

The Creber model included other establishment carbon costs including nursery tree production, chemical (herbicide, pesticide and fertiliser) application, tree protection and fencing. For the purposes of comparing model outputs, inputs were simplified to maintain consistency with the Vanguelova model, which excluded the "carbon management impact" (i.e., losses) of these establishment costs, to balance the data-model comparisons. All forest establishment carbon change parameters in the Creber model, other than soil carbon change and biomass carbon change, were excluded to enable model comparison. All outputs from the Creber model have been converted from t CO_2 e to t C by using a conversion factor of 3.67 (t CO_2 e/t C).

Planting density for the native broadleaf (NBL) was directly comparable to the productive conifer at 2500 stems per hectare and represents high density productive broadleaf forests, whereas mixed native woodlands are typically at a planting density of 1100-1600 stems per hectare. A reduction in planting density to 1600 stems would reduce volume of soil disturbed by MO cultivation by ~36%, which would reduce total net soil C loss by a lesser extent due to reduced litter accumulation. The rate of soil carbon accumulation from leaf litter was modelled for YC 14 Sitka spruce in conifer scenarios and a conversion factor of 0.8 was applied for native broadleaf scenarios (Keith et al., 2015).

Forest Yield

For each disturbance scenario (cultivation methods and O horizon depths) representative yield classes were assigned for Sitka spruce as 'commercial conifer' and birch/oak mix (2:1) as 'native broadleaf'. Timber volume was derived from ForestYield – a digital version of the Forestry Commission Forest Yield Tables (Edwards, 1981). The ForestYield model (Matthews, 2016) is built upon an extensive UK permanent sample plot network and has been reviewed against a stand level dynamic growth model (Lonsdale et al., 2015) and shown to perform well for Scots pine (Lonsdale et al., 2015). These values are then used to estimate the mass of carbon stored in the woody biomass (Morison, 2012). The standing volume model outputs are scaled using well-established and robust allometric relationships (Levy et al., 2004; Randle et al. (2014)) and modified by wood density (Lavers and Moore, 1983) to convert wood volumes to dry weight, 50% of which is assumed to be carbon (Matthews, 1993). Biomass C accumulation used included all tree carbon both above and below ground.

Carbon Balance

The cumulative total net carbon stock change was calculated independently by both modelling approaches at five-year intervals from year 0 to 40, and then combined with temporal outputs for total woody biomass, which were then converted to t C, from the ForestYield model. The two empirical soil C stock models then underpinned total modelled site carbon stock change, for each scenario. A 3rd degree polynomial regression was fitted to carbon change data under each scenario. The intercept of the polynomial regression was calculated to assess the years from planting when a given scenario achieved a positive carbon balance, i.e., where the net cumulative total carbon stock exceeds pre-afforestation carbon stock. This can be used to assess the short to medium term contribution of woodland creation scenarios to NetZero climate targets.

6.3.5 Biochar Carbon

Biochar was incorporated into both models by applying adjustment factors to temporal values across carbon pools: biomass accumulation, direct soil carbon storage and soil carbon priming. The growth improvement from specified biochar application to Sitka spruce was applied to yield modes for the early establishment phase (year 0-5). This was calculated using data from the studies presented in Chapter 3 & Chapter 4 and was adjusted according to yield class (a factor of 1.8 was used in YC 10 and 1.4 YC 14). Growth improvements were not included at YC 16 and above, these yield classes are only achieved where soil nutrient regime is not poor to very poor (VP1 to VP3). The difference at year 5 between biochar treated and untreated trees was added to the cumulative biomass carbon curve. As broadleaf species were not tested in experimental work and due to the lack of data, these scenarios were not modelled. The direct carbon storage of biochar was applied at year 0. Total biochar carbon storage was applied at 0.847 t C t biochar^{-1} (calculated from the total carbon content of specified biochar). The carbon stored by biochar was then decreased to 0.708 t C t biochar^{-1} (2.6 t CO_2 t biochar^{-1}) at year 5, representing the loss of the biochar labile carbon pool. The intermediate targeted biochar application rate of 25 g seedling^{-1} (from Chapter 3 and Chapter 4) was used, equating to 0.062 t ha^{-1}. The effect of biochar on soil carbon priming used reduction factors from data reported by Wang et al. (2016) & Ding et al. (2018). These were selected to represent the modelled soil properties, biochar properties and disturbance intensity (adjustment factors of -0.03 to 0.1 were used), models outputs were not sensitive to variation in priming effect (0.43 % variation between priming scenarios following sensitivity analysis).

6.4 Results

6.4.1 Soil Carbon Change

Total soil carbon change at year 40 following afforestation was highly consistent for both models across all cultivation scenarios at O horizon depths of ≥20 cm (Table 6-2 & Figure 6-2). Soil carbon change at year 40 for scenario outputs, where the O horizon is ≥20 cm, varied between models from -2.77 to 4.19 t C ha^{-1} (Table 6-2). Both models show high disturbance cultivation methods (shallow plough) reduces total soil carbon stocks by over 10 t C ha^{-1} at year 40 at horizon depths ≥20 cm. Low disturbance cultivation (mound) scenarios result in net soil carbon accumulation, through leaf litter, at all O depths <35 cm.

Table 6-2 – Total cumulative soil carbon change at year 40 (following planting) for selected cultivation scenarios for peaty gley organo-mineral soils. Soil carbon change includes soil carbon loss from disturbed cultivated soil and the soil carbon change resulting from the alteration of cover vegetation (including carbon accumulation from leaf litter). Soil carbon change values exclude root biomass carbon accumulation, which is included in Table 6-3.

	Shallow Plough			Hinge Mound			Direct Plant		
	t C ha^{-1} soil carbon loss			t C ha^{-1} soil carbon loss			t C ha^{-1} soil carbon loss		
O Depth (cm)	Vang.	Creb.	Diff.	Vang.	Creb.	Diff.	Vang.	Creb.	Diff.
5	14.60	7.76	6.84	20.87	14.47	6.40	22.43	15.71	6.73
10	4.81	-1.00	5.81	17.60	12.42	5.18	20.80	14.90	5.91
20	-15.24	-18.51	3.26	10.92	8.33	2.59	17.46	13.27	4.19
30	-35.56	-36.02	0.46	4.15	4.24	-0.09	14.07	11.65	2.42
40	-55.88	-53.54	-2.34	-2.63	0.14	-2.77	10.69	10.03	0.66

Both models show that the impact of low disturbance cultivation on 36-40 cm O horizon depth peaty gleys on total soil carbon change at year 40 is equivalent to high disturbance scenarios on 10-13 cm O horizon depth. There is a slight increase in model divergence for soils with an O horizon depth <20 cm, with the difference between models peaking at 6.40 - 6.85 t C ha^{-1} in soils with a 5 cm O horizon. This difference can be explained by the higher proportion of soil C released from mineral soil horizons (E/B), which is a factor only contained within the Creber model, thus higher total carbon loss was observed in shallow O horizon soils.

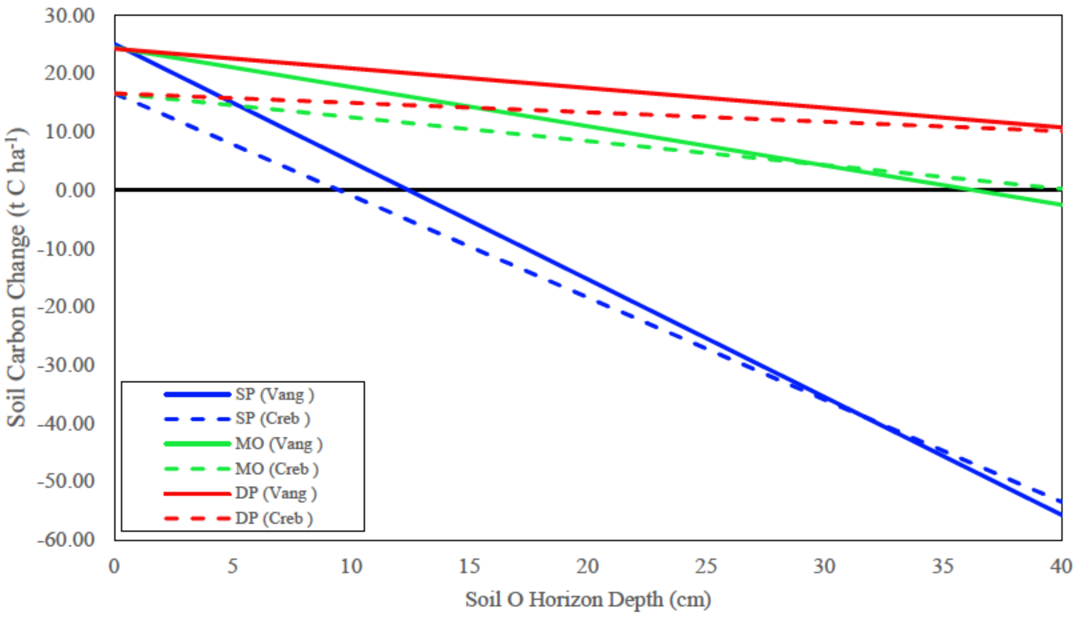

Figure 6-2 - Year 40 soil carbon stock change under different soil cultivation scenarios. High disturbance shallow plough (SP) is shown in blue, low disturbance mound (MO) is shown in green and no disturbance direct plant (DP) is shown in red respectively. The outputs of the Vanguelova (Vang.) model are displayed in the solid regression lines and the Creber (Creb.) model are displayed in the dashed linear regressions (R^2>0.99).

6.4.2 Total Site Carbon Balance

in the cumulative net soil carbon stock change estimates under high disturbance scenarios differs between models (Figure 6-3). Total soil carbon stocks over the first rotation (40 years) were found to decline in soils where O horizon depths exceeded 15 cm with a high disturbance SP cultivation prescription. Low disturbance cultivation in soils with a 40 cm O horizon resulted in no change to soil C stock after 40 years, with both modelling approaches showing consistent total soil carbon stock at year 40. The Vanguelova model approach applies a constant rate of soil carbon change over the first rotation, resulting in a linear change in total soil carbon stock over time. The Creber model approach applies a non-linear rate of soil carbon loss, with most of the soil carbon loss occurring up to canopy closure. This non-linear rate of soil carbon change shows a rapid loss of directly disturbed labile carbon from cultivation, followed by a steady accumulation of soil carbon as leaf litter accumulation exceeds soil carbon loss. This difference between models is most pronounced in high disturbance or deep (<30 cm) O horizon scenarios. Both models provide evidence that high disturbance cultivation in soils with a shallow O horizon (≤5 cm) and low disturbance cultivation in soils with an O horizon ≤30 cm result in an increase in total soil carbon stocks by year 40. Soil C recovers from a period of net

SOC loss in low disturbance cultivation at year <20 in peaty gleys <20 cm and <5 cm for high disturbance cultivation in the Creber model, where a non-linear model of soil C loss is used. Low disturbance cultivation on soils with an O depth of 40 cm results in considerable soil C loss (- 17 t C ha^{-1}) at year 20 in the Creber model, whereas soil C stock remains relatively stable in the Vanguelova model. The rate of tree C accumulation can be significantly increased with biochar application during the early establishment period (5 years), which will impact cumulative tree C storage to canopy closure (Figure 6-4). In yield scenarios comparable the expected yield classes in the control treatment (no biochar application) used in Chapter 4, total standing carbon increased by a maximum of 5 t CO_2 ha^{-1} (year 8) in Sitka spruce (YC10) and Scots pine (year 15).

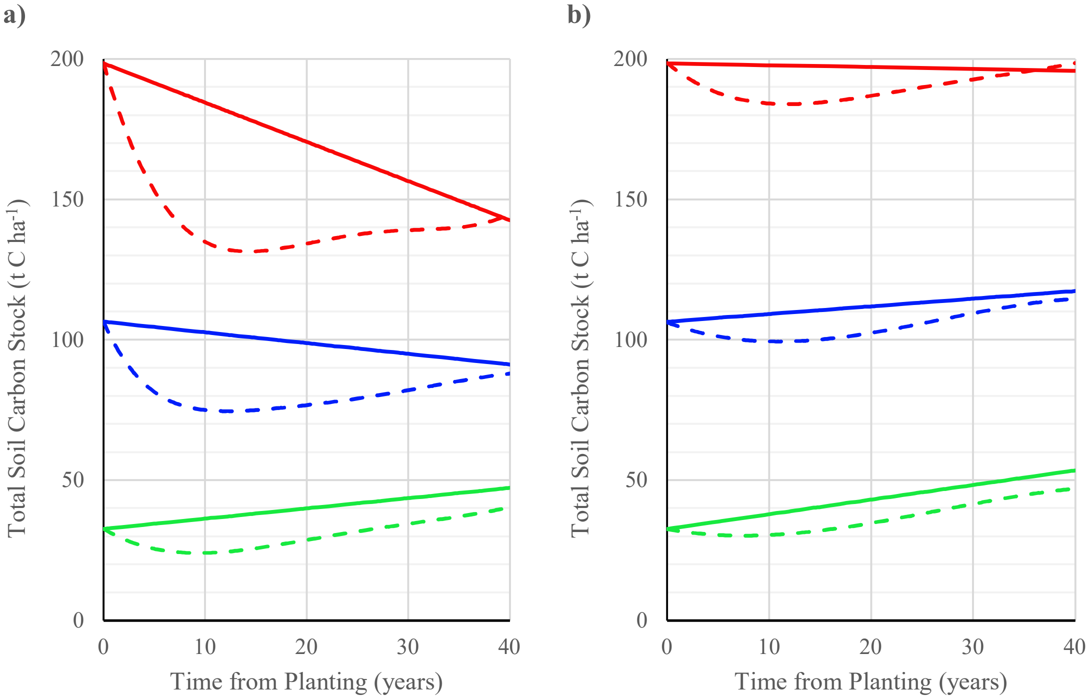

Figure 6-3 – Total soil carbon stock change model comparison for 5 cm, 20 cm and 40 cm O horizon depths in peaty gley organo-mineral soils. High disturbance cultivation (shallow plough) is displayed in graph a) and low disturbance cultivation (mound) in graph b). Polynomial regressions (3rd degree) fitted was to scenario data (R^2>0.99). A linear rate of soil carbon change is applied in the Vanguelova model (Vang.), Creber (Creb.) model includes temporal soil change dynamics over the establishment period and is therefore non-linear. Theses modelled soil carbon change values can be used to calculate the required blanket applications of biochar required to mitigate soil carbon loss (using Year 20 values).

Cumulative total carbon balance change (tree biomass and soil carbon) for selected scenarios is similar for both models under low disturbance cultivation (Figure 6-5), whereas there is considerable differences between regression curves in high disturbance scenarios (Figure 6-6).

Despite similarity between regression curves between models for low disturbance scenarios, the low slope at the intersect with 0 t C ha^{-1} change (year of positive carbon balance) results in a difference between the year of positive carbon balance for different disturbance scenarios (Table 6-3). For low disturbance scenarios with a 40 cm O depth, the year of positive carbon balance varies between models by 10 – 12 years according to tree species and yield. Low dose targeted biochar application, and the resultant improvements to early establishment yield, resulted in improved carbon balance over the establishment period. All low disturbance scenarios where the O horizon was <15 cm and targeted biochar was included resulted in no negative carbon change during establishment, with the growth improvements exceeding soil C loss. In low yield scenarios this can reduce the year of net positive carbon balance by 3-6 years. Only the Creber model showed negative cumulative total carbon stock during establishment (due to improved soil dynamics modelling) in biochar scenarios. A positive carbon balance was reached at year 6 (30 cm O horizon) and year 10 (40 cm O horizon), reducing the period of net carbon loss by ~5 years because of biochar application.

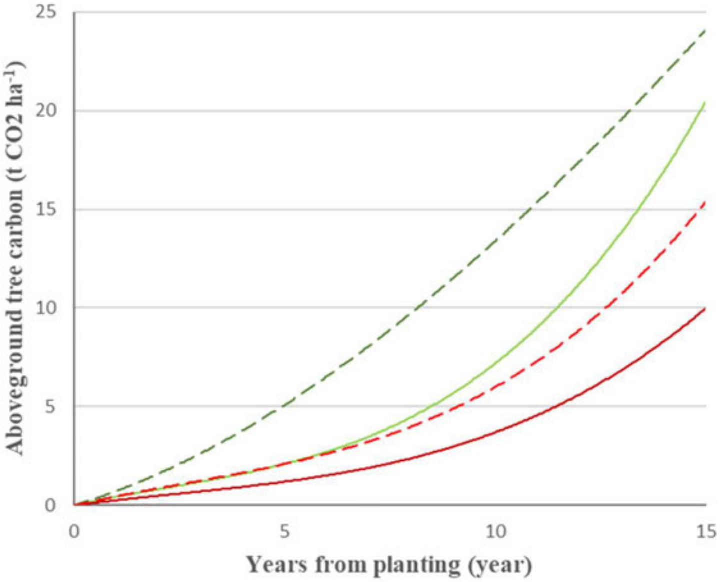

Figure 6-4- Tree biomass cumulative carbon from planting to canopy closure, the modelled biochar carbon curves (solid) can be compared to yield model curves (dashed). Modelled curves represent the expected Scots pine (red) and Sitka spruce (green) yield classes in Chapter 4 (YC 8 and YC 10 respectively). End of rotation carbon total is the same as year 15 (Sitka spruce) and year 22 (Scots pine), however this difference is smaller as a proportion of total carbon.

Year 40 total carbon stock increased by 6 – 20 t C ha^{-1}, in low disturbance scenarios where specified biochar was applied. This can be attributed to the decreased time to canopy closure. The impact of faster establishment is most pronounced during the period of highest annual yield increment, where a one-year growth improvement can equal a 4 t C ha^{-1} difference. When growth slows as trees reach maturity and annual increment decreases, the difference between biochar scenarios and untreated scenarios declines to <1% total C stock.

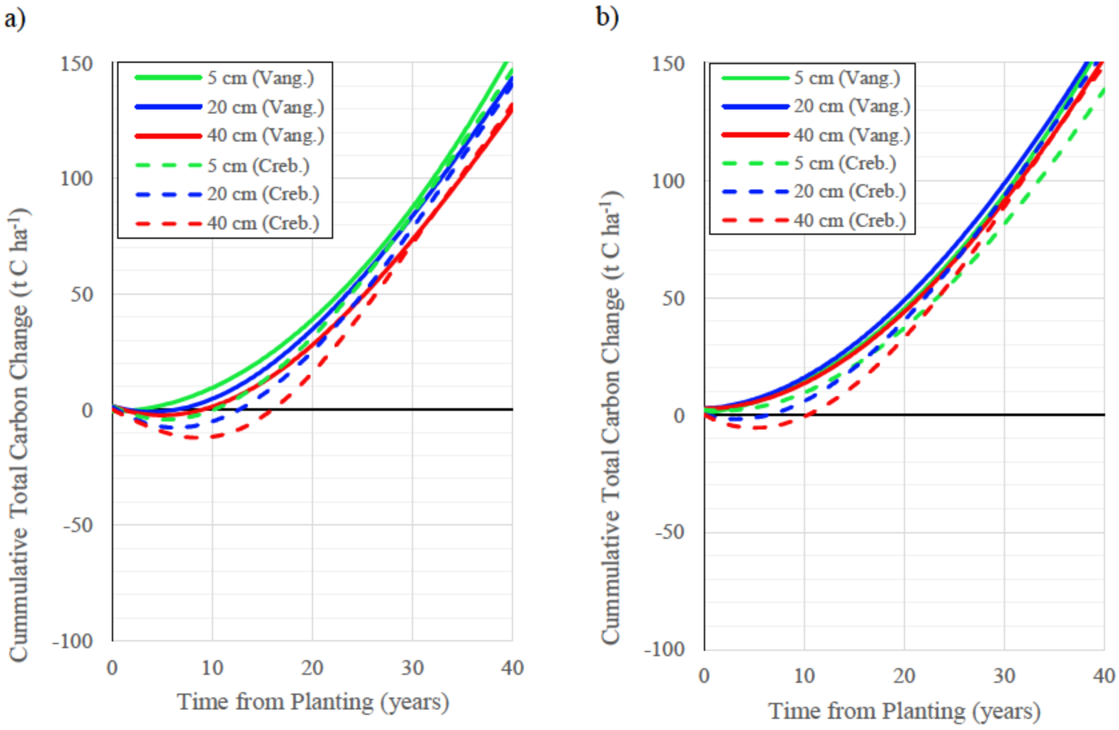

Figure 6-5 – Sitka spruce YC 14 (SS 14) model comparison of cumulative total carbon change (soil carbon and biomass accumulation) curves for selected peat depths for low disturbance mound cultivation scenarios, using expected yield class for nutrient poor organo-mineral soils. Vanguelova (Vang.) is displayed by solid curves and Creber (Creb.) is displayed by dashed curves. Polynomial regressions (3rd degree) fitted was to scenario data (R^2>0.99). a) displays model outputs for untreated establishment scenarios. b) model outputs for targeted VCZ application scenarios using both Creb. & Vang. Soil models.

High disturbance cultivation scenarios show similar variation between the year of positive carbon balance produced by the two models, despite greater discrepancy in early carbon balance. Due to the high rate of C loss from the soil in high disturbance scenarios, the modest carbon increase from biochar improved establishment growth and survival but has little impact on total cumulative carbon storage. In high disturbance scenarios where the O horizon >15cm biochar will only advance the time to a positive carbon balance is reached by 1 year. The

Vanguelova model likely overestimated the influence of biochar amendment on establishment due to the linear rate of soil loss used, resulting in a lower proportion of total carbon loss attributed to year 1 to 20 compared to the Creber model. The regression slope of both models is lower at the intersect (where net zero carbon balance is reached) in low disturbance scenarios and the slope further reduced in low yield native broadleaf scenarios where carbon accumulation rates are slow (Figure 6-7). This contributes to the relatively high differences in the year of positive carbon balance between models under low yield scenarios.

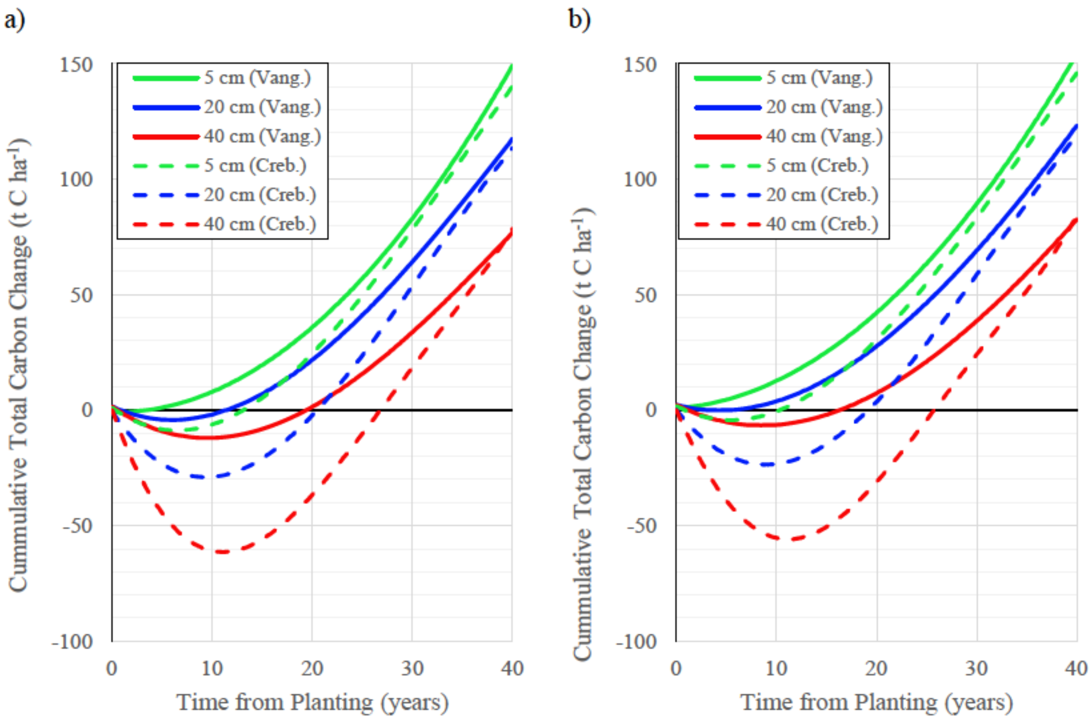

Figure 6-6- Sitka spruce YC 14 (SS 14) model comparison of cumulative total carbon change (soil carbon and biomass accumulation) curves for selected peat depths high disturbance cultivation scenarios, using expected yield class for nutrient poor organo-mineral soils. Vanguelova (Vang.) is displayed by solid curves and Creber (Creb.) is displayed by dashed curves. Polynomial regressions (3rd degree) fitted was to scenario data (R2>0.99). a) displays model outputs for untreated establishment scenarios. b) model outputs for targeted VCZ application scenarios using both Creb. & Vang. Soil models.

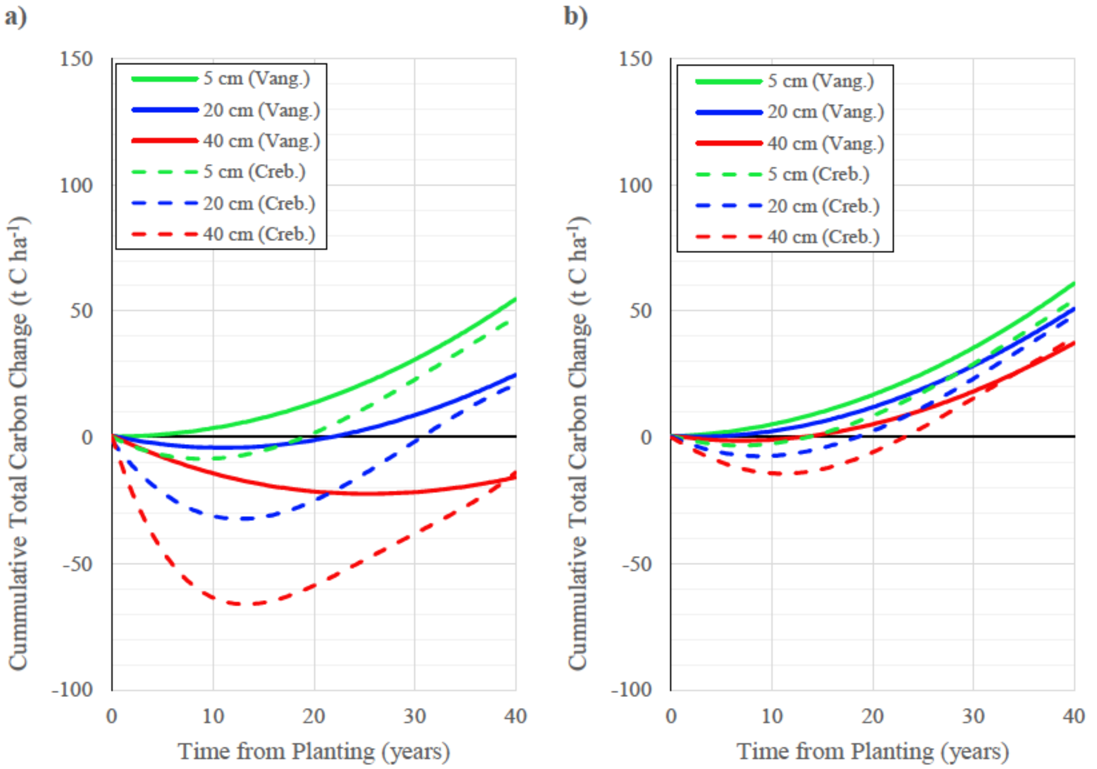

Figure 6-7 – Native broadleaf YC 2 (NBL 2) model comparison of cumulative total carbon change (soil carbon and biomass accumulation) curves for selected peat depths. Vanguelova (Vang.) is displayed by solid curves and Creber (Creb.) is displayed by dashed curves. High disturbance (shallow plough) scenarios are shown in graph a) and low disturbance (mound) is shown in graph b). Polynomial regressions (3rd degree) were fitted to scenario data (R^2>0.99).

Total afforestation carbon balance (soil carbon change and biomass accumulation) between models at year 40 showed similar cumulative soil carbon changes (Table 6-3). Model outputs showed consistent total carbon change at year 40 for all scenarios, with <10 t C ha^{-1} difference. All native broadleaf (YC 2) scenarios had lower year 40 carbon balance than YC 14 Sitka spruce, with high disturbance cultivation resulting in a net carbon loss of -16.1 (Vanguelova model) and -13.8 (Creber model) t C ha^{-1} at year 40 on soils with an O horizon of 40 cm. Both models show that high disturbance cultivation reduces end of rotation C stock by 53 t C ha^{-1} on soils with a 40 cm O horizon soil compared to a total reduction of 6 t C ha^{-1} on soils with a 5 cm O horizon. Whilst year 40 model outputs are consistent, the differences in soil carbon loss at year 20 carbon balance between models suggest that soil carbon dynamics need to be considered when calculating the year of positive carbon balance.

Table 6-3 – Scenario cumulative total carbon change results at year 20 & year 40 (t C ha^{-1}) shown for peaty gley O horizon depths. Scenarios parameters are split for species type, cultivation method and model. Species codes refer to species (SS (Sitka spruce) and NBL (native broadleaf)) and modelled yield class (YC). Cultivation method includes high disturbance shallow plough (SP), low disturbance mound (MO) & no disturbance direct plant (DP). The years from planting when woodland creation leads to a net positive site carbon balance (soil carbon change and biomass carbon accumulation) is shown, calculated from cumulative carbon change curves.

O Depth			5 cm			10 cm			20 cm			30 cm			40 cm		
Species	Cultivation Method	Model	Year 20	Year 40	Year of C Balance	Year 20	Year 40	Year of C Balance	Year 20	Year 40	Year of C Balance	Year 20	Year 40	Year of C Balance	Year 20	Year 40	Year of C Balance
SS 14	SP	Vang.	34.7	146.7	1	29.8	136 9	3	19.7	116.9	13	9.6	96.6	16	-0.6	76.3	21
SS 14	SP	Creb.	23.4	139.9	13	14.8	131 1	16	-2.4	113.6	21	-19.5	96.1	24	-37.2	78.6	27
SS 14	MO	Vang.	37.8	153.0	1	36.2	149.7	1	32.8	143.0	2	29.4	136.3	3	26.1	129.5	5
SS 14	MO	Creb.	29.9	146.6	8	28.0	144.6	10	24.0	140.5	11	20.1	136.4	13	16.0	132.3	16
SS 10	SP	Vang.	20.6	104.0	1	15.7	94.3	3	5.7	74.2	16	-4.5	53.9	22	-14.7	33.6	27
SS 10	SP	Creb.	9.3	97.2	16	0.7	88.4	19	-16.4	70.9	24	-33.6	53.4	28	-51.2	35.9	33
SS 10	MO	Vang.	23.7	110.3	1	22.1	107.0	1	18.7	100.4	2	15.4	93.6	4	12.0	86.8	7
SS 10	MO	Creb.	15.9	103.9	12	13.9	101 9	13	9.9	97.8	15	6.0	93.7	17	1.9	89.6	19
NBL 2	SP	Vang.	12.6	54.3	1	7.7	44.5	3	-2.3	24.5	23	-12.5	4 2	35	-22.7	-16.1	>40
NBL 2	SP	Creb.	1.3	47.5	14	-7.3	38.7	23	-24.4	21.2	32	-41.6	3.7	37	-59.2	-13.8	44
NBL 2	MO	Vang.	15.7	60.6	1	14.1	57.3	1	10.7	50.7	1	7.4	43.9	2	4.0	37.1	13
NBL 2	MO	Creb.	7.9	54.2	13	5.9	52.2	15	1.9	48.1	18	-2.0	44.0	21	-6.1	39.9	23

The Creber model accounts for soil carbon change dynamics over the initial establishment phase and provides an indication of the year of positive carbon balance following planting when woodland creation contributes to net GGR (Figure 6-8). High disturbance cultivation on organo-mineral soils >17 cm delays the year that a positive carbon balance is achieved until after 2045, if afforestation is implemented ~2025, for modelled species and yields. Low disturbance cultivation with low yield native broadleaf planting was similarly unviable with O horizons <25 cm. As NetZero 2045 commitments are neared, less woodland creation scenarios will contribute to NetZero target dates, despite long-term afforestation GGR benefits. Moderate yield (YC14) Sitka spruce remains viable as a GGR option for 2045 up to a planting date of 2037, where O horizon depth <10 cm and low disturbance cultivation is used. Targeted specified biochar application can markedly reduce the modelled time to net zero for low disturbance scenarios, or low O horizon depth. In the context of NetZero targets this can improve the viability of woodland creation in contributing to net carbon removal as target dates are approached. With biochar application, low disturbance planting can viably contribute towards net GGR in 2045 if planted by 2040 (YC 14, O horizon <15cm). Figure 6-8 can be used to calculate the expected latest viable planting year for modelled scenarios to achieve a positive net carbon balance by NetZero target years. Increased forest yield, minimal cultivation disturbance, shallow O horizon and biochar application planting scenarios provide the shortest delay to the year of positive carbon balance and may be viable for net carbon removal contribution closer to NetZero target dates.

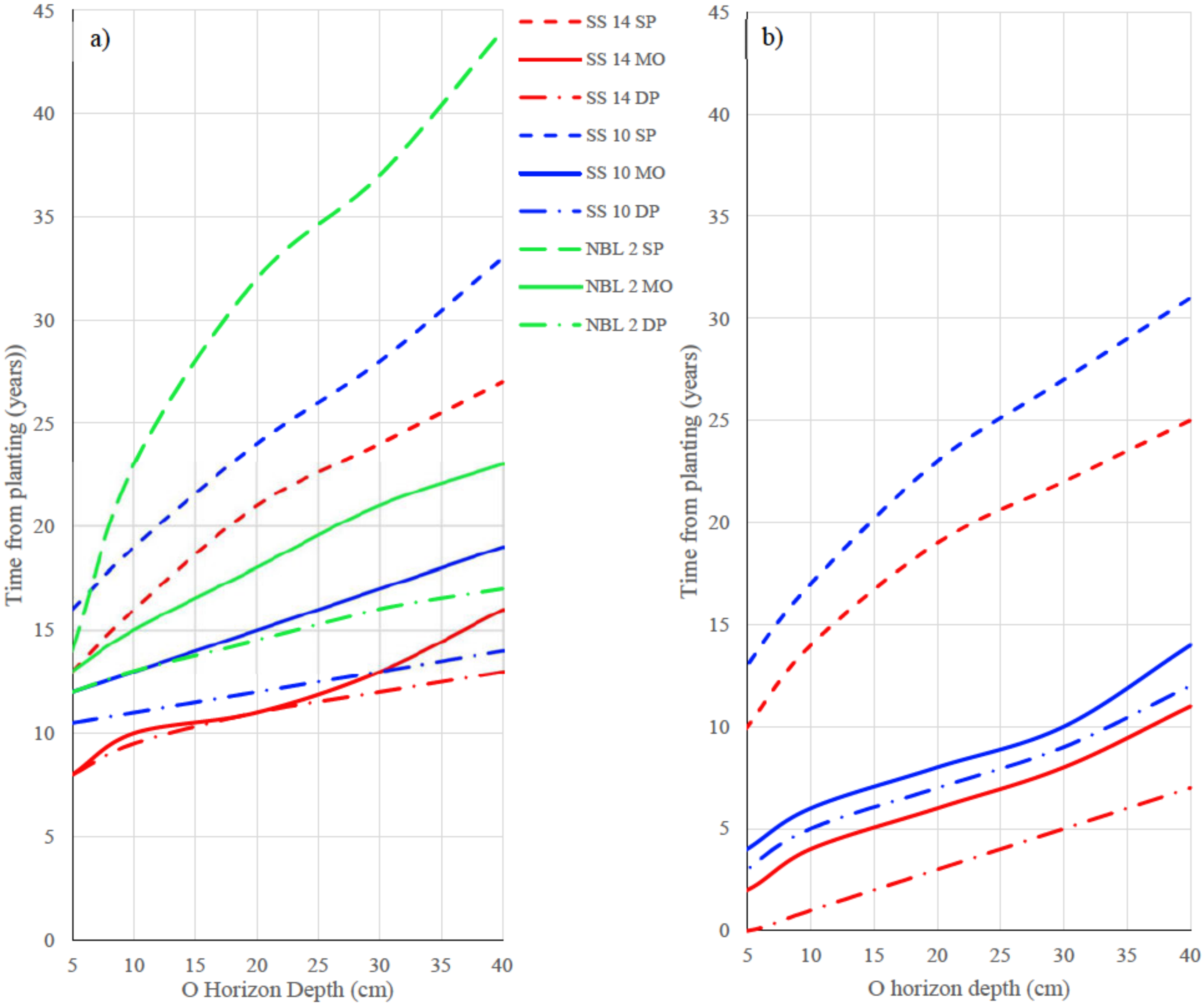

Figure 6-8- Year of positive carbon balance, when cumulative net total carbon change increases compared to pre afforestation carbon stock (years from planting) for expected YC on nutrient poor planting sites. Modelled scenarios are displayed across O horizon depths (at 5 cm intervals from 5 cm to 40 cm) according to species, yield class and cultivation method (high disturbance (SP), low disturbance (MO) & no disturbance (DP). Only results derived from Creber model are displayed as soil C change dynamics are included. a) shows modelled output for untreated seedlings. b) shows modelled outputs for VCZ treated targeted application in Sitka spruce only.

The potential of biochar application in forest establishment can be optimised to provide optimal silvicultureal benefits with minimal application. The practical biochar use scenario described in Chapter 4 uses low dose targeted biochar application to improve growth rather than maximise direct soil carbon storage. Blanket biochar appication, which has less silvicultural value and practical challenges in a forestry context, could be used to entirely offset soil carbon emmissions and maintain a positive soil carbon stock change during woodland creation. Table 6-4 shows the required specified biochar application dose required to prevent net soil carbon loss during the establishment period.

Table 6-4- Net soil carbon change at Year 10 (t C ha^{-1}), which is the maximum negative soil carbon balance in the Creber model. The required biochar dose (specified biochar) to directly offset soil carbon loss (excluding soil carbon priming effects) is included, this would prevent any net decline in soil carbon during woodland creation. Scenarios were modelled for high and low disturbance cultivation techniques across various O horizon depths. Some required biochar doses are extreme and would be detrimental to soil.

	High disturbance		Low disturbance	
Soil O horizon depth	Soil Carbon Change	Biochar Dose	Soil Carbon Change	Biochar Dose
cm	t C ha^{-1}	t ha^{-1}	t C ha^{-1}	t ha^{-1}
5	-13.2	18.7	-2.8	4.0
10	-20.1	28.2	-4.6	6.5
20	-34.5	48.0	-8.2	11.6
30	-51.3	72.0	-12	16.9
40	-68.0	96.1	-15.8	22.3

6.5 Discussion

6.5.1 Changes in Soil Carbon and Total Carbon Stock

Both modelling approaches confirm that there is a considerable negative impact of high disturbance cultivation on soil carbon stocks in the first rotation post afforestation. Comparing model outcomes highlights that high disturbance cultivation on peaty gley soils with an O horizon of ≥14 cm (Vanguelova model), and ≥10 cm (Creber model), results in a net reduction to soil carbon stocks in the first 40 years following planting. Low disturbance cultivation methods reduce the volume of disturbed soil and provides a partial buffer to rapid fluctuations in soil conditions, reducing carbon mineralization compared with high disturbance scenarios. Both models show that low and no disturbance cultivation reduces loss of carbon stocks and results in net accumulation of SOC in organo-mineral soils with an O horizon depth ≤35 cm at year 40 following planting. Under certain scenarios for both models, biomass C accumulation through afforestation does not compensate soil C losses at year 20 & 40. The end of rotation total afforestation carbon stock is highly variable across scenarios (and consistent between models) depending on soil, cultivation, and growth parameters, with modelled scenarios ranging between -16 t C ha^{-1} and 153 t C ha^{-1}. This supports Matthews et al. (2020) who showed that productive land classes have the potential to deliver greater net carbon storage (C ha^{-1}) than low yielding trees on low-quality planting ground and that disturbance of organo-mineral soils can result in net C emissions from afforestation that persist for decades.

The net carbon balance output of both the Vanguelova and Creber models at year 40, which show a negative or no total carbon change with afforestation in high disturbance low yield afforestation scenarios, is consistent with ecosystem carbon measurements at year 39 by Friggens et al. (2020). Low dose biochar application has no direct effect on year 40 soil carbon stocks, due to the low application rate (0.0625 t ha^{-1}). The effects of biochar on soil C cycles and SOC priming were not modelled in this study. The effect of biochar on negative or positive carbon priming is variable and poorly understood ((Wang et al., 2016). The indirect effects of biochar on soil carbon through altering soil C cycles creates some uncertainty in the modelled biochar outputs. This uncertainty in modelled soil carbon change from biochar application highlights the requirement for model validation, which was outside the scope of this study. There has been no research on the long term soil carbon fluxes from low dose targeted specified biochar application, although it has been suggested that biochar does not alter soil C fluxes in productive conifer forests (Palviainen et al., 2018).

Blanket biochar applications could alter end of rotation carbon balance, where the dose rate exceeds 10 t ha^{-1}, the effects of high doses on forest productivity and biomass accumulation are unknown and further research is required. Root and rhizosphere respiration has been found to be a primary contributor to variation in soil carbon balance in conifer woodland (Korhonen et al., 2009). Biochar has been found to increase root biomass during establishment (Chapter 3), however the long-term effects of biochar on conifer root structure and rhizosphere respiration rates are not known. Model outputs for low yield native-broadleaf scenarios show higher rates of SOC accumulation through leaf litter, which accounts for the slight increase in year 40 carbon balance compared to Friggens et al. (2020). This suggests that the models could be refined by reducing SOC accumulation rates for broadleaf afforestation, where broadleaf leaf litter contributes to less SOC development and higher C turnover in humic horizons.

The differences between year 40 soil carbon change between models can be attributed to three differences between calculation methods: the rate of soil carbon accumulation through leaf litter, the SOC carbon content and stability of the O horizon and SOC change in mineral soil horizons. Accumulation of SOC arising from leaf litter decomposition is applied at a rate of 0.6 t C ha^{-1} yr^{-1} for YC 10-14 Sitka spruce, rising to 0.8 t C ha^{-1} yr^{-1} YC $\geq$16, from year 15 in the Creber model. The Vanguelova model applies a fixed leaf litter SOC accumulation rate of 0.6 t C ha^{-1} yr^{-1}, which accounts for 8.4 t C increase in total C change at year 40 between models. Peaty gley soils contain a detectable clay content in the O horizon, which reduces the labile C pool by stabilising SOC stocks (Singh et al., 2018). Stable O horizon C stock development improves the resilience of soils to soil carbon loss due to cultivation disturbance, compared to other soil mineralogy. The stabilisation effect of clay mineralogy is included in the Creber model, which accounts in part for the slight reduction in total soil C loss at higher O horizon depths. High clay content soils have also been reported to benefit from biochar application from the negative carbon priming effect from biochar (Wang et al., 2016). Biochar could further stabilise SOC pools, however this would likely only alter model comparisons at high dose rates. Dystrophic blanket peat soils are highly vulnerable to increased mineralization of labile carbon pools resulting from improved aerobicity through drying by drainage, land-use change or climate change (Turetsky et al., 2015). The SOC content and proportion of labile carbon in the O horizon of organo-mineral soils is lower than on dystrophic blanket peat soils and therefore organo-mineral soils are expected to have a lower rate of SOC loss through comparable aerobicity increases. Rates of soil carbon loss for afforestation modelled from data derived from organic soils should be adjusted and validated for woodland creation on organo-mineral soils, which exhibit slightly different soil carbon loss dynamics and rates.

There has been minimal research into the potential application of biochar on blanket peat soils. Biochar could effectively add stable carbon to degraded organic soils during peatland restoration. Care should be taken to limit improvements (or impediment in this context) to soil water and pH properties, which could increase soil aerobic and pH, increasing C mineralisation. Biochar properties could be optimised for use in this context, such as high density, low pH and low porosity pelletised biochar (Edeh & Mašek, 2022). This suggests the potential of biochar additionality in other land based GGR techniques.

Whilst the majority of carbon stock is held within the O horizon in organo-mineral soils, mineral horizons contain some elemental carbon vulnerable to mineralization due to disturbance during afforestation (Bárcena et al., 2014; Vesterdal et al., 2002). Interaction between carbon and clay particles in mineral horizons in peaty gleys stabilises much of this carbon, improving resilience of the labile carbon pool to increased microbial action (Singh et al., 2018). The inclusion of soil carbon loss from mineral soil horizons in the Creber model, and its exclusion in the Vanguelova model, contributes to the differences in soil carbon loss between models at shallow O depth scenarios. This also partially accounts for the reduction to regression slope in the Creber model, as the proportion of soil carbon in mineral horizons decreases with O horizon depth. According to the Creber model, up to 50% of total soil carbon loss during afforestation in shallow O horizon organo-mineral soils can be attributed to mineralisation of SOC in the mineral horizons, potentially improving the accuracy of the Creber model. This partially explains the increased potential of biochar in improving soil carbon stocks and carbon balance in low O horizon scenarios using the Creber model. Consideration of soil C loss from mineral horizons should not be excluded from woodland soil carbon stock change and carbon balance calculations, particularly under high disturbance, shallow O horizon scenarios. This also highlights the importance of considering the impact of cultivation soil C loss on afforestation carbon balance and GGR for low yield planting on mineral soils.

When considering the viability of high disturbance cultivation as a management practice from a carbon perspective on organo-mineral soils, it is likely that low or no disturbance cultivation will be a more effective management prescription in enhancing soil carbon stocks during the first rotation. High disturbance site preparation prescriptions are further compromised by concerns with linear cultivation methods, which are likely to exacerbate soil erosion (Guerra et al., 2017; Vladimír & Lukasz, 2019), nutrient leaching/runoff (Malhi et al., 2018) and negative soil albedo effects (Cierniewski et al., 2018). This supports the adoption of low to no disturbance cultivation during afforestation establishment. Blanket biochar applications to offset soil carbon loss should therefore not be used to justify high disturbance cultivation methods on nutrient poor sites, or sites vulnerable to soil erosion. Historic cultivation of un-improved grasslands, and improved rotational grassland, is widespread on organo-mineral soils in the UK which are managed under low density grazing. Research and soil survey data show that historical cultivation as part of grassland management can significantly degrade the labile soil C pool, which can reduce the elemental C content of the O horizon to ~15 % C, compared to ~42 % C in semi-natural uncultivated peaty gleys (Chapman et al., 2013; Lilly et al., 2020). Historically cultivated peaty gley soils were not included in modelled scenarios but will likely contribute to the reduction of soil carbon loss through cultivation and afforestation due to the decrease of SOC in the O horizon due to past management practices. If the decreased soil carbon loss is proportional to total SOC, historically cultivated peaty gleys would have less than half the soil carbon mineralized during the first rotation when compared with semi-natural soils. This variability in potential soil C loss due to afforestation should be considered when assessing the NetZero contribution and carbon balance of new woodland creation.

6.5.2 Soil Carbon Change Dynamics and Carbon Balance

The scenarios assessed by both models for carbon balance and end of rotation carbon stock showed that under certain site conditions, management prescriptions and expected YC, woodland creation does not increase the net carbon stock of land on decadal timescales. This supports the findings by Friggens et al. (2020), who investigated similar scenarios to those which were modelled. However, the study by Friggens et al. (2020) did not include afforestation on shallow O horizon organo-mineral and high yield classes, therefore the stated negative carbon balance on decadal timescales should not be extrapolated to these scenarios. The initial high stocking density, high mortality post establishment and the restructuring of the site to birch only makes the comparison with the Friggens et al. (2020) study more difficult to compare with the results of this study. The growth improvement from targeted specified biochar application could partially mitigate low productivity induced negative carbon stocks, as shown in model scenarios. Whilst afforestation scenarios with low yield, high disturbance and/or high O horizon depth require decadal timescales to achieve positive carbon balance, afforestation can be an effective short-term (<10 years) GGR method on organo-mineral soils providing soil C loss in mitigated and adequate forest yield (YC ≥14) is achieved, potentially through biochar utilisation. High dose blanket biochar applications (which exceed 10 t ha^{-1}) do not increase plant growth and extreme doses can be detrimental (Ye et al., 2020). Blanket application could be considered as a method to maintain soil carbon stock through establishment in low disturbance scenarios where the O horizon is <20 cm. This will only be achievable where site conditions support productive woodlands and where soil carbon loss can be adequately reduced or offset using low disturbance cultivation methods or blanket biochar application.

The carbon balance of woodland creation over the first rotation on organo-mineral soils is dominated by the balance between soil carbon loss and biomass accumulation. At low O horizon depth and average YC (≥14), most of the cumulative total C change can be accounted for as biomass accumulation. There are therefore similar carbon balance curves across both models, which strongly follow YC curves. Where there are relatively high SOC stocks in the O horizon or slow biomass accumulation at low YCs the difference between model regression curves is considerable. The Vanguelova and Creber models both suggest that high disturbance cultivation on peaty gley soils with an O horizon depth >10 cm is unviable when considering woodland creation as short term GGR technology, as <YC14 has a negative carbon balance over decadal timescales. The difference of year of positive carbon balance between models is more pronounced in low disturbance cultivation scenarios, which have comparatively low SOC loss during establishment, due to the low slope of regression curves at the year of net zero carbon balance. High disturbance scenarios on deep (40cm) organo-mineral soils has a 37 t C ha^{-1} variation between models at the year of positive carbon balance. There is a delay of 7 years to achieve positive carbon balance between models (with the Creber model showing a longer time to the year of positive carbon balance). This can be compared to low disturbance cultivation which has a 10 t C ha^{-1} difference between models with a corresponding proportionally higher delay of 6 years for high yield scenarios. This highlights the importance of accurately modelled soil C loss dynamics, particularly in low SOC loss scenarios when assessing the net carbon removal of establishing woodlands on all soils. Both models show comparable carbon balance improvements in terms of year of net zero. However, there are issues in interpreting the soil carbon balance results for biochar scenarios in the Vanguelova model for the early establishment phase due to the linear rate of soil carbon change applied.

The inclusion of a non-liner rate of soil carbon loss in the Creber model, to account for soil C loss dynamics, results in a longer time to positive carbon balance in all scenarios. The applied non-linear rate of soil carbon loss form high disturbance scenarios in the Creber model is similar to the 9% total soil C loss reported by Karhu et al. (2011) at year 20 of afforestation on organo-mineral soils. High disturbance cultivation for all scenarios on peaty-gley soils resulted in a considerable delay in the year of positive carbon balance when a non-linear soil carbon loss rate was applied. Applying a linear rate of soil C change (net soil carbon loss and accumulation) over the first rotation provides an accurate end of rotation carbon balance for woodland creation scenarios, however underestimates soil carbon loss over the first ~20 years and overestimates soil carbon loss in the last ~20 years of the rotation. Other soil C change models, such as ECCOSE (Smith et al., 2007) & RothC (Coleman & Jenkinson, 1996), account for soil carbon dynamics and show similar proportional carbon changes to the soil C loss dynamics of the Creber model. Accounting for soil carbon change dynamics is critical when assessing the impact of biochar induces growth changes, which can only currently be empirically modelled for the early establishment period. The undercalculation of soil carbon change in the initial period of rotation in linear soil carbon change models overemphasises the effects of biochar induced growth improvements and carbon storage on carbon balance. This means that modelled carbon balance can appear net positive for year 0, despite significant soil C lost identified by a non-linear model, due to the lower proportional soil carbon loss in early establishment.

The direct disturbance of soil during cultivation leads to rapid changes to the soil environment by increasing soil temperature, aerobicity and reducing soil moisture. Almost complete rapid mineralization of the labile carbon pool in directly disturbed soil can be expected following cultivation and subsequent drying (Rodriguez et al., 2021). In nutrient poor soils rates of soil carbon loss would be expected to increase with fertiliser application due to carbon priming effects and elevated soil N status (Butterly et al., 2018). Cultivated soil is less resilient to extended dry periods due to elevated position, undecomposed plant matter and lack of vegetation cover, which may enhance SOC decomposition upon rewetting (Unger et al., 2010). The application of biochar could be effective in mitigating this process (Edeh et al., 2020). Soil carbon loss from the bulk soil (soil not disturbed during cultivation) during the first rotation following afforestation is also exhibits potentially rapid SOC mineralisation (Hernandez-Ramirez et al., 2021). During the early establishment the rate of soil carbon loss from the undisturbed soil is dependent on drainage implementation, through either linear cultivation or direct drain creation (Harris et al., 2018). The rate of soil carbon loss from peaty gley soils due to drainage is comparable to deep peat soils and the carbon loss is directly correlated to drain depth (Leiber-Sauheitl et al., 2014). The rate of soil carbon loss gradually increases, peaking at canopy closure, before declining as the soil environment stabilises and organic input to soil increases. Targeted biochar application would not influence soil drying rates, however the impact on soil moisture in a woodland creation scenario from blanket biochar application is unknown. High rates of biochar could increase the rate of drying and create a larger flux in soil carbon emissions (Edeh et al., 2020). During woodland establishment, up to the stage of canopy closure, cover vegetation changes alter soil conditions driving SOC mineralization. As herbaceous or ericaceous vegetation is replaced by trees the below ground biomass of these plant decomposes, which may stimulate the further decay of labile SOC and increase SOC loss. Growth improvements from biochar application would be expected to accelerate the rate of shade suppression and death of understory plants as the time to canopy closure decreases. The rhizosphere development improvements from biochar application (discussed in Appendix 2 and Chapter 5) could result in improves nutrient capture from decaying vegetation and enhance above-ground biomass growth, improving total carbon storage.

Below ground biomass accumulation led to an accelerated release of soil carbon from the bulk soil in shallow rooting species. It can be expected that as yield class increases, bulk soil in the O horizon is both disturbed and aerated by root development at a greater rate in the first half of the first rotation following afforestation. Carbon mineralization rates, from decomposition of SOC, are promoted through direct drying on the O horizon by root water uptake and through improved soil aerobicity from reduced soil moisture and root disturbance. These mineralisation processes could be increased due to the increase in root development and associated ectomycorrhizal fungi following in response to targeted biochar application (Lei et al., 2022; Palansooriya et al., 2019; Warnock et al., 2007). This may contribute soil carbon loss from soil priming in certain scenarios. Wang et al. (2016) have shown that sandy soils are particularly vulnerable to carbon priming from biochar application, however the rate is expected to not influence soil carbon content. The effect of cultivation methods on seedling tree growth also lacks adequate data to be included in models. It is generally accepted that cultivation improves early stage growth (Paterson, 1999) and reduced the time to canopy closure, however specific research is limited.

Model scenario outputs support studies (Friggens et al., 2020; Matthews et al., 2020) which suggest that area-based planting targets require carbon balance assessment in order meet GGR policy objectives. Bias in afforestation towards unimproved grassland and heathland where organo-mineral soils are extensive in Scotland (Brown et al., 2014) results in reduced net carbon balance and GGR potential compared to more productive sites, where less soil C loss is expected. Improvements in establishment success because of biochar application are expected to reduce on higher productivity sites where soil nutrient regime does not limit growth. Other establishment costs accounted for in the full Creber LCA model, and the Woodland Carbon Code, further reduce woodland creation carbon balance by 2 – 10 t C ha^{-1} and should also be considered. The impact of biochar was not included with other management practices, such as chemical application and tree protection for comparability between models tested in this study. Targeted biochar application has the potential to have a limited fertilisation effect on herbaceous plants compared to conventional fertiliser (and therefore may reduce chemical weeding intensity, compared to scenarios where NPK fertiliser is applied) (Ye et al., 2020). Nutrients held within biochar can be applied directly to seedling roots at planting, allowing preferential uptake by seedlings over annual weeds, whereas surface applied fertiliser may deliver nutrients to herbaceous weeds and increase herbicide requirement. Further research is needed before the inclusion of such terms in LCA modelling.

As NetZero commitments focus on delivery in Scotland to 2045 and 2050 for the rest of the U.K., the contribution of woodland creation to GGR will reduce and become negative (with net emissions), scenario dependent, as these years are approached. For current woodland creation (planted ~2025) to have a net carbon benefit by 2045, which is the NetZero ambition for the Scottish Government (UK government has 2050 benchmark), the year that a positive carbon balance is achieved must be <20 years from planting. Productive woodland creation (YC>10) will contribute to net GGR at year 20 on organo mineral soils with an O depth <35 cm and low disturbance cultivation. Low yield native broadleaf planting under all cultivation methods, is not viable as a short-term (<20 year) GGR method for all cultivation methods on organo mineral soils. High disturbance cultivation on O depths <10 cm, regardless of modelled yield is also not viable. Biochar has little impact on carbon balance in high disturbance cultivation scenarios or high carbon soils, however, can be an effect technology in the context of improving the short-term carbon balance of woodland creation in low yield scenarios. Biochar can provide greater carbon balance improvements at low O horizon depths, when high disturbance cultivation is used, due to the lower soil C loss. It is unlikely that nutrient poor establishment sites will be suitable for high disturbance cultivation. Targeted biochar application can extend the viable planting periods, where woodland will have a net positive carbon balance by 3 to 6 years in low disturbance planting. Whilst the viability of woodland creation as a short term GGR technology is highly variable, medium term benefit carbon storage must be also considered. NetZero targets should not be used to influence reduce afforestation rates due to short term negative carbon balance (net emissions) as the year of NetZero is approached and afforestation scenarios become net emitters during 2045 and 2050.

High disturbance cultivation as a management practice on organo-mineral soils should be discourage due to the negative impact on soil carbon, particularly during the establishment phase, and the subsequent impact on woodland creation carbon balance. The silvicultural benefits of all manual cultivation on deep (≥40 cm) organo-mineral soils should be considered in relation to soil C change. Where soil C dynamics over the establishment phase are modelled, reduced net soil C stocks in the first 20 years following planting can be expected with low disturbance cultivation on organo-mineral soils ≥40 cm. This supports recent U.K. afforestation cultivation guidance, which has been partly developed using the Vanguelova model. The

Creber model provides strengthened evidence on cultivation method and establishment carbon balance by the inclusion of soil carbon loss dynamics. Woodland creation policy in relation to climate change mitigation and NetZero should prioritise carbon stability, forest yield and incentivise the planting of higher quality land. The woodland creation application process could be improved to assess the carbon contribution of new afforestation in relation to NetZero and include other GGR technologies, such as biochar. Targeted specified biochar application can be considered an effective management intervention in improving the short-term carbon storage potential of establishment and reduce the time to GGR contribution by delivering some carbon storage and improving early growth in the establishment phase. Blanket biochar application can effectively offset total soil carbon loss during land use change to forestry, however modelling is required to calculate application rates and assess the potential silvicultural impact on crop productivity and soil health. Biochar as a GGR technology has additionality to other land-based carbon storage methods, with the modelled combination of biochar and forestry showing improved carbon storage potential compared to when these methods are assessed independently. The optimisation of GGR technologies can help accelerate carbon storage and further their contribution to NetZero.

6.6 Contributions

Part of this chapter was produced by the author as a manuscript for Scottish Forestry under the title; Disturbance is key: time to delivery of net climate mitigation from woodland creation. Insights from two empirical soil carbon models. The co-authors were Dr Mike Perks[2], Dr Saran Sohi[1], Dr Robert Matthews[2] & Dr Elena Vanguelova[2] ([1] University of Edinburgh [2] Forest Research). Co-authors contribution was as follows; Dr Elena Vanguelova provided access to her soil carbon model and provided methods relating to her model, Dr Robert Matthews provided access to forest yield models and information for the methods, Dr Mike Perks provided advice for the soil modelling and provided some text for the forest yield method and Dr Saran Sohi provided advice during model development. Dr Mike Perks and Dr Elena Vanguelova provided comment on the draft manuscript. The full LCA which has not been included, as it is not directly relevant to this thesis and partly under a NDA (for the forest nursery section), was developed during a placement with Tilhill Forestry as part of the NERC studentship. Data used for forest establishment emissions and nursery production was provided as part of this and the author would like to thank Peter Whitfield (ret.), Andrew Vaughn and Tim Liddon (ret.) at Tilhill Forestry for facilitating this.

Chapter 7

Biochar synergies in U.K. forest establishment

The aim of the studies conducted as part of this PhD (Chapters 3-6) were to investigate the and quantify the potential of low dose biochar applications as a method to improve seedling nutrition in nutrient poor soils and investigate the wider silvicultural implications of targeted biochar application during early forest establishment. This work was driven by the challenges facing upland forest establishment, namely soil nutrient decline and increased abiotic stress factors resulting from climate change. There is extensive research into the agronomic application of biochar, however little attention has been specifically given to productive forestry. This thesis provides a foundation for future research in this area and identifies the potential of specified biochar in improving establishment success and seedling abiotic stress resilience. The effect of biochar on nutrient availability and accessibility is discussed, along with underpinning mechanisms. A range of distinct biochar types were compared to inorganic mineral derived phosphorus (P) fertilisers, including a specified biochar which was developed to address identified soil limitations to seedling growth. This chapter provides a summary of key findings, as presented in Chapters 3 to 6. The primary effects of biochar on nutrient and water accessibility and availability are then discussed, along with the physiological response by seedling to biochar application. Synergies between these factors are then explored. The silvicultural potential and pathway to implementation of biochar by the forestry sector are examined, including the implications of biochar applications on forest carbon. To conclude, future research priorities are highlighted and the limitations of thesis outcomes for practical implementation are identified.

7.1 Key Research Findings

Seedling Growth

The potential of targeted specified biochar application to provide tree nutrition to Sitka spruce (*Picea sitchensis*), Douglas fir (*Pseudotsuga menziesii*) and Scots pine (*Pinus sylvestris*) was tested in in this study. The study described in Chapter 3 tested the observed seedling responses to biochar application in a controlled environment, the Chapter 4 study tested biochar use on seedling establishment in a field environment. A range of distinct biochar types were applied at planting to Sitka spruce (*Picea sitchensis*), and Scots pine (*Pinus sylvestris*) seedlings at planting on a nutrient limited, P deficient soil or growing media. Biochar was found to increase early establishment growth compared to no application and inorganic mineral derived P fertiliser in both studies. Above ground biomass was significantly higher in some biochar treatments (notably VCZ biochar) compared with control and mineral fertiliser treatments of the experiment described in Chapter 3. End of experiment biomass was not assessed in the field experiment (Chapter 4), preventing direct assessment of yield class change. The specified vascular cambium derived biochar (VCZ), which has ideal physical and chemical properties for use in P limited forest soils, outperformed other biochar types in both studies. Optimal biochar treatment dose rate tested in the controlled environment study (Chapter 3) was species specific. Direct species comparison was difficult due to single origin planting stock.

Seedling mortality was high in Year 1 of both studies (Chapter 3 and 4) in seedlings treated with high available P treatments (P infused biochar and mineral P fertilisers). Rapidly declining soil pH from mineral derived P following application may have contributed to increased mortality, however temporal soil pH change was not assessed. Scots pine seedlings showed higher year 1 mortality in the controlled environment study and the lower mortality field experiment compared to Sitka spruce. This contradictory finding may be due to phenological differences between Sitka spruce planting stock or lower soil buffering capacity in enclosed pots used in the controlled environment study. There were low seedling mortality rates after Year 1 across all species and treatments in both studies. This may in part be attributable to planting shock and highlights the sensitivity of newly planted seedlings to environment factors.

Inorganic mineral derived P fertilisers are not effective in improving overall seedling growth compared to the control treatment but can provide a short-term nutrient source. Biochar application was found to improve early establishment seedling growth, compared to no application and inorganic mineral derived P fertiliser, outperforming conventional fertiliser in both studies. Seedling growth rate trends over the experimental period, in response to application treatments, provided insight into longer term impact of biochar on establishment. Growth rates show a gradual decline over the experimental period with no application, high available P treatments showed a highly variable growth rate across years (suggesting low resilience to abiotic stress factors) and VCZ treatments showed a consistent high growth rate.

Root development was assessed in Chapter 3 and Appendix 2. Root biomass and lateral root density was different between treatments in all species. High available P fertiliser applications have a profound impact on root development and morphology, reducing root biomass and lateral root density. Limited rhizosphere development can limit key soil nutrient bioavailability. This may result in decreased nutrient uptake and subsequently limit growth rate. The suppression of root development may contribute to the lower growth rates observed in this study in mineral fertiliser treated trees, compared to comparatively low available P biochars. Biochar was found to increase the accessibility of P, K and Cu, particularly in VCZ biochar, evidenced by higher foliar nutrient concentration. This may have been a factor in the increased aboveground biomass growth and accumulation in all tested species, as the soil nutrient content was a limiting factor to seedling growth in both studies. Specified VCZ biochar, which contains moderate available P, increased root biomass and lateral density, potentially improving nutrient bioavailability. Improvements in foliar nutrient concentration with biochar application were more modest in Scots pine treatments. This may be due to the tolerance of the species to low nutrient soils and the suitability of the species on highly acidic sites (which may have contributed to the lower observed root mortality compared to other species, improving nutrient bioavailability). The results of this experiment highlight the potential of novel fertilisers in improving early growth in the establishment phase, in turn perhaps expanding the range of viable species which can be established on nutrient poor environments. Chlorophyll fluorescence was used to assess photosynthesis and can be used as a proxy for assessing seedling stress response. Biochar was found to limit the decrease in seedling photochemistry following planting in nutrient limited soil or growing media in both studies. This suggests that biochar may reduce the stress response of seedlings, improving resilience to stress factors. Soil nutrient availability was likely the principal environmental factor in limiting seedling photochemistry in the controlled environment study described in Chapter 3, as temperature and water stresses were controlled. The environmental factors which contributed to changes to photochemistry in the field study (Chapter 4) are difficult to quantify. The effects of biochar on soil water availability and nutrient availability likely contributed to the differences in photochemistry in outplanted seedlings, suggesting improved resilience to abiotic stress factors. Results of this study suggest that targeted biochar, when correctly used, has the potential to help mitigate declining forest productivity caused by climate change and soil nutrient loss.

The results of this study show that biochar can improve growth, biomass accumulation, root development and Chlorophyll fluorescence in conifer seedlings grown in low nutrient conditions. This is likely due to the increased nutrient uptake of seedlings following application of biochar. Results from the study described in Chapter 3 show that needle macronutrient concentrations (NPK) and total foliar nutrient pools were increased at certain dose rates with biochar application in all tested species compared to the control, however this was principally confined to VCZ treatments. Mineral P fertilisers were found to significantly increase needle Cd concentration, providing evidence of direct uptake from the nutrient source (which is high in Cd), Needle Cd concentration remained below the threshold where Cd concentration would be consider limiting plant growth through toxicity. These differences in foliar macronutrient concentrations were not observed in the results from the field study described in chapter 4, with no differences between treatments in both tested species. This provides further indication that other environmental factors, such as water availability, may have contributed to the observed differences in growth and photochemistry response of seedlings to biochar amendment. Environmental heterogeneity in the field trial and seedling phenological may have increased the likelihood of type II error in the field experiment results. Despite no differences between seedling foliar nutrient concentrations between treatments, biochar was found to increase mean needle macro nutrient concentrations above the threshold where trees are considered to be nutrients deficient in some VCZ treatments. This suggests that biochar may be able to partly mitigate seedling nutrient deficiency in the early establishment phase. The improvements to seedling growth during the early establishment phase is therefore likely not only from mitigating nutrient deficiency.

The increased foliar nutrient concentration and growth responses of seedling to biochar suggest that biochar can be effective at delivering nutrients to seedlings. VCZ biochar was found to outperform conventional fertiliser applications, in terms of growth rate improvements and foliar nutrient uptake, despite relative low nutrient concentration. Biochar can directly deliver available nutrients to soil but can also result in a root and mycorrhizal response, potentially increase nutrient accessibility and uptake. This study found that biochar can increase root biomass (Chapter 3), stimulate P foraging by seedling roots (Appendix 2), increased fungi abundance (Chapter 5) and biochar particles may be exploited by mycorrhizal fungi (Chapter 5). Rhizosphere interaction with the charosphere is deterministic to seeding nutrient uptake from biochar particles and the accessibility of available nutrients. Specified VCZ biochar increased ECM abundance in surrounding growing media, compared to all other treatments, indicating potential hyphal nutrient foraging for P and subsequent ectomycorrhizal extramatrical mycelium (EEM) proliferation. High available P concentration treatments (inorganic mineral derived P fertiliser and P infused biochar) profoundly reduced ECM abundance, show a likely avoidance and suppression effects. Scots pine root response to treatments showed the same response (Appendix 2), suggesting similar mechanisms or a symbiotic response.

Biochar structure was found to determine the extent of ECM colonisation of biochar particles, and the morphology of the EEM. Pelletised feedstock restricted internal hyphal growth to fissures between pellet aggregates, which may limit nutrient accessibility by physically immobilising nutrients held within pellet aggregates. Surface growth followed depressions and fissures in the surface forming undifferentiated strands. P infused biochar reduced ECM abundance, possible due to a suppression effect from high available P concentrations. ECM fungi were able to utilise the cellular structure of unprocessed (non-pelleted) wood derived biochar to colonise internal spaces entering biochar through large diameter axial tracheids and resin canals. This may allow ECM fungi to exploit nutrient replete areas within biochar particles. Hyphae were found to proliferate throughout the internal structure of unprocessed biochar; however, surface entry points were limited to pores <20µm in diameter. The EEM formed a dense labyrinthine morphology on unprocessed biochar until surface enter points were found. These results suggest that biochar structure may be critical to the exploitation of internal nutrients by ECM fungi. The physical structure of biochar, and nutrient accessibility, likely accounts for the some of the observed differences in seedling response to tested biochars observed in Chapter 3 and Chapter 4. Increased mycorrhizal and root surface area from biochar application may also improve bioavailable nutrients and water from the soil, benefitting seedling establishment, The interaction between the rhizosphere and charosphere determines the nutrient accessibility to the host tree, which influences the efficacy of biochar for forest nutrition.

The growth results from Chapter 3 and Chapter 4 were used to model establishment carbon balance. This provided an indicative assessment of the potential impact of improved establishment growth and survival from biochar application on the net carbon storage of afforestation on nutrient poor organo mineral planting sites. Soil carbon changes were modelled using an empirical soil carbon model that was compared to an existing forest soil carbon model, used to develop cultivation forest policy. The comparison of two independent empirical models for soil C change in organo-mineral soils following afforestation show consistent total change in end of first rotation carbon balance, for all modelled scenarios. Results suggest that woodland creation on organo-mineral soils should not be conducted under the assumption of delivery of short-term net carbon sequestration. Reducing cultivation disturbance on all O horizon depths in organo-mineral soils can improve end of rotation total net carbon balance and considerably reduce the year to positive carbon balance by limiting SOC mineralisation during establishment. Model variation in the rate of soil carbon loss (comparing linear and modified Boltzmann sigmoidal models for soil C loss) highlights the importance of soil carbon loss dynamics over the establishment period when assessing the year to positive carbon balance for woodland creation, at which point afforestation improves the carbon stock of land and contributes to greenhouse gas removal (GGR).

Overall, modelled biochar benefits were found to increase forest carbon stocks over the establishment phase, and this could offset soil carbon loss in some scenarios. The potential improvements during growth in the establishment phase due to targeted biochar application was found to quicken the year to positive carbon balance for establishment and compensate for soil carbon loss from mounding in nutrient limited organo mineral soils. In addition to target biochar applications, blanket biochar application during woodland creation can also be used to ensure that the carbon balance of woodland creation remains positive, and the required dose rate can be calculated from the developed soil carbon models. There are potential trade-offs between carbon storage and soil productivity linked to blanket biochar use on nutrient poor forest soils. This study found that the efficacy of biochar types is highly dependent on physical and chemical properties. This study demonstrated that practical biochar applications can be effectively introduced to the forestry sector to improve forest early establishment growth and may improve net carbon storage in the establishment phase on nutrient poor planting sites. The results highlight the need to optimise biochar properties to address particular soil parameters and that targeted low dose biochar application can be effective. Modelled biochar scenarios were based on the specific biochar type, dose and soil tested in this study. Further work is required to validate any modelled carbon changes due to biochar application and modelled biochar carbon is an indicative analysis at this stage.

This study has shown that biochar can improve early growth in the establishment phase and effectively mitigate abiotic stress factors. Biochar application may be an effective management intervention during forest establishment on nutrient poor planting sites. There is a clear research and policy pathway for biochar adoption by the forest industry, which would enable biochar to become a recognised management tool for adapting U.K. forestry to increased climate pressures and soil P decline.

7.2 Targeted Biochar use in Early Establishment

The effects of biochar on the rhizosphere, wider soil ecosystem, nutrient cycles and seedling development are synergistic and mutalistic, which likely accounts for the increased silvicultural benefit of biochar compared to mineral fertiliser found in this study. The principal interactions and effects of biochar in a nutrient limited plant-soil system are shown in Figure 7-1. Biochar can enhance synergies between soil ecosystem functions which can positively affect above ground biomass growth and resilience. Biochar can influence mycorrhizal fungi, soil properties, root systems and soil biology through multiple mechanisms. The extent to synergies, and trade-offs, are highly dependent on biochar properties (chemical and physical), application rate, application frequency and the soil environment (Joseph et al., 2021). Targeted biochar amendment creates localised changes to soil properties, which can stimulate fungal, microbial and plant activity, resulting in broader alteration of the soil environment. Rapid localised changes to soil properties resulting from specified targeted biochar application has the potential to create distinct soil "hotspots" with improved soil properties which act primers for the development of wider soil improvement through multiple synergistic mechanisms. This section will describe an overarching vision of how specified biochar application can alter soil properties in nutrient poor acidic forest soils and how the synergies of these alterations can influence seedling growth and resilience to abiotic stress factors. It is these mechanisms which likely result in the observed growth response of seedlings to biochar application found in this study. Three themes will be discussed, these are: *i*) the effect of biochar on soil nutrient and water availability, *ii*) how biochar application can alter soil nutrient and water accessibility through rhizosphere responses and *iii*) the implications of changes to availability and accessibility of soil nutrients and water on seedling physiology.

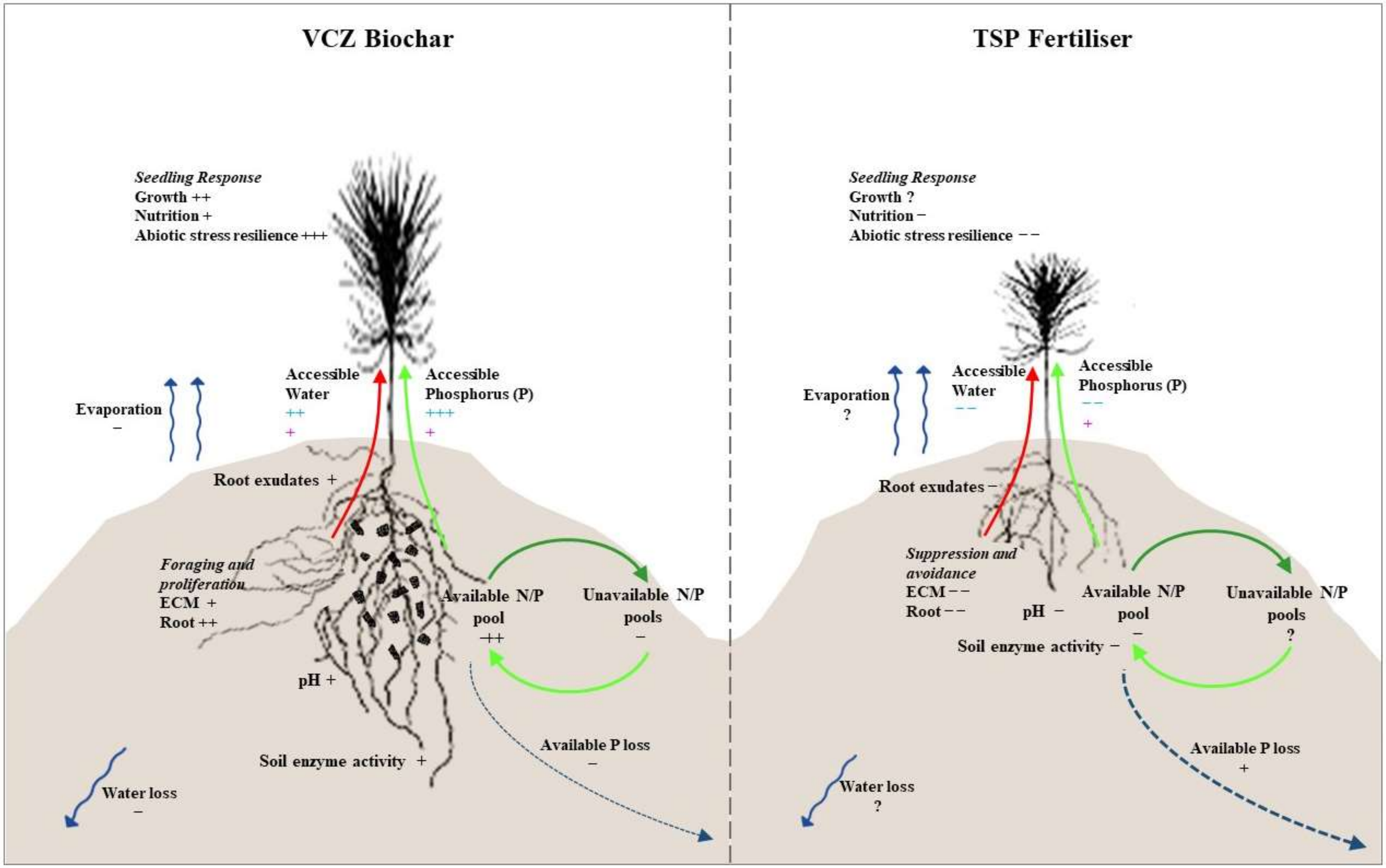

Figure 7-1- Concept illustration on the effects of specified VCZ biochar application and TSP fertiliser application in a nutrient limited podzol. Positive or negative effects (+/-) are compared to the control (no application), blue indicates indirect effects and purple direct, uncertain effects or interactions are displayed (?).

7.2.1 Seedling Responses to Biochar

In this study, constrained to the tested environmental parameters, specified biochar application increased seedling growth and establishment success in the early establishment phase. The rhizosphere was also enhanced, through root proliferation and increased ECM associations. Biochar was found to improve seedling nutrition, ameliorating local soil nutrient deficiency which could be exploited by seedlings. Seedling photochemistry was also found to be improved following biochar application, suggesting the biochar may limit plant stress potentially due to drought stress factors. The strong correlation between above ground growth and below ground biomass can result in a positive feedback cycle in biochar treated seedlings. As biochar stimulated root development increases soil nutrient availability and accessibility, seedling nutrition improves and above ground biomass accumulation accelerated, this creates more resources which can be allocated to root and EEM growth, in turn further improving nutrient and water accessibility. This feedback loop is likely responsible for the prevention of seedling establishment check (where growth rate declines due to nutrient deficiency and interspecific competition) in biochar treatments. Biochar induced establishment improvements are due to numerous interlinked mechanisms detailed in this chapter, however the principal effect can be suggested as the improvement of soil plant accessible P, in the tested P limited soils. The synergistic effect of biochar on rhizosphere development and soil nutrient availability probably results in increased uptake efficiency, suggested by the increased foliar nutrient concentration in biochar treated seedlings, and total available nutrient supply Biochar is unlikely to improve establishment seedling growth in soils which have a high soil nutrient regime. Deb et al. (2016) state that soil P-status is the principal variable which determines biochar induced growth improvements over biochar specification or crop species.

The synergies between biochar and the soil ecosystem may result in increased plant deliverable nutrients compared with higher nutrient concentration of directly applied inorganic mineral derived fertiliser and enhances the accessibility of these nutrients. The relative increase to plant uptake of soil nutrients with specified biochar application likely contributed to the observed increase in seedling nutrition compared to conventional fertiliser application. Specified biochar can enhance ecosystem functions, which in turn may improve forest growth in the establishment phase and resilience.

7.2.2 Soil Nutrient Availability

The effect of specified biochar application on soil nutrient availability can be attributed to beneficial alterations to soil ecology and soil chemistry. The multifunctionality on biochar improves soil available nutrients and water through a number of mechanisms which interact mutualistically.

Nutrient availability - Soil chemistry

Biochar can directly deliver available nutrients held within the structure of biochar to soil. During pyrolysis a high proportion of nitrogen (N) is lost, however P is retained and concentrated. The specified VCZ biochar was found have an available P concentration of 3.68 ± 0.49 mg g^{-1}, which directly delivered 92 mg of available P per seedling at the intermediate tested biochar dose rate. This is a relatively low direct available P dose and is not enough to prevent P deficiency in the tested soils. VCZ biochar was found to alleviate P deficiency at low targeted dose rates, resulting in optimal foliar P concentrations, which may be due to the indirect increase in soil available P due to biochar interaction with the soil P cycle. The relatively high pH of VCZ can locally increase soil pH to optimal levels for P availability in highly acidic soils. Increased soil pH from biochar application limit immobilisation of available P by adsorption onto Al^{3+} and Fe^{3+} molecules, which is one of the key mechanisms limiting available P in nutrient poor podzolic soils (which have a high Al^{3+} and Fe^{3+} content). This mechanism increases the retention and concentration of soil available P by slowing the cycling of mineralised organic P and desorbed P back into unavailable P pools. The temporary increase in local soil acidity with high P fertiliser application, may increase the adsorption and immobilisation of soil available P, which was indicated by relatively low foliar P concentration in some inorganic mineral derived fertiliser treatments.

Nutrient availability - Soil microbial activity and mycorrhizal fungi

Localised "nutrient hot spots" created within and around biochar particles may stimulate wider nutrient mineralisation from the causal sequence of increased microbial activity and ECM fungal activity dispersing outward from biochar hot spots. Biochar can also stimulate soil disturbance from root growth and increase soil fauna abundance, in turn accelerating nutrient cycling. These factors can cause biochar to indirectly increase soil available nutrients. The extent of nutrient availability alterations from biochar induced changes to the soil ecosystem is highly dependent on soil properties and biochar properties. These is a clear synergy between the direct effect of biochar on soil chemistry and the degree of available nutrient improvement through microbial, root and fungal activity.

Phosphate solubilising microorganisms

Soil amendment with biochar increases soil microbial biomass and microbial activity (Pokharel et al., 2020; Zhang et al., 2018). Biochar incorporation into soil increases phosphate solubilising microorganism (PSM) activity and can provide suitable conditions for increased colonization of phosphate solubilising bacteria (PSB) and mycorrhizal fungi, (principally ECM fungi in U.K. productive conifer forest ecosystems) which are considered the predominant PSMs (Ali et al., 2020). Phosphate solubilising microorganisms (PSMs) improve solubilisation of soil phosphorus, increasing soil available phosphorus, without increasing soil total phosphorus (Siddiqui et al., 2016). ECM fungi has also been shown to forage for organic P and convert organic P to available P (Rousseau & Reid, 1991). Biochar may therefore increase soil available phosphorus through both direct phosphorus delivery and through increasing soil available phosphorus by increasing PSM activity. The properties of VCZ biochar are consistent with the proposed mechanisms which increase PSM activity, and this could increase soil available phosphorus indirectly from biochar application. The relatively high pH of VCZ biochar partially neutralises local soil pH and increase the growth and colonization of PSB and mycorrhizal fungi. The high surface area and pore structure of VCZ biochar match the suitable habitat for PSM colonization described by (Yang et al., 2019), which is partially confirmed in Chapter 5 (PSBs were not assessed). This contributed to the increased nutrient uptake by VCZ treated seedlings, in effect highlighting the biological fertilisation value of VCZ biochar. There has also been evidence of synergies between biochar, PSB and the increased desorption of P bound to Al^{3+} and Fe^{3+} (Han et al., 2022).

Soil N Bioavailability

Biochar has been found to improve seedling N nutrition, despite containing little to no available N. This is due to the effect of biochar on soil N cycling. In some cases biochar application can supress the accumulation of NH_4^+-N and NO_3^--N (Liang et al., 2016; Schomberg et al., 2012; Spokas et al., 2012), which can protect the total N pool from degradation due to reducing NO_3^- leaching through the soil profile (Borchard et al., 2019). NH_4^+-N adsorbed onto biochar is bioavailable, which could contribute to improved available N retention and subsequent seedling nutrition (Taghizadeh-Toosi et al., 2012). Biochar application was found to increase foliar N concentration in this study, suggesting that biochar improves N availability in the soil (despite only trace native available N content). The low N concentration of biochar suggests the effect of biochar on soil microbial communities, mycorrhizal associations and root development can enhance bioavailable N through mineralisation of organic nitrogen pools and could contribute to improved plant uptake. Biochar can promote microbial and mycorrhizal fungi, which will improve the biofertilisation value of biochar for N availability. This contradicts the findings of Pingree et al. (2022), who found no evidence of increased microbial activity following biochar application to boreal forest soils and suggested that the plant growth response is not due to soil biological processes. The trace N concentration in biochar, increase in available soil N and the increase in foliar N found in this study strongly suggest an increase N mineralisation due to increased microbial activity.

Soil Enzyme Activity

Soil enzymes play a major role in soil biochemical functions; thus their activities affect the changes of soil C decomposition, and nutrient cycling (N and P). Biochar decreases C-cycling enzyme activities, but biochar application has been found to increase the N and P cycling enzyme activities in soils (Zhang et al., 2019). Increased N and P cycling is most pronounced in biochar produced at low temperatures and with high nutrient content feedstock materials and biochar applications in acidic soils. VCZ biochar application in acidic forest soils may contribute to improved P and N cycling enzyme activities. This suggests that VCZ biochar can improve soil health and increase soil available nutrients through increased enzyme acquisition and cycling (Fan et al., 2018; Itziar Alkorta et al., 2003). Studies have also found that inorganic mineral derived P fertilisers can inhibit soil enzyme activity in conifer forest soils (Fatemi et al., 2016).

Root Nutrient Cycling

It is widely accepted that the roots of conifer species can exude compounds (phosphatases and organic acids) that can mineralise soil organic P and mobilise mineral soil P pools (through desorption and dissolution), locally increasing soil available P (Attiwill & Adams, 1993). This has shown to be strengthened by ECM associations (Püschel et al., 2021). The significance of this ECM mediated nutritional benefit mechanism has not previously been tested in a nutrient limited forest establishment context. The proportional increase in root biomass and lateral root density in VCZ biochar application treatments expands the area of the rhizosphere. It is likely therefore that the improved P cycling from organic and mineral P pools to available P through root exudates will increase with biochar application. The fine root suppression observed in high dose available P treatments (including inorganic mineral derived P fertiliser) may limit nutrient cycling in the rhizosphere and could degrade soil available P pools (Hendricks et al., 1993).

Soil fauna

Soil invertebrates play an important role in soil nutrient cycling (Reichle, 1977). Below-ground nutrient turnover and cycling is increased with higher invertebrate abundance and diversity. This transfers N and P from inactive pools into biologically active components, which will add to the available P pool (Coleman et al., 1983). Soil invertebrates were observed migrating into VCZ biochar particles, as refugia. This has the potential to move nutrients into biochar particles, locally increasing biochar P, which can then be exploited by ECM hyphae.

Synergies between nutrient availability factors

Nutrient cycling in forest souls is highly complex and the chemical and biological factors which biochar can influence interact synergistically (Sharma & Sharma, 2004) (Foster & Bhatti, 2006). The combined net beneficial effect of VCZ biochar on factors which increase the soil available nutrient pools may result in improved soil nutrient cycling with biochar effectively functioning as a bio-fertiliser. Target biochar application can locally increase soil available nutrients in the rhizosphere through indirect and direct processes, this can result in a greater increase in available nutrients than the native nutrient content of wood derived biochar. Conversely, conventional fertilisers can detrimentally limit biological nutrient cycling processes, causing a decline in available P from organic and mineral pools, whilst also increasing available P adsorption to Al^{3+} and Fe^{3+}. Synergies between biochar and nutrient cycling by belowground biota and biochar chemistry partially explains the relative seedling growth improvement, and disproportionate soil available P improvement, when low dose rates of biochar is target applied to nutrient poor soils, when compared to no application or high available P conventional fertilisers.

7.2.3 Soil Nutrient and Water Accessibility

The increased seedling growth, and improved photochemistry from biochar application observed in this study may be due to improvements to both seedling nutrition and plant accessible soil water. Improvement to measured seedling establishment parameters from targeted biochar application cannot be simply attributed to the direct and indirect enhancement of soil available water and available nutrients. The effect on seedling physiology is the result of the combination of improved soil properties coupled with increased root and mycorrhizal development which improves the accessibility of soil water and nutrients.

Nutrient Accessibility - rhizosphere development

This thesis has shown that biochar can effectively increase seedling foliar nutrient concentration compared to conventional fertiliser and no application, suggesting that biochar can enhance the accessibility and uptake of soil micro and macronutrients by treated seedlings. This could be achieved by three principal factors which are a result of many interconnected mechanisms. Biochar increases root and mycorrhizal development in proximity to nutrient replete biochar particles, biochar stimulates the development of fine roots and filamentous ECM hyphae, and biochar application can result in improved seedling nutrition which can promote further rhizosphere development.

Root interactions with the charosphere could be a key factor in plants accessing biochar-derived P (Chen et al., 2021)). Seedling roots were found to preferentially forage for biochar particles and fine roots exploited longitudinal fissures in biochar particles in order increase root-biochar contact (likely contributing to the improved nutrient transfer and uptake). The porous structure of biochar derived from unprocessed wood-derived biochar makes it easier for roots and hyphae to penetrate and colonize biochar particles, increasing the accessibility of nutrients held within biochar internal spaces, which is consistent with findings by Jaafar et al. (2014). The high internal and external surface area of biochar provides greater opportunity for nutrient transfer (Prendergast-Miller et al., 2014). The proliferation of mycorrhizal hyphae inside biochar through fissures and cellular pore structures was found to stimulate further hyphal growth and colonisation of internal biochar spaces, improving the accessibility of nutrient held in biochar particles. This supported in similar studies, which assessed arbuscular mycorrhizae (AM)) colonisation (Hammer et al., 2014; Jaafar, 2014; Jaafar et al., 2014).

The improved growth and seedling nutrition resulting from biochar application, and the associated increase in nutrient accessibility and availability, will accelerate root development. Below-ground biomass is strongly correlated to above-ground biomass and as yield curves are followed growth exponential increases until the maximum mean annual increments. This will create a positive feedback loop, as increased aboveground growth will result in greater energy resource for rhizosphere development. In turn this will increase nutrient accessibility due to root and ECM growth through the bulk soil.

Water Accessibility

Biochar can directly alter soil water content held at field capacity (FC), wilting point (WP) and plant available water content (AW). A meta-analysis by Razzaghi et al. (2020) found that biochar significantly increased AW by 45% in sandy soils, comparable to the soil of the experimental field site (Chapter 4), compared to the loamy and clay textured soils (by 21% and 14%, respectively). This suggests that biochar can effectively improve water availability in drought sensitive soils. The effect of biochar on direct changes to soil water properties is highly dependent on biochar properties and doses (Edeh et al., 2020). The physical structural properties of VCZ biochar match the optimal biochar properties for improving soil water properties, hydrophilic with high intra-porosity. The low bulk density and high micro-porosity of VCZ biochar has the potential to increase water holding capacity in free draining soils and reduce the wilting point in imperfectly drained soils. Application method and dose is highly determinant of the effect of biochar on soil water properties. The targeted application of biochar used in this study directly delivers biochar into the rhizosphere of seedlings, potentially improving soil water properties in the rhizosphere at low dose biochar application rates.

The mechanisms which contribute to the increased rhizosphere development in response to biochar application discussed in relation to nutrient accessibility contribute to improved water accessibility. Biochar application can improve plant accessible water content through stimulating root growth, particularly root hair density, which directly increases the ability of plants to uptake soil water (Razzaghi et al., 2020). The increase in the ratio between below ground biomass and above ground biomass, following biochar application, increases the relative capacity of root water uptake. Mycorrhizal fungi also increase potential water uptake for host plants via the direct uptake and transfer of water and nutrients through the fungal hyphae to the host plants (Sohrabi et al., 2012). Biochar has been shown to increase hyphal density, within and contiguous to biochar particles which in turn will allow associated plants to access water held within biochar. This effect of biochar on ECM development is expected to be greater in restock establishment sites, where mycorrhizal mycelium are extensive and well established, compared to woodland creation sites where there is likely less potential for rapid ECM colonisation (Flynn et al., 1998; Mah et al., 2001). The mechanisms which stimulate root and ECM growth, through foraging and proliferation, are likely in response to nutrient acquisition. Biochar is therefore less likely to improve water accessibility on nutrient rich establishment sites, where biochar will have a weaker effect on rhizosphere development.

Edeh and Mašek (2022) identified that biochar small intra-pore diameter (pores within biochar particles) can be hard for plants to access. The ECM colonisation of biochar may improve the ability of seedling to access available water held within biochar particles. More research is required to assess the efficiency and water transfer pathways in ECM colonised biochar. Low-dose targeted biochar application will have no measurable effect on bulk soil water properties or drought resilience in established woodlands, however, has a profound impact on the drought resilience of seedlings during establishment

7.3 Biochar and Forest Carbon

The effect of biochar on seedling growth, due to improves water and nutrient availability and accessibility, directly implicates forest carbon balance. The improved rate of biomass accumulation resulting from biochar application may help compensate soil carbon loss during planting and reduce the time for establishment to become net carbon positive (and contributing to net carbon storage). The improved resilience of seedling to abiotic stress factors can also ensure that expected carbon accumulation during planting is achieved (as growth rates are maintained). Biochar application also directly stores recalcitrant carbon, although in low dose application this has a minor contribution to the total carbon pool. Biochar also has the potential to alter soil carbon pools, though carbon priming processes (Wang et al., 2016). Biochar carbon priming is generally synergistic with nutrient mineralisation from organic pools and is interlinked with nutrient availability (Wang & Tang, 2017). The seedling growth response to enhanced nutrient availability due to microbial processes, which also increase soil C priming in some biochar use systems, may result in higher net carbon storage (with the increased biomass carbon outweighing soil carbon loss). The silvicultural benefits of biochar are vital in optimising the positive effect of biochar on forest carbon stocks. Silvicultural benefits are determined by the direct and indirect impact of biochar soil nutrient and water availability and accessibility.

7.4 Provisional Recommendations

Biochar has been found to improve seedling establishment success, early growth in the establishment phase and seedling resilience to abiotic stress factors using targeted specified biochar application on a nutrient poor soil. Targeted biochar could be considered as an appropriate intervention where Ecological Site Classification (ESC) field based assessments or other decision support system (DSS) modelling identifies soil nutrient regime and soil moisture regime as limiting environmental factors to growth (Pyatt et al., 2003). This thesis evidence on the effect of targeted biochar use on establishment of three commonly planted conifer species (Sitka spruce, Douglas fir and Scots pine), the effects of biochar use out with these species, or similar species, and long-term effects have not been studied. The silvicultural effects of targeted biochar application are also not known on nutrient rich soils. Unprocessed woody biochar which has a moderate nutrient content (VCZ biochar) was found to be the most effective across all tested species and should be considered for during planting. Optimal dose rate was species specific. The optimal tested dose rate for the species used in this study are Scots pine at 25 g biochar seedling^{-1} (low dose sensitivity), Douglas fir at 15 g seedling^{-1} (high dose sensitivity) and Sitka spruce at 40 g seedling^{-1} (moderate dose sensitivity). Targeted biochar application could also be considered to improve the resilience of newly planted seedlings to abiotic stress factors (such as drought). Biochar application is complimentary to good silvicultural practices and should not be used to mitigate bad practice, poor species choice or facilitate poor nutrient management (such as brash harvesting). Biochar could be applied to improve the range of suitable species and encourage diversification, although more data would be required to accurate model how species suitability would be altered with biochar application.

The efficacy of biochar application in a forestry context is highly dependent on biochar characteristics, soil type and target species. This should be considered when prescribing biochar for seedling nutrition and biochar properties should match target soil functions. There are often trade-offs between biochar functions and specified properties. Utilisation of forest derived low value feedstocks, such as sawmill co-products, will recycle nutrients back to forest land and improve circularity, closing nutrient loops. Seedling growth increases in forest establishment from biochar amendment is likely limited to P deficient soils, however biochar application could still be a viable silvicultural intervention on other soils due to the effect on other soil functions, particularly in drought prone regions of the U.K.

High nutrient concentration fertilisers (including inorganic mineral derived P fertilisers) were found to be ineffective at delivering seedling nutrition benefits, partly due to the rhizosphere response. Current fertilisation practices also do not deliver sufficient nutrients to mitigate site nutrient loss during harvesting. Encouraging improved rhizosphere development and nutrient cycling should be favoured to high concentration nutrient applications. As forest soils are sensitive to rapid changes care should be taken when considering blanket biochar application. The effect of blanket applications on trees species adapted to acid low nutrient soils is not yet understood and conservative doses should be used <10 t ha^{-1}.

7.5 Implications of Research on the Forestry Industry

There are a number of barriers to industry adoption that can broadly be categorised as policy challenges, supply constraints and industry conservatism. Biochar is not included in any U.K. forest policy (including the U.K. Forestry Standard (UKFS), U.K. and the Woodland Assurance Standard). The fourth edition of the UKFS identifies the need avoid mineral fertiliser use where possible and focus on appropriate species selection, however biochar does not fit the definition of chemical fertiliser and should not be included as a fertiliser in the context of UKFS guidelines (Forestry Commission, 2017). It also recognises that there are sites of low nutrient availability which may require additional fertiliser input for the second rotation. Targeted biochar application adheres to these stipulations.

To supplement the UKFS, the document “Adapting forest and woodland management to the changing climate UK Forestry Standard Practice Guide” was released in 2022. This policy documents details management practices which may lead to a reduction in risks to forests associated with climate change. Ascension of biochar into policy documents, as a method to improve seedling resilience to climate pressures and improve soil productivity, is required before widespread industry adoption. Inclusion of biochar as a soil management option in the ESC model can could be used to identify planting scenarios where biochar application is effective and appropriate. The efficacy of biochar in improving woodland creation carbon balance, and the acceptance of biochar as a greenhouse gas removal (GGR) method, highlight the potential for biochar inclusion in the Woodland Carbon Code. This could incentivise industry adaption of biochar for carbon storage.

The wider forest industry is unlikely to extensively adopt biochar without inclusion in forestry planting guidance or addition to forestry grant scheme. Further research is needed before the implications of biochar use in a range of forestry scenarios is understood. This will be required before it can be confidently advised as best practice by public bodies or grant funded. Low dose biochar application adheres to environmental legislation provided virgin woody feedstock is used and application rates do not exceed 1 t ha^{-1} yr^{-1}. A position statement specific for biochar use in forestry by the Scottish Environmental Protection Agency (SEPA), the Environment Agency (EA), the Department for the Environment and Rural Affairs (DEFRA), Scottish Natural Heritage (SNH) and Natural England would provide a pathway to industry acceptance.

The U.K. biochar industry is still in its infancy and there is a lack of biochar supply. As the use of biochar becomes more widespread in agriculture, opportunities for use in forestry will emerge. Produces should consider developing specific biochar products for use in nutrient poor forest systems, where a narrow range of biochar properties yield strong results.

7.6 Future Challenges and Research Priorities

This thesis studied the effects of targeted biochar application on nutrient poor freely drained podzolic planting sites for a range of productive conifer species. Resource and time limitations prevented investigating the response of seedlings to biochar in other common establishment scenarios or from quantitively investigating nutrient transfer pathways hypothesised in this study. Four broad research themes have been identified:

i) *Expanding the tested parameters and scenarios of biochar utilisation in forestry management*

Theses parameters should include high nutrient status soils and gleyed soils. Broadleaf species should also be assessed. Blanket biochar application in forest soils could also be investigated. The use of biochar in peatland restoration to rapidly increase soil carbon, following deforestation or peatland degradation through drainage, would be an interesting research field in which balancing biochar properties with soil functions would be critical. The longer-term impact of biochar should also be investigated through continuation of the field experiment.

ii) *Improve understanding of establishment practices on rhizosphere development and nutrient cycling*

Little quantitative research has been done on the effects of forest establishment practices on rhizosphere development and nutrient cycling, or cultivation on localised soil nutrient availability. The potential of biochar in containerised seedling production as a novel solution for out-planting fertilisation and improving seedling rhizosphere development is another research priority.

iii) *Understand nutrient transfer pathways between biochar and trees, including ectomycorrhizal fungi*

Quantifying the nutrient and carbon pathways identified in this study, requires further research. Radio isotope tracing (^{14}C and ^{15}N) could be used to measure and map the transfer of nutrients within biochar particles and establish whether sequestered carbon is transferred to hyphal hotspots around biochar particles. This could also indicate the nutrient transfer efficiency across colonisation strategies and the biochar types tested in this study. As part of this work, the fertilisation effects of biochar could be proportioned into direct nutrient addition and indirect improvements to nutrient availability through biological processes. A similar rhizobox experimental design to Appendix 2 could be used.

iv) *Fiscal economics (and LCA assessment) of targeted biochar production and the net carbon benefit of production versus storage in deliver of goals for GGR*

A full LCA is required to understand the carbon storage potential for biochar application scenarios in the forestry industry, expanded to assess the economic feasibility and prospective subsidisation. This would ideally include piloting biochar production and use.

7.7 Concluding Remarks

Biochar application may be an effective management intervention during forest establishment on nutrient poor planting sites. The results of this study suggest that specified biochar application can be used to improve seedling establishment success, enhance seedling growth and strengthen seedling resilience to abiotic stress factors. Biochar can effectively improve soil nutrient availability and accessibility by altering soil nutrient cycles through indirect and direct mechanisms. The quantified growth establishment improvements observed in this study can significantly alter the early establishment carbon balance of low yield scenarios. Low dose biochar application can improve the early carbon contribution of woodland creation to Net Zero and mitigate soil carbon loss. Whilst further research is needed to understand biochar use in a wider U.K. forestry context, it should be considered as a carbon negative fertiliser which provide additional benefits through biological fertilisation.

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9.1 Appendix 1 – Supplementary Information

Appendix 1 contains;

Chapter 3

- Chlorophyll fluorescence boxplots for F_v/F_m - Maximum quantum efficiency of PSII photochemistry, ϕPSII is not presented as there was no variability between treatments or species.
- Inter species comparisons for VCZ treatments (which had the highest biomass accumulation results), with data adjusted to enable comparisons across species by presenting the difference in biomass from the CON treatment for each species.

Chapter 4

- Inter species comparisons for all treatments, with data adjusted to enable comparisons across species by presenting the difference in annual height growth from the CON treatment for each species. Year 3 and Year 4 are presented separately.

Chapter 5

- Visual assessment of EEM coverage on the surface of biochar particles, using optical microscopy. 1 cm^3 particles were selected for each replicated across species and biochar treatments.

9.1.1 Chapter 3 - *Biochar and seedling growth*

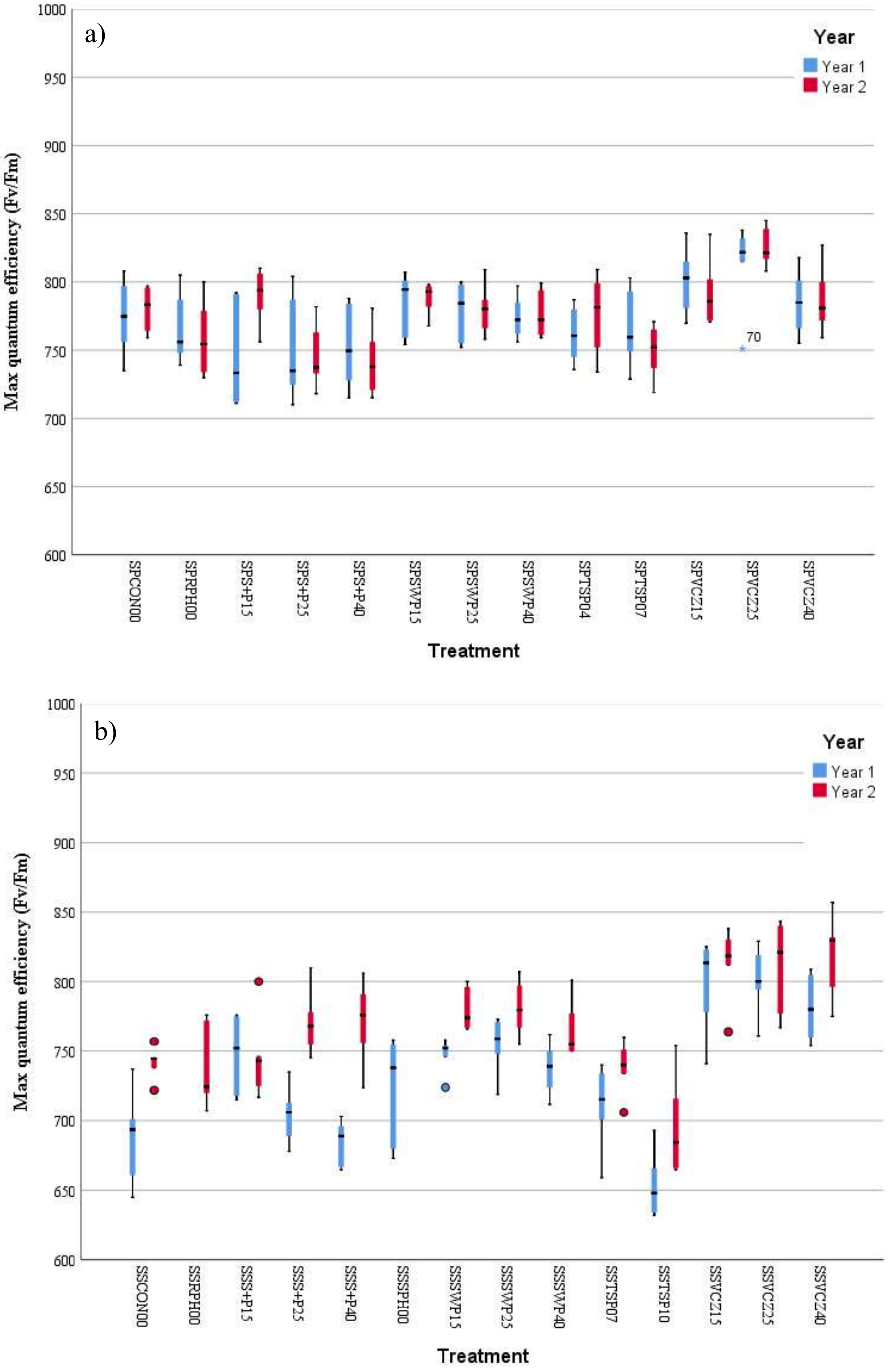

Figure 9-1- Boxplots for chlorophyll fluorescence measurements obtained for sample seedling in the Chapter 3 experiment for a) Scots pine and b) Sitka spruce, data is for F_v/F_m - Maximum quantum efficiency of PSII photochemistry, *n=6.*

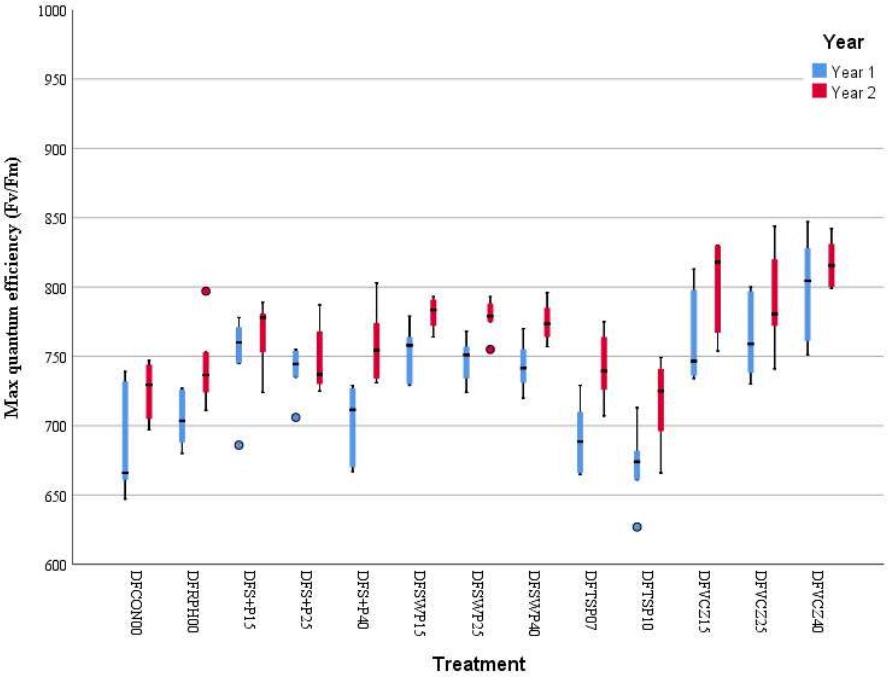

Figure 9-2- Boxplots for chlorophyll fluorescence measurements obtained for sample seedling in the Chapter 3 experiment for Douglas fir, data is for F_v/F_m - Maximum quantum efficiency of PSII photochemistry, *n=6.*

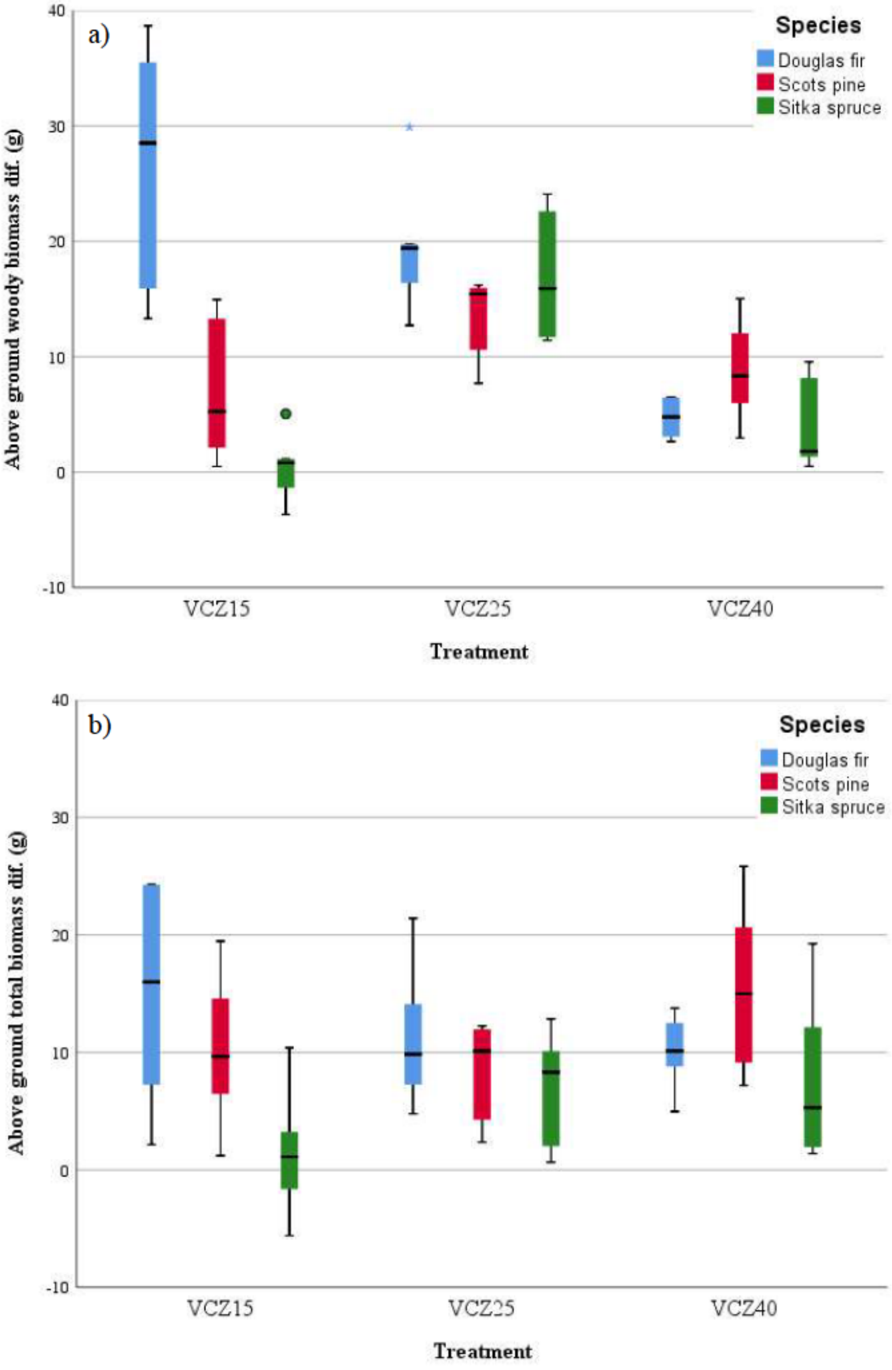

Figure 9-3- Inter-species comparison for VCZ treatments (which displayed highest growth response for each species). Above ground woody biomass (a) and above ground total biomass (b) display the difference for each treatment from the CON treatment mean for the relevant species. The mean control (CON) biomass was subtracted from the biomass measurements for each replicate in order to correct variation between species and enable comparisons. Most effective treatments for each species are displayed and the relative efficacy compared to the CON is shown, *n=6*.

9.1.2 Chapter 4 - *Field experiment*

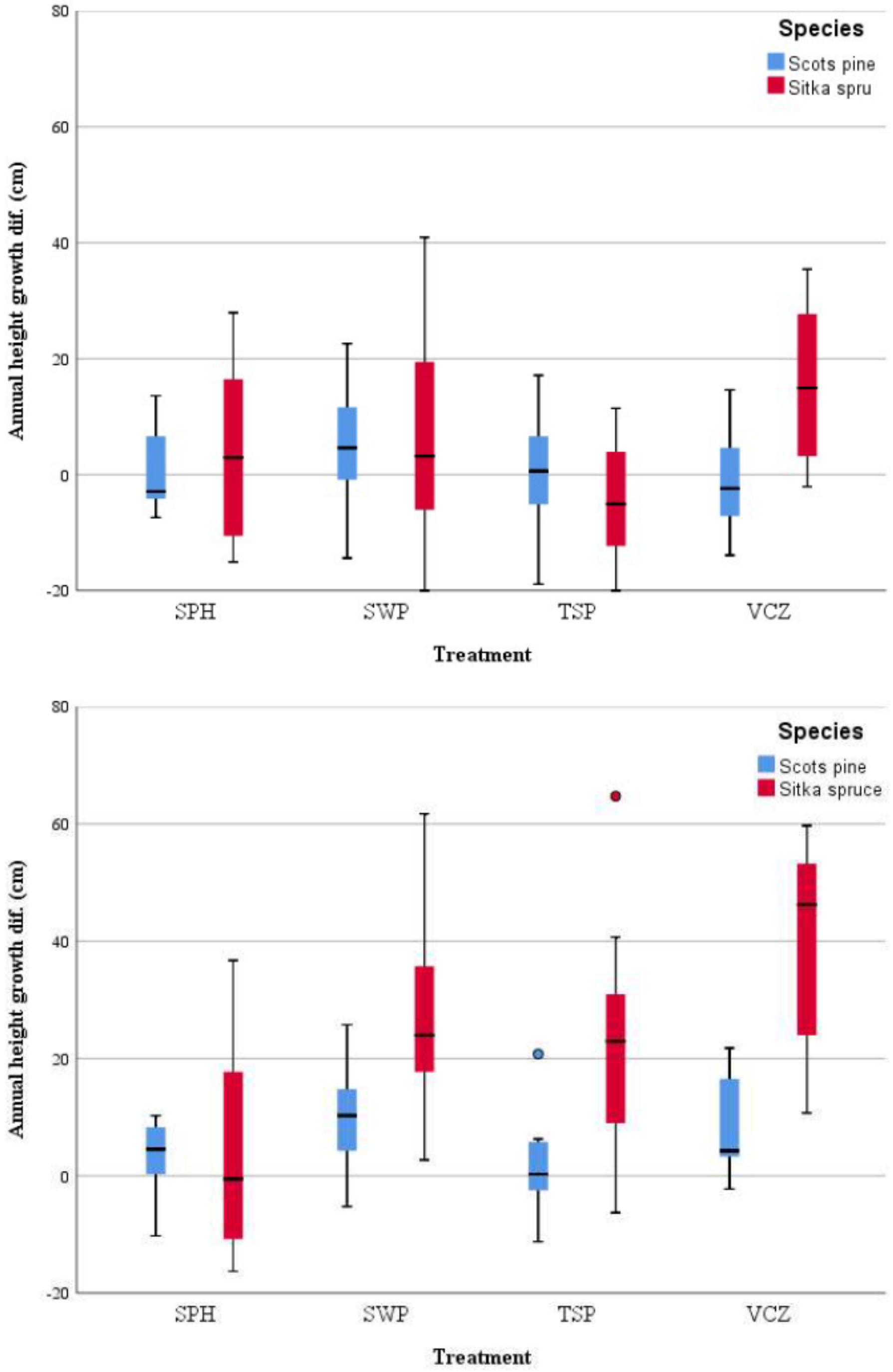

Figure 9-4- Inter-species comparison for all treatments, during: a) year three and b) year four. Boxplots display the difference for each treatment from the CON treatment mean for the relevant species. The mean control (CON) annual height growth (cm) was subtracted from annual height growth for each replicate in order to correct variation between species and enable comparisons. Most effective treatments for each species are displayed and the relative efficacy compared to the CON is shown, *n=75*.

9.1.3 Chapter 5 - *Rhizosphere and charosphere interaction*

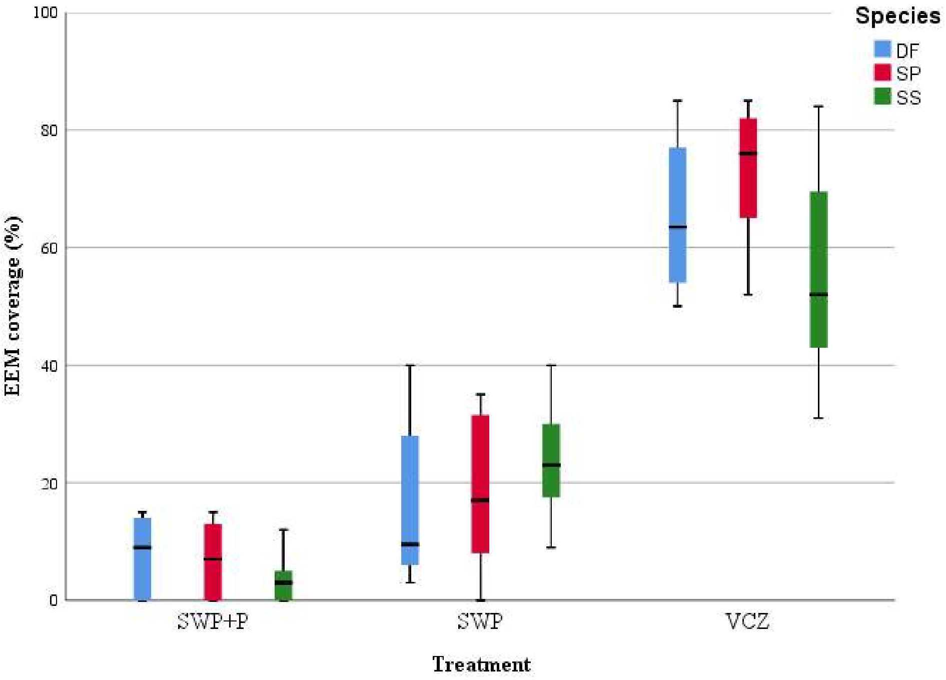

Figure 9-5- Assessment of percentage EEM cover on biochar particles (~1 cm^3) for the three tested biochars (SWP, VCZ and SWP+P), *n=24*.

9.2 **Appendix 2 -** Preferences of Pinus sylvestris seedling roots for different phosphorus sources under phosphorus-deficient conditions

This appendix contains the published paper from the supplementary experiment to this thesis, which was conceived and designed by the author to compliment this project and published: K. Lei, H. Creber, R. Bol, A. Tietema and S. P. Sohi (2022). "Preferences of Pinus sylvestris seedling roots for different phosphorus sources under phosphorus-deficient conditions." Plant and Soil. (DOI: https://doi.org/10.1007/s11104-022-05682-0).

The experiment was completed as part of a MSc by Kaiyu Lei (who was lead author in the publication), which was overseen by the author, who also assisted with data collection and analysis. The novel method of rhizosphere and root trait analysis was developed by the author. The experimental treatments were consistent with those tested in this thesis.

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RESEARCH ARTICLE

Preferences of *Pinus sylvestris* seedling roots for different phosphorus sources under phosphorus-deficient conditions

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Albert Tietema · Saran P. Sohi**

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Abstract
Purpose Phosphorus (P) is a limiting nutrient in many managed forests. To further understand the risks and benefits of biochars as sustainable P source in forest management, an improved mechanistic understanding of its interactions in root systems is required.
Methods A rhizobox experiment was conducted to observe root response of *P. sylvestris* (Scots pine) seedlings to different biochars in comparison to triple superphosphate (TSP) fertiliser as a P source. Three types of wood-derived biochar were compared: biochar from mixed softwood pellets ("Reference biochar"); from the vascular cambium zone of *Picea sitchensis* ("VCZ biochar") and from mixed softwood pellets infused with TSP ("Processed biochar"). These biochars presented a range of available P from low to high. Seedling root development was spatially analysed using GIS software.
Results The total length of *P. sylvestris* roots did not significantly differ between treatments. However, seedling roots showed strong preference for soil proximal to VCZ biochar and strong avoidance to TSP fertiliser. There was a milder avoidance effect for Processed biochar. Differences in root responses could be explained by available P: roots favored a moderate, sustained P source and avoided high available P sources. The avoidance effect can be attributed partially to lower soil pH around TSP fertiliser.
Conclusion The extent concentration and duration of P availability affects the root response of *P. sylverstris* to P sources. Under P-deficient conditions, *P. sylvestris* root growth was markedly improved by introducing biochar with a certain P concentration, and VCZ biochar has potential as an effective source of P in forest establishment.

Keywords Biochar · Roots · Phosphorus · Rhizosphere · Spatial analysis

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Introduction

Phosphorus (P) is a key limiting nutrient in the growth and establishment of seedlings in forest ecosystems. The increasing atmospheric nitrogen (N) deposition and resulting N accumulation and saturation causes P to replace N as the limitation of tree growth (Attiwill and Adams 1993; Crowley et al. 2012). Under P-deficient conditions, a tight P 'recycling system' develops, closing the P cycling loop. This maximizes the use-efficiency of the P pool and sustains the P supply within mature forest systems (Lang et al. 2016). In a mycotrophic forest, a 'recycling system' depends on mycorrhizal fungi as well as root morphological alterations (Attiwill and Adams 1993; Lang et al. 2016). However, clear-fell silvicultural systems may disrupt the closed P cycling system, leading to net P loss from the ecosystem P pool by leaching and lateral transport, reducing the soil available P during replanting (Kaila et al. 2014; Nieminen 2003; Rodgers et al. 2010).

Soil P content and plants' endogenous P status controls root morphology (Neumann et al. 2000), with enhanced root hair development and generate denser root clusters under deficient conditions (Lambers et al. 2006; Ma et al. 2001). Root clusters capture available P in soils by exudation of carboxylates through an anion channel which increases P availability (Lambers et al. 2013). In a mycotrophic forest, the P recycling system, mycorrhizal fungi as well as root morphological alterations play a role (Attiwill and Adams 1993; Lang et al. 2016). Mycorrhizal fungi in symbiosis with roots extend the P depletion zones and accelerate available P transformation through the excretion of organic acids (Cairney 2011) while accessing complex moieties in the soil organic matter (Cánovas 2019; Lindahl and Tunlid 2014).

Biochar is the manufactured product of thermochemical biomass conversion, mainly pyrolysis, and consists of various combination of carbon and mineral nutrients (Sohi et al. 2009). Biochar from woody sawmill residues contains a low concentration of P when compared to biochar produced from animal wastes and even arable crop residues (Hossain et al. 2020; Zhang et al. 2016). Relatively low concentrations of P could limit the agronomic value of wood-derived biochar, particularly when compared with mineral P fertilisers where concentrated P is rapidly disseminated in the soil. Biochar derived from ring debarking residues exhibits higher P concentration compared to other wood-derived biochars due to the relatively high nutrient concentration in the vicinity of the vascular cambium (Rathnayake et al. 2021). Generally, this particular feedstock may provide biochar with higher agronomic value than wood-derived biochars.

Leaving forest residues in-situ following clear-fell harvesting has been reported to mitigate P leaching at the same time as decreasing greenhouse gas emissions (Palviainen et al. 2004; Kaila et al. 2014). The application of wood-derived biochar following clear-fell harvesting has the advantage of stabilizing carbon as well as (Saarela et al. 2020) recycling P from harvested timber. Although there is volatilization of N during pyrolysis, the potential N gap is small and could be matched by N deposition (Crowley et al. 2012). The release mechanism of P from biochar is dependent on multiple factors (Qian et al. 2013; Uchimiya et al. 2010), affected by soil conditions as well as the self-promotion of root development (Abiven et al. 2015; Joseph et al. 2021; Lehmann et al. 2011; Xiang et al. 2017). Indeed, root promotion in pine (*Pinus spp.*) has been also observed for certain forest systems exposed to wildfire charcoal (Wardle et al. 1998).

Root interactions with the charosphere could be a key factor in plants accessing biochar-derived P (Chen et al. 2021). The porous structure of biochar derived from unprocessed woods makes it easier for roots and hyphae to penetrate and colonize biochar particles, increasing opportunity for nutrient transfer (Prendergast-Miller et al. 2014). The proliferation of mycorrhizal hyphae inside biochar through cracks and pores seems to stimulate root development as well as nutrient uptake (Ascough et al. 2010; Hammer et al. 2014; Jaafar et al. 2014; Solaiman et al. 2010).

The present study investigates the potential of biochar as a sustainable fertiliser for seedling establishment in P-deficient forest ecosystems, focusing on the interactions between the charosphere and rhizosphere. Three types of biochar were assessed as P sources in the establishment of *P. sylvestris* seedlings alongside mineral P fertiliser. The response of *P. sylvestris* root systems to the presence of these biochars of increasing P concentration and availability was assessed for low-P conditions using rhizoboxes (Fig. 1) and a bespoke growing medium (Table 1). Root system responses to the biochars was compared to mineral P fertiliser and to no P addition at all. Our study tests

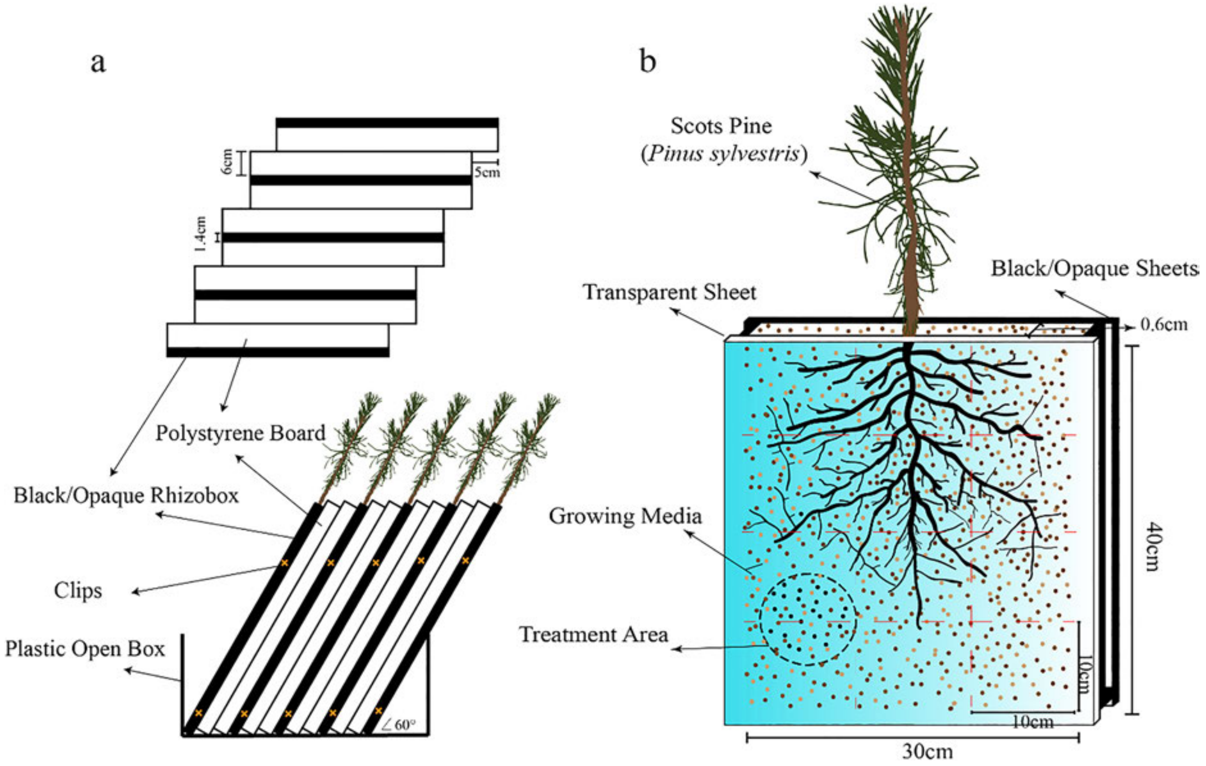

Fig. 1 Schematic diagram of rhizobox experiment. (**a**) Placement of a group of rhizoboxes stacked at an angle of 60° in the greenhouse; (**b**) Rhizobox with effective size of 40 cm × 30 cm × 0.6 cm

the hypotheses that the acquisition of P affects the spatial distribution of new root growth in the early establishment phase of *P. sylvestris* seedlings.

Material and methods

Biochar production and properties

The three biochars applied in this study were "Reference biochar" (low in P), "VCZ biochar" (derived from vascular cambium, naturally higher in P) and "Processed biochar" (high in P, artificially enhanced P content). All three biochars used were derived from woody biomass feedstock and produced by the UK Biochar Research Centre (UKBRC; University of Edinburgh, Edinburgh, UK). The equipment used was the Stage III system, a horizontal continuous feed pyrolysis kiln, manufactured by Ansac Pvt Ltd (Bunbury, Australia). The feedstock was pyrolyzed at a nominal highest heating temperature (HTT) of 550 ± 5 °C with a heating rate of 78 °C min^{-1}. Mean residence time in the kiln was 12 min, with 3.9 min at HTT. Details of the Stage III pyrolysis system can be found in Mašek et al. (2018).

The feedstock for VCZ biochar was the product of ring debarking of *Picea sitchensis* (Sitka spruce) (Rathnayake et al. 2021). This is a sawmill process that removes the outer 5 mm of timber logs, yielding 40% wood and 60% bark by volume, incorporating the vascular cambium. The VCZ feedstock from this study was obtained from a sawmill in south-east Scotland (BSW, Petersmuir, Scotland).

Reference biochar was prepared using standard biochar SWP550, a material widely adopted by the research community and readily available from UKBRC (Mašek et al. 2018). The SWP550 biochar was produced from mixed softwood pellets (Puffin Wood Fuels, Insch, Scotland) using the Stage III pyrolysis system previously mentioned. To provide comparison with Processed biochar that was soaked in P solution and dried afterwards, the SWP550

Table 1 Key chemical properties of (a) supply treatment materials and (b) growing medium/treatment mix in rhizobox treatment area (TA). Data are mean ± SD, $n = 3$ in a and b except for the Blank, $n = 5$. Replicates are technical replicates

a)

Treatments	VCZ biochar	Reference biochar	Processed biochar	TSP fertiliser
pH	10.0 ± 0.01	7.42 ± 0.02	3.60 ± 0.02	2.74 ± 0.40
NH_4^+-N (mg g^{-1})	< 0.01	< 0.01	0.401 ± 0.09	< 0.01
NO_3^--N (mg g^{-1})	< 0.02	< 0.02	0.08 ± 0.01	< 0.02
Available P (mg g^{-1})	2.24 ± 0.84	0.542 ± 0.010	258 ± 6	602 ± 3
Fe (mg kg^{-1})	22.9 ± 11.3	66.4 ± 5.7	29.1 ± 0.5	189 ± 3
Al (mg kg^{-1})	21.4 ± 10.4	42.4 ± 3.6	148 ± 8	387 ± 6
Ca (g kg^{-1})	12.4 ± 5.0	2.71 ± 0.05	19.7 ± 0.5	53.2 ± 0.9
Cd (mg kg^{-1})	0.06 ± 0.01	0.05 ± 0.01	1.49 ± 0.04	7.61 ± 0.20

b)

Treatments	Blank	VCZ biochar	Reference biochar	Processed biochar	TSP fertiliser
pH	4.41 ± 0.02	8.12 ± 0.01	5.58 ± 0.01	3.65 ± 0.02	2.9 ± 0.02
NH_4^+-N (mg g^{-1})	1.41 ± 0.06	0.798 ± 0.047	0.742 ± 0.031	0.754 ± 0.087	1.12 ± 0.11
NO_3^--N (mg g^{-1})	1.04 ± 0.02	0.654 ± 0.029	0.423 ± 0.044	0.201 ± 0.009	0.722 ± 0.053
Available P (mg g^{-1})	0.046 ± 0.020	1.24 ± 0.86	0.314 ± 0.048	156 ± 24	590 ± 37
Fe (mg kg^{-1})	64.9 ± 3.0	20.8 ± 1.2	8.91 ± 0.23	23.5 ± 1.2	173 ± 4
Al (mg kg^{-1})	9.52 ± 0.54	15.6 ± 0.5	11.2 ± 0.4	107 ± 8	365 ± 2
Ca (g kg^{-1})	0.135 ± 0.007	4.72 ± 0.19	1.21 ± 0.03	11.2 ± 0.7	50.2 ± 1.0
Cd (mg kg^{-1})	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	1.11 ± 0.04	7.12 ± 0.33

biochar was soaked in deionized water for 72 h and oven dried at 50 °C.

To create Processed biochar, the SWP550 biochar was infused with dissolved P solution with a concentration of 13.2 mol P l^{-1} prepared from commercial TSP fertiliser (46% phosphate, Progreen Ltd, Bourne, England). The infusion was undertaken (1:2 w/v) over a period of 72 h to ensure even distribution through the biochar particles. Processed biochar was oven dried at 50 °C.

The composition and properties of the biochars and TSP fertiliser are shown alongside those of the growing medium used in the rhizoboxes in Table 1.

Rhizobox experiment design

Rhizoboxes were made of 0.6 cm thickness acrylic (sides and back) and polycarbonate (front) sheets. The effective size was 40 cm height × 30 cm width × 0.6 cm deep (Fig. 1). The separation was customized for one-year-old *P. sylvestris* seedlings, considering the diameter of the root collar and the expected length and density of their root systems. The rhizobox comprised of five parts in total including the sides and base, which had machined grooves along joins to provide additional strength and prevent potential slippage. The transparent front sheet was detachable and designed for observation and photographic imaging. Black opaque sheets were used for the other sides and were secure along the exterior joins using polyurethane grab adhesive (Gorilla Glue, Sharonville, Ohio). Rhizoboxes were set for 24 h to ensure that the skeleton of each rhizobox was stable. All rhizoboxes were rinsed, alcohol-sterilized and dried before use.

Bespoke growing medium was prepared and sterilized (at 85 °C) 2 weeks prior to the set-up. It comprised 60% peat and 40% washed quartz sand by volume with no nutrients added, to ensure that key nutrients were initially at a deficient level (Table 1). These conditions represent the nutrient regime of poor forest soil (Pyatt et al. 2001) and typical P-poor plantation forests in the UK (Lang et al. 2017). The growing medium was passed through a 2-mm sieve prior to filling the prepared rhizoboxes. If the measured pH (McLean 1983) was outside the range 4.5 ± 0.5 it was adjusted using 0.01 mol l^{-1} NaOH solution and 0.01 mol l^{-1} H_2SO_4 sprayed on the surface of growing medium and mixed afterwards. Approximately 720 g of growing medium was added into each rhizobox. The variation of added growing medium was no more than 10% to maintain a bulk density of 1.0 ± 0.10 kg m^{-3} to provide a comparable growing environment at the outset.

The 30 cm × 40 cm frontal area of the rhizoboxes was divided into twelve unit-sectors with a size of 10 cm width × 10 cm height which were marked onto the front sheet to create a visual grid. After the growing medium was added, the four treatment materials with complete structures were applied at differential mass dose based on the recommended dose of P fertilisation for conifer seedlings in P deficient soils (Taylor 1991). These amounts were 45 g rock phosphate/m^2, adjusted for targeted application and growing medium volume, to maintain equivalent TSP:growing medium ratio: 0.96 g TSP fertiliser, 4.56 g Processed biochar, 3.60 g Reference biochar and 3.60 g VCZ biochar. The difference in mass dose of Processed biochar compared to other biochar was due to the mass of infused TSP within the material: 3.60 g Reference biochar + 0.96 g TSP fertiliser. The treatments were introduced centrally in the lower left sector without any mesh exclusion. No movement of treatment areas occurred during the experiment. The diameter of the treatment area (TA) for TSP fertiliser, Processed biochar, Reference biochar and VCZ biochar was 2 cm, 4 cm, 5.5 cm and 7.8 cm, respectively and implemented using prepared paper templates. The original growing medium was removed from the TA before the treatment materials were added. Treatment materials were mixed with growing medium (1:1) prior to application in order to prevent airspaces between treatment particles and simulate forest fertilisation conditions. Each treatment was arranged with five replicates. A blank control ("Blank") comprising growing medium was included, resulting in 25 rhizoboxes in total. The growing medium and treatment materials in the rhizoboxes were fully watered (until point of saturation) using distilled water and allowed to equilibrate for 48 h before planting the seedlings.

One-year-old container grown *P. sylvestris* seedlings were obtained from Forest Research, Northern Research Station, Roslin, Scotland. *P. sylvestris* was selected as it is the most tolerant and suitable conifer species in sandy P-deficient soils in the UK and has a high commercial profit and ecological benefits (Savill 2019). These seedlings had been grown from seed and all seedlings were in a dormant state. The seedlings had been grown under non-deficient nutrient conditions and all displayed root systems 20–30 cm in length. Seedlings were carefully removed from their containers, growing medium manually removed, and roots carefully rinsed to avoid transfer of nutrients. The seedlings were planted into the rhizoboxes in the central position, gently separating entangled roots. To diminish the possibility of 'transplant shock', the roots were not sterilized or trimmed. To ensure roots could consistently detect the added P sources, one root in each rhizobox was positioned 2 cm adjacent to the edge of the TA, oriented towards the TA center. Each rhizobox was watered weekly by removing the front sheet and evenly spraying the surface medium with distilled water using a mist sprayer. The watering regime was flexible to ensure that the growing medium was moist but with no excess water or vertical water movement or pooling, preventing lateral P movement from TAs.

The front (colorless) polycarbonate sheets were clipped to the treated, planted rhizoboxes. Polystyrene sheets cut to the same size and shape were used to exclude light and maintain consistent internal conditions. They were also used to loosely cover the surface of the growing medium to reduce soil surface light penetration and temperature gradients, while ensuring respiration was not impeded. The temperature and relative humidity in the glasshouse were between 17.0–27.2 °C and 36.5–100.0%, respectively during the 10 weeks experimental period, detailed data can be found in Supplementary Information 2. The experiment duration was determined by the time required for full colonisation of the rhizoboxes by the seedling roots.

The experiment was arranged using a randomized block design, with one replicate of each treatment randomly placed in each block. The rhizoboxes in each block were stacked at an angle of 60°, slightly staggering the boxes (vertically and horizontally) to ensure the above ground parts of the seedlings did not interfere (Fig. 1).

Sampling and chemical analysis

At the conclusion to the experiment (after 10 weeks), the root system was separated from the seedling at the first root branch and was gently rinsed, dried in an oven at 90 °C and weighed on an electronic balance. New leader needles were picked individually from the top and oven dried at 90 °C before further total P analysis. The needles were digested with concentrated H_2SO_4 and 30% H_2O_2 and analysed by autoanalyser (Bran Luebbe AA3, Seal Analytical, Norderstedt, Germany).

Each treatment material was analysed before use. The same analysis was conducted on the growing medium at the outset and on TA material at the beginning and end of the growing period (Table 1). For the initial analysis of TA, material was pooled across replicates. After 10 weeks the TA was sampled, together with TA3 (the zone at the edge of the TA extending outwards into the growing medium for 3 cm, Fig. S3). The growing medium were sampled separately from each replicate rhizobox, using prepared paper templates to ensure correct positioning. The material sampled within each TA was then thoroughly mixed to reduce intra-sample variation and transferred to a refrigerator below 4 °C. Part of each fresh sample was used for NH_4^+-N, NO_3^-N and available P analysis. The rest of each samples was subsequently oven dried at 90 °C and milled in a ball grinder before further analysis for Fe, Al and Ca.

Chemical analysis of the growing medium and TA was undertaken to elucidate potential factors influencing root-biochar interactions. For the pooled pre-planting samples, the extraction or digestion procedure was replicated 3 times (as technical replicates). The final samples (after 10 weeks) were only extracted or digested once, i.e. without technical replicates. Extraction for NH_4^+-N and NO_3^--N was undertaken using 1:10 w/v 1 M KCl and analysed by autoanalyser (as above). Available P was analysed through water extraction (Prendergast-Miller et al. 2014) and subsequent analysis of the extracts by autoanalyser (as above). Fe, Al and Ca was assessed using a modified dry-ash method for biochar. Samples were heated in a muffle furnace (500 °C, 8 h) and placed into a steam bath upon cooling. Concentrated nitric acid (HNO_3) was added and evaporated to dryness, followed by 1:4 HNO_3 and H_2O_2 which was evaporated to dryness. All residues were transferred through Whatman Grade 4 filter paper, using HNO_3 and deionized water, into a volumetric flask. The elemental composition of the filtrate was measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo-iCAP 6300, Thermo Electron, Waltham, USA). The pH of the growing medium was assessed for TA3 after the 10-week experiment period using a soil:water ratio of 1:2 (McLean 1983).

Morphological monitoring and analysis of root growth

The root system in each rhizobox was imaged photographically once each week (7 days interval). A lightbox was constructed from PVC sheet enveloped with thick photographic curtain. A port was created at the top of the box to provide access for a camera lens. The box was illuminated internally with LED symmetrical lighting strips orientated in four evenly distributed parallel lines across the top of the box, while thick black felt was used to line the base and sides to eliminate the ingress of natural light. The rhizoboxes were imaged in turn, with a fixed camera position in the light sealed port and with a precisely determined position for the rhizobox that ensured consistency in image positions. The rhizoboxes were imaged weekly using a DSLR camera at a native resolution of 7360 × 4912 pixels.

A method to spatially track and analyse root growth was developed using ArcGIS Pro 2.6 software (ESRI, Redlands, USA). The first step was to map each image to a referenced 30 cm × 40 cm physical area (the effective rhizobox area). This provided the coordination required for ArcGIS Pro 2.6 to define the absolute position feature layer which was created to manually track the growth of individual roots, assisted by the association between root color and increasing root age. A line feature layer along with corresponding point feature layer was created afterwards. The "Summarize Within" tool in ArcGIS was used to calculate the length and density of new roots. Kernel density is widely used in visualizing spatial data (Kalinic and Krisp 2018). Since root development in the interior surface of rhizoboxes could be regarded as a two-dimensional space, Kernel Density analysis was assessed in ArcGIS to analyse the cluster of root data (Kalinic and Krisp 2018; Okabe et al. 2009). Both the cell size and search radius used were default values calculated by the tool. The Gi* Hot Spot Analysis was conducted along with Kernel Density using similar procedure (Kalinic and Krisp 2018) (Fig. S2), visualising statistical information. New roots developed on the interior surface of the rhizoboxes owing to geotropism and the availability of water and air. Only new roots growing on the surface of front sheet were assessed and counted.

Weekly images were analysed as separate segments for the same rhizobox image. Overlaying the images in this way allowed an integrated root growth feature layer to be created and summarized for each rhizobox. This provided the calculated length and density of cumulative root growth. The analysis also allowed length, density and growth rate to be visualised and analysed. Root growth was separately assessed for the whole rhizobox, the TA and TA3.

Statistical analysis

All data were tested for normality (K-S test) and homogeneity of variance (Levene test, $p = 0.05$). One-way analysis of variance (ANOVA) was conducted using Python 3.8 (Scotts Valley, CA) and with $p = 0.05$ as the threshold for statistical significance to compare the root density, root density difference, available P, and total Al, Fe and Ca between treatments. Post-hoc pairwise comparison was conducted using Tukey tests. Considering the F-test results and the distribution of residuals, a square root transformation-linear model was applied to root density data to distribute slight skew and reduce heteroscedasticity of the residuals (Osborne 2002) (Fig. 2). Both the simple linear model and linear model after square root transformation (Osborne 2002) were developed using Python 3.8 (Scotts Valley, CA).

Results

Root morphological responses to different treatment materials

After 10 weeks, there was no statistically significant effect of treatment material on total growth of new roots or total root biomass (Table S2). One seedling in the Blank treatment did not survive transplanting and this replicate was excluded from further analyses. All roots within 2 cm of TSP TA exhibited complete death of root tissues. Similar root mortality was found in one rhizobox where the treatment patch contained Processed biochar.

Linear regression revealed similar growth trends of roots throughout the rhizobox between the Blank, Reference and VCZ biochar treatments after 10 weeks (Fig. S1). Root growth in Blank treatment was much higher than Processed biochar and TSP treatment. In TA3, root growth was significantly higher for rhizoboxes with TAs containing Reference biochar or VCZ biochar. Detailed fitting data was listed in Table S3. Total root growth between treatments was quite similar at the beginning (<4 weeks). The slope between treatments was fairly consistent and no significant difference could be observed in the short term, which was in accordance with One-way ANOVA results (Table S1). The limitations of available nutrients and rhizobox space in the rhizoboxes preclude prediction in the long term. In TA3 (Fig. 2f–j), the root growth for Reference biochar and VCZ biochar treatments was consistent with the growth trend in the whole rhizoboxes and much greater than that in Processed biochar and TSP fertiliser treatments. Root growth for Processed biochar and TSP fertiliser was at a consistent, extremely low level. The higher slope k of linear fitting indicates that the root growth for TA3 in Reference biochar (0.075) and VCZ biochar (0.077) treatments was greater than other treatments (0.036 in Blank treatment, 0.026 in Processed biochar treatment) prior to Week 8.

According to model fitting results (Fig. 2) as well as Kernel Density analysis results (Fig. 3), *P. sylvestris* seedling roots have a distinct growth preference for VCZ biochar TA. The mean root density in the 10th week in the VCZ TA and TA3 was 4.2 mm cm^{-2} and 4.1 mm cm^{-2} greater than the Blank treatment, respectively. The TA in TSP treatment showed a clear limiting effect on seedling root density, with a complete absence of root growth in 4 replicates. A similar but milder limiting effect on root development was observed in Processed biochar treatment. *P. sylvestris* roots show undifferentiated growth in the Blank treatment. It was notable that the area of densest root growth for VCZ biochar was located at the boundary of the TAs and that root density was significantly higher than the corresponding areas in the Blank treatment.

By comparing root density in TA3 with the mirrored area on the opposite side of the rhizobox, a quantitative left-to-right comparison can be made throughout the experimental period for each treatment based on weekly images (Fig. 4). During the first 7 weeks, there was no significant preference for roots associated with any TAs, although avoidance of TSP was visually apparent. From week 8, the left-to-right

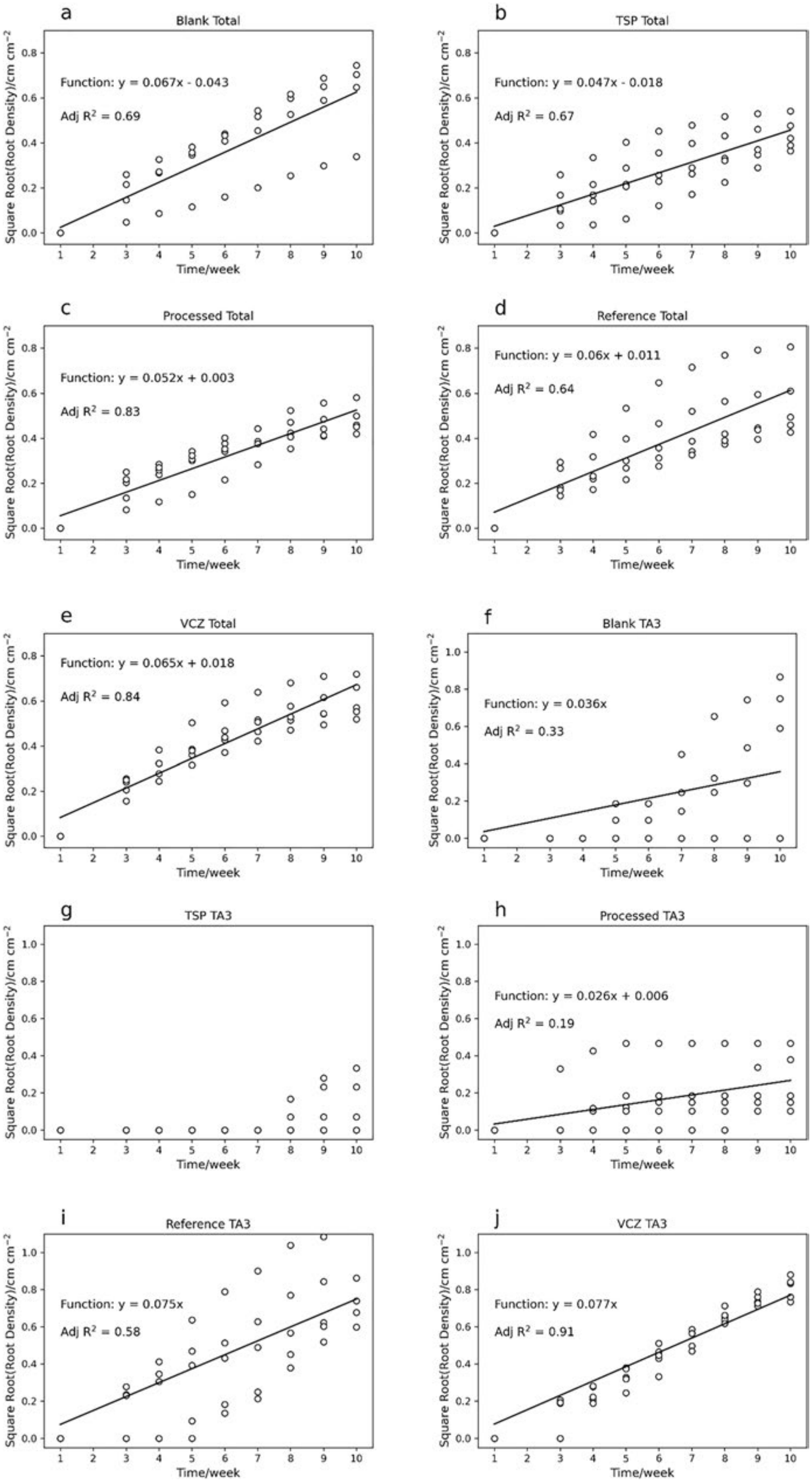

Fig. 2 Square root transformation-linear model fitting root results for different treatments over 10 weeks. Root density in the whole rhizobox for (**a**) Blank; (**b**) TSP; (**c**) Processed; (**d**) Reference and (**e**) VCZ treatments. Root density of TA3 in (**f**) Blank; (**g**) TSP; (**h**) Processed; (**i**) Reference and (**j**) VCZ treatments. Detailed results see Table S3, $n = 4$ in Blank and $n = 5$ in other treatment. Plots with no trend line indicate non-significance according to the linear regression model

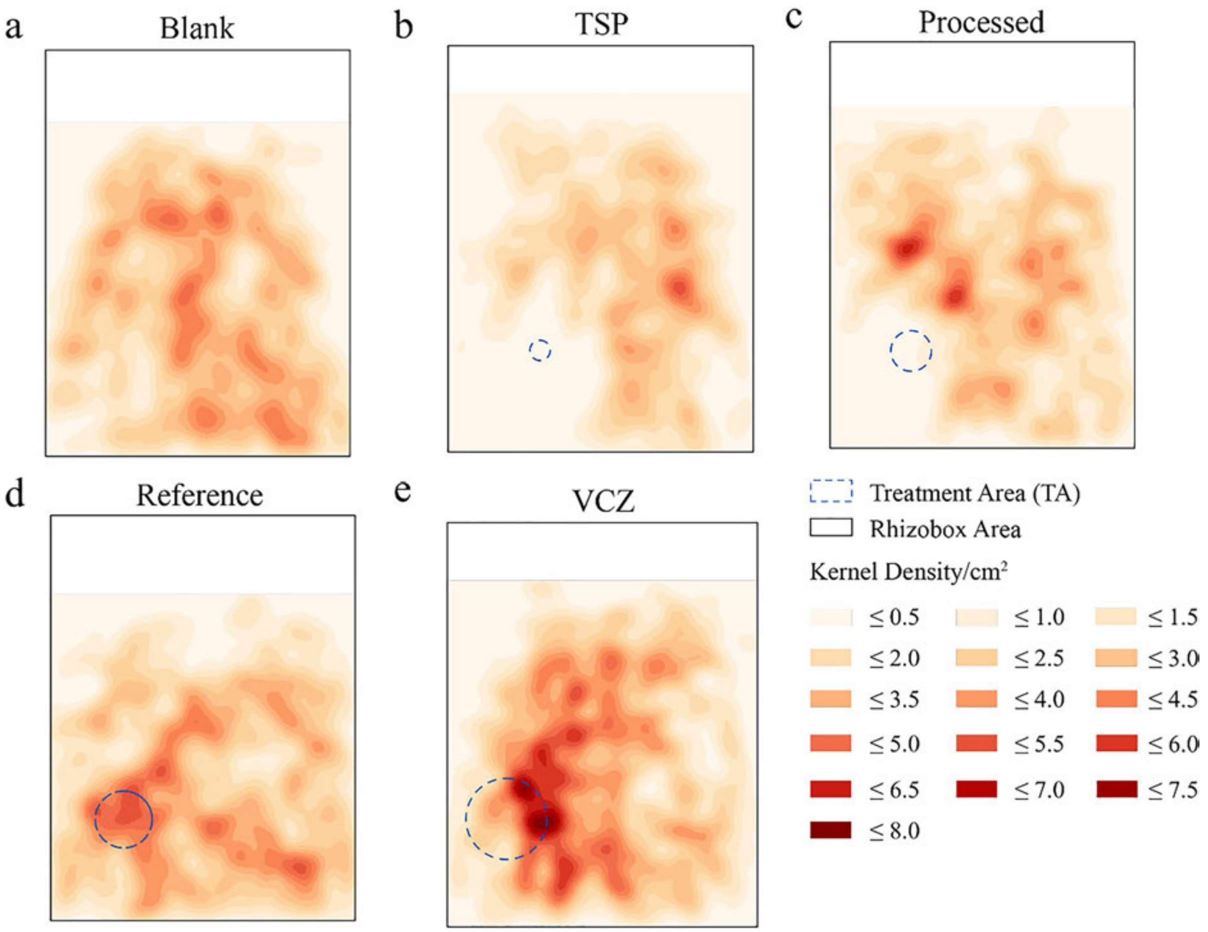

Fig. 3 Kernel Density (cm^2) of summarized new roots in different treatments. (**a**) Blank; (**b**) TSP fertiliser; (**c**) Processed biochar; (**d**) Reference biochar; (**e**) VCZ biochar. Data used for Kernel Density are the new roots across five replicates of each treatment after 10 weeks experimental period

comparison became significant for TSP fertiliser and VCZ biochar treatments. Subsequently, from week 8, the trends began to clearly diverge (Fig. 4). In VCZ biochar and Reference biochar treatments, root growth became concentrated within the TA indicating the emergence of seedling root development preferences. Conversely, an avoidance emerged in Processed biochar and TSP fertiliser treatments. In the Blank treatment, a weak imbalance emerges during the initial growth (week 0–7), but convergence on a left-to-right equilibrium indicates that the contribution of new roots in the Blank treatment gradually becomes uniform and undifferentiated (Fig. 4). Overall, the differences demonstrate that there was a strong preference for *P. sylvestris* roots to forage around VCZ biochar and a milder corresponding preference for Reference biochar. There was a strong avoidance for TSP fertiliser and a milder avoidance for Processed biochar. Around Processed biochar, a high density of new roots was concentrated on the outer edge of the TA (Fig. 3).

Roots response to different pH

The growing medium was alkalised by VCZ biochar and Reference biochar, and acidified by Processed biochar and TSP fertiliser (Table 1). After 10 weeks, pH in areas within 3 cm of the TA stabilized between 5.2 to 6.2, with pH 5.7–6.2 (approx.) in TA3 of the Blank, VCZ biochar and Reference biochar treatments, and pH 5.2–5.7 in TA3 of Processed biochar and TSP fertiliser treatments. The difference between

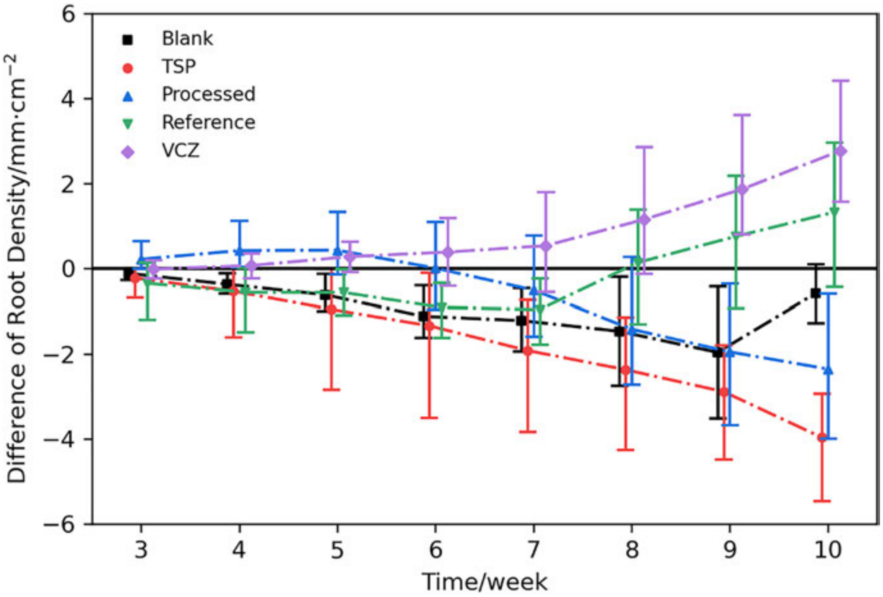

Fig. 4 Cumulative weekly root density difference between TA3 and corresponding areas on the opposite side of the rhizobox for 10 weeks. A positive number means that the density in TA3 was greater, while a negative number means density in TA3 was lower. A positive number indicates greater new root growth in TA3, a negative number indicates greater new growth in the opposite side. Data are mean ± SE, *n* = 5 except for Blank where *n* = 4

the Blank treatment and both Processed biochar and TSP fertiliser was significant (Table S5), even in areas at a greater distance (4.5 cm) from TA3 (Fig. S3).

Roots response to different available P

The available P in TA ranged from extremely low in the Blank treatment to extremely high in TSP fertiliser treatments. TSP fertiliser has extremely high available P (and low pH).

Regardless of source, the P available in TAs declined drastically after 10 weeks (Fig. 5a). Proportional available P decline was not significantly different between TSP fertiliser, Processed biochar and Reference biochar treatments, while the difference between the Blank treatment and other treatments was significant. The VCZ biochar treatment showed the lowest proportional decline (92.3%) in available P over the duration of the experiment (apart from the Blank treatment, where the decrease was 44%). The decrease in available P for VCZ biochar is significantly lower than in TSP fertiliser treatments (97.9%). The mean available P decline in Reference biochar and Processed biochar treatments was 95.6% and 96.1%, respectively.

There was significantly higher Ca, Fe and Al in TAs of TSP treatment (Fig. 5b). The Fe and Al concentration in the TA of VCZ biochar treatment was lower than that in TSP fertiliser treatment, but similar to Reference biochar treatment. Processed biochar treatment showed a significantly higher Al concentration in the TA than that of the Blank treatment.

Total P concentration in new growth leader needles was significantly higher for TSP fertiliser treatments than the Blank and VCZ biochar treatments, but did not differ between the Blank and VCZ biochar or Reference biochar treatments (Table S6).

Discussion

P. sylvestris seedlings show altered root development strategies in response to distinct P sources. The morphological root response in the acquisition of P from different sources determines the spatial distribution of new root growth in the early establishment phase. There are several potential mechanisms to account for these differences, which either enhance or limit root development.

Morphological and spatial root responses to sources of phosphorus

The length of roots is known to be affected by many factors, including soil nutrient content (López-Bucio et al. 2003), pH (Hinsinger et al. 2003; Vanguelova et al 2007) and soil physical structure (Dexter 2004). Some effects of P-deficient conditions are the stimulation of

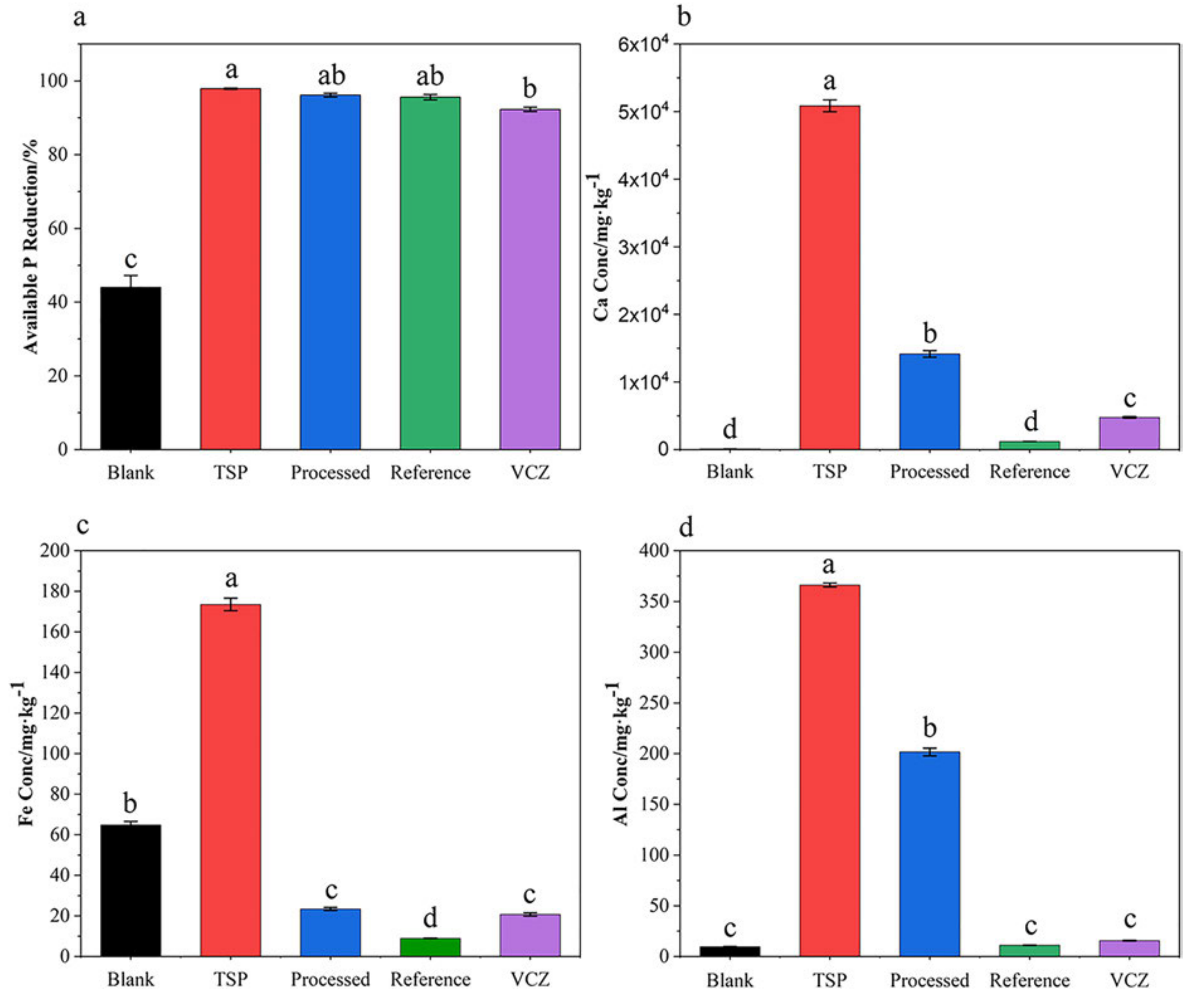

Fig. 5 After 10 weeks experimental period, (**a**) Available P reduction in TA, $p < 0.05$. Data are mean ± SE, $n = 3$ (technical replicates) at the beginning, $n = 5$ after 10 weeks (except for Blank where $n = 4$); (**b-d**) Ca, Fe and Al concentration in TA. Data are mean ± SE, $n = 3$ (technical replicates) at the beginning (except for Blank where $n = 5$)

primary root growth and the exploration of a larger soil volume (Ma et al. 2001). Roots under these conditions may also exude carboxylates to facilitate the mobilization of P (Lambers et al. 2013). Calvaruso et al. (2013) showed that *P. sylvestris* Scots pine can accelerate apatite dissolution through organic acid. In the present study, the roots of *P. sylvestris* showed clear preference for VCZ biochar compared to the Blank treatment, and a milder preference for Reference biochar (Fig. 3). These preferences indicate high delivery of P present in the biochar. Deliverable P could be expressed as the available P in soil which plants can actually access, which is limited by root health and root accessibility to available P-rich zones. Mineral nutrients associated with the interior and exterior surfaces of biochar may be mobilized by exposure to soil solution, providing a supply of assimilable P in otherwise deficient soils (Joseph et al. 2021). Phosphorus in biochar originates from the feedstock biomass and in general, displays low concentrations. The vascular cambium has a higher concentration (Risopatron et al. 2010), extending to the zones used to create the VCZ biochar from *Picea sitchensis* (Sitka spruce) biomass.

Root aggregation occurs around the boundary of the VCZ biochar TA (Fig. 3e), suggesting that VCZ biochar could provide more available P to seedlings compared with Reference biochar and growing medium without additional P source. VCZ biochar concentrated root development in TA and limited foraging in areas that are low in P. Antithetical to VCZ biochar, Reference biochar provided low available P, resulting in a sparser but wider root system in order to explore a large volume of growing medium to satisfy P demand (Fig. 3d).

In addition to the negative effect of low pH on root density and biomass of *P. sylvestris* seedlings (Vanguelova et al. 2007), alteration of soil pH can result in phosphorus fixation in growing medium, where Al, Fe and Ca competitively bind to free phosphate compounds, decreasing the P that is available in soils (Schlesinger 2005). The P pool in the Blank treatment was probably at equilibrium with respect to pH. The TSP fertiliser lowered soil pH and contributed to accelerating decline of available P after application as the soluble phosphate may be sorbed by Al, Fe and Ca within comparatively a short period of time (Penn and Camberato 2019) (Fig. 5a). Although acidic conditions have a negative long-term impact on P acquisition provision in a forest system, extremely high available P can directly inhibit root growth and potentially cause root mortality (Ma et al. 2001). While TSP fertilisers guarantee P supply for *P. sylvestris*, the concentration of P can still inhibit wider foraging, leading to root structures that are potentially detrimental to mature stands. This may adversely impact the deliverability of P to the seedling and limit the potential uptake of P from bulk soil once the additional P was sorbed, due to poorly developed root systems. The actual effect of TSP fertiliser on root development and foraging ability is related to the application regime and rates which should be carefully considered when applied to natural stands as indirect topical application of a 1:1 ratio of TSP to growing medium has caused root mortality and root forage inhibition (Fig. 2g).

Application of biochars showed variable impacts on the pH of the external growing medium, conversely, the external pH will have also affected the availability of the P present in different amounts in different biochars. These factors interact to determine the P that is available inside and around biochar TA. High levels of Ca could limit the release of P from the high-pH interior of VCZ and Reference biochars (Buss et al. 2018). The proportional decrease in available P from VCZ biochar treatments was, however, significantly smaller than for TSP fertiliser (Fig. 5a). The pH conditions at the interface of alkaline biochar and acidic growing medium could be critical, providing for the mobilization of P bonded with Ca but without Fe phosphate that form below pH ~ 6 (Penn and Camberato 2019). It is likely that the availability of P was affected indirectly by pH, altering the labile P pool in the growing medium and the total P released from the TA. Comparison of root biomass in the TA of contrasting pH revealed no significant difference, suggesting that direct effects of pH were not a major factor.

In addition to the chemical mobilization of P that occurs along a pH gradient, physical changes could be relevant. VCZ biochar is friable, has a low bulk density, high porosity and a large internal surface area (Rathnayake et al. 2021). This supports relatively easy access by roots and ectomycorrhizal fungi that facilitate P acquisition. The folding, compression and heating that occurs in the pelleting of biomass for Reference biochar and Processed biochar creates structures that are less conducive to such interactions. Pelletisation of biomass destroys the cellular structure of wood, decreasing the porosity and surface area of potential biochar, while conversely increasing its potential bulk density (Stelte et al. 2012). Surface colonization by ectomycorrhizal fungi was visually apparent on VCZ and Reference biochar particles (Fig. S4). These fungi could provide a mycotrophic alternative for the access and acquisition of P by *P. sylvestris* (Joseph et al. 2021). On the other hand, the potential for these microorganisms to assist in the uptake of P within the timeframe of the rhizobox study is uncertain.

Analysis for metals revealed concentrations of Cd in TSP fertiliser (Table 1) that could potentially limit the root growth and levels of Cd in plant tissue that present toxicity issues (Yazici et al. 2021). Although direct toxic effects on *P. sylvestris* root systems and above-ground depends on the free Cd^{2+} concentration in plant tissues, which was not analysed, the Cd concentration measured in TSP fertiliser was approximately an order of magnitude higher than the 5 mg kg^{-1} threshold proposed by Ismael et al. (2019) and set for fertiliser products in the Netherlands (7 mg kg^{-1}) (Crommentuijn et al. 2000), although within the threshold of 60 mg kg^{-1} set by the EU (Council regulation 2019). Since most of the P added in Processed biochar originated from TSP, it was not surprising that Cd concentrations in Processed biochar also exceeded some fertilizer thresholds.

Potential of biochar in phosphorus management in temperate forest systems

The available P in bulk soil is critical to seedling establishment success in the first stage of forest regeneration (Ceccon et al. 2003) and is often the limiting factor during seedling establishment in sandy upland planting sites in the UK. *P. sylvestris* is widely planted in semi-natural and commercial forests (Savill 2019) as a tolerant species that can survive and grow in nutrient poor sandy soils where other species would be unsuitable. Seedling root development is important as it influences the root architecture of the mature tree, with high lateral root growth of improving tree stability and mature growth. Wind damage is the primary factor in determining the terminal height of trees in Scotland and the primary cause of tree damage in the UK. Targeted application of fertiliser P can lead to under-developed or asymmetric root architecture in mature trees, affecting crop stability (Krišāns et al. 2020) and deliverability of P. The stimulation of root development from biochar amendment could potentially improve the structure of roots in mature stands, although further research is required. Improved crop stability may be important to the future resilience of forests in the face of climate change (Alongi 2008).

Biochar has been recognized as an option for improved management of agricultural systems, including the UK (Dobbie et al. 2011). The specific use of biochar in silviculture has been less widely considered. The results of the present study indicate that biochar can be selected to efficiently deliver small doses of P to tree seedlings, manipulating pH in the charosphere and potentially stimulating symbioses with fungi, VCZ biochar has similar available P and porous structure to charcoal collected in coniferous forests after wildfires (Gundale and DeLuca 2007; Santín et al. 2017) and Wardle et al. (1998) which have demonstrated positive effects on germination and growth of trees in pine forest. *Pinus* spp. are generally fire-adapted and other studies have shown a significant improvement of plant growth after applying charcoal derived from wildfires (DeLuca et al. 2006; Gundale and DeLuca 2007).

Co-products of timber processing such as the cambial zone of *P. sitchensis* contain nutrient P that could be usefully and effectively cycled back into forestry at nutrient limited establishment sites. Supplying wood-derived P to P-deficient forest using biochar would expand local P pools and assist in closure of the P cycle, contributing to the sustainability of forestry operations (Palviainen et al. 2004; Kaila et al. 2014).

Conclusion

Growth of roots in *Pinus sylvestris* (Scots pine) seedlings showed strong preference for growing medium proximal to biochar made from the vascular cambial zone (VCZ) of *Picea stichensis* (Sitka spruce) and strong avoidance to TSP fertiliser. The preference and avoidance effects for biochar from softwood pellets with and without additional infused phosphorus were milder. These different preferences reflected the concentration and duration of P available from the materials, which was probably a function of pH at the soil interface, led to suppression of root growth and decrease the availability and deliverability of P. VCZ biochar has good potential as a source of P in establishment of forests on P-deficient soils.

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Author contributions All authors conttributed to the study conception, espically Hamish Creber. The biochars, seedlings and growing medium were prepared by Hamish Creber and Kaiyu Lei. The rhizoboxes were designed and assembled by Hamish Creber and Kaiyu Lei. Specimen collection and analysis was performed by Kaiyu Lei and Hamish Creber. Kaiyu Lei collected and analysed the data and worte the first draft of the manuscript. All authors commented on previous verisons and did some modifications. All authors read and approved the final manuscript.

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Data availability The dataset generated during this study are available from the corresponding author on reasonable request.

Declarations

Competing interests We declare there is no competing financial interest.

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