CZECH UNIVERSITY OF LIFE SCIENCES IN PRAGUE

FACULTY OF ENVIRONMENT SCIENCE

DEPARTMENT OF APPLIED ECOLOGY

THE INFLUNCE OF GREY WATER AND BIOCHAR TREATMENTS ON PLANT GROWTH CHARACTERISTICS

BACHELOR THESIS

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

BACHELOR THESIS ASSIGNMENT

Martin Roe

Environmental Data Science
Ecology

Thesis title

The influence of grey water and biochar treatments on plant growth characteristics

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Objectives of thesis

The main goal of the thesis is to evaluate how watering plants by grey water in combination with biochar soil additives influences the growth and development of plant characteristics. The thesis is based on a greenhouse experiment.

Methodology

A greenhouse experiment will be conducted using four different treatments – inert substrate, substrate with addition of biochar and irrigation by tap and grey water. Variables which will be controlled are: light exposure, depth of substrate, temperature of the greenhouse and application of nutrients. Treatments, and their combination, will be compared as well as the effects on different planted species. The data will be statistically analysed. Based on the results, the suitability of plant species for planting in green walls and roofs will be evaluated.

The proposed extent of the thesis

50 pages, 2 figures

Keywords

grey water, biochar, green walls, plant ecology

Recommended information sources

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Prague on 27. 03. 2022

Declaration:

I hereby declare that I have independently elaborated the diploma/final thesis with the

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Abstract:

The bachelor's thesis was entitled the influence of grey water and biochar treatments on plant growth characteristics. Plant growth characteristics included: growth rate, development of leaves and development of flowers. Over a two-month period, data was collected on plant growth and plant characteristics and then samples were separated into: leaves, flowers and roots and the dry mass was measured.

In the research part, the influence of plant growth due to biochar and grey water was investigated, as well as how such factors had been applied to green roofs and living walls. The experimental part took place in a greenhouse, with controls on temperature and light.

Data was collected from the experiment on paper and was then entered into excel. Statistical analysis was made with R (r project). A three-way analysis of variance (ANOVA) was done on mean growth rates, with a significant level of $p<0.05$, followed by Tukey's HSD test. For the number of flowers and number of leaves, a Poisson general linear model was used to evaluate the data. Dry and water weights were also assessed with a two-way analysis of variance (ANOVA), on total mass, with a significant level of $p<0.05$, followed by Tukey's HSD test.

The results showed biochar significantly affected mean growth rates of *A. maritima*, as well as the dry biomass and water mass. Grey water had no significant effects on growth rate. This suggests grey water and biochar may be useful factors when designing or retrofitting green roofs and living walls.

Further work could be completed on assessing chemical composition of dry biomass, to help understand growth mechanisms and if certain uptake of nutrients may be limited or in excess because of the influence of biochar and grey water.

Keywords: Biochar, Grey water, Ecosystems services, green roofs

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1. Introduction

Urbanisation has been a growing trend, with a greater proportion of people living in cities than at any time in human history (Zhang, 2016). Many cities have continued to grow and sprawl outwards and as people have become more affluent; people desire greater amounts of space to live in. Human populations have also continued to grow, intensifying urban sprawl. Nevertheless, these trends have had significant effects on the natural world, with many ecosystems destroyed for urban development. Population growth and rising affluence have also created demand for more clean water. This pressure on clean water supplies is further exacerbated by changing weather patterns across the world due to climate change and aerosols, with extreme weather events such as flooding and droughts becoming more frequent (Stott, 2016). In this bachelor thesis, grey water - a by-product from hand washing and other non-excretory bathroom streams, was utilised to investigate its impacts on plant growth and plant development, relative to clean water. Furthermore, plants were grown in two mediums, normal soil, and biochar. This research will add value to the debates around green infrastructure on buildings, so that plants may be grown in urban environments, such as roofs or walls, while using resources which are abundant, such as grey water. Two plant species *A. maritima* and *G. macrorrhizum* were tested during the experiment.

Growing plants in urban areas would be a valuable ecosystem service for local communities. Loss in biodiversity – due to human activities, as analysed by the planetary boundaries framework is seen as in the second worst position only to Novel Entities (Azote, 2022), hence being able to reverse this trend by growing a large diversity of plant species, along with knock on effects on improving local pollination (Langemeyer, 2020), would be a positive ecosystem service. Plants could reduce flash flooding, at times of intense rainfall, due to being able to hold water in roots (Cuong T.N. Cao, 2014). However, it has been discussed that there may be issues with runoff water quality, with high levels of nitrogen and phosphorus in storm water runoff (Kuoppamäki, 2016). Green infrastructure could also cool urban environments, hence, may reduce issues with urban heat island effects and higher temperatures due to climate change (H. Saaroni, 2018). Green infrastructure on buildings could also have benefits on people’s mental health and wellbeing (Andreucci, 2019) and offer a source of cultural and educational opportunities for residents.

2. Objectives of thesis

The principal question being investigated is whether rates of plant growth and plant development were different when grey water is used as a replacement for tap water and biochar is used as a soil amendment as opposed to normal soil.

The independent variables were:
1. Type of water used to water plants
2. Type of soil medium plants were grown in
3. Plant species used

The dependent variables were:
1. Height of plant
2. Number of leaves on the plant
3. Number of flowers (closed and sprouted)

The control factors under experimental conditions were:
1. Depth of soil medium
2. Light taken up by plants
3. Temperature and humidity of greenhouse that plants were grown in
4. Total volume of water supplied to each plant
5. Nutrients supplied to plants

3. Introduction - Theoretical part

3.1 Ecosystem services

Green walls and roofs can serve a range of ecosystem services, including mitigating urban heat island effects, reducing storm water runoff, improving air quality, storing carbon, reducing energy needs for a building, sound mitigation, supporting biodiversity and pollination, aesthetically improving urban areas and helping to improve resident's mental wellbeing (J.-C. Lata, 2018).

3.1.1 Urban heat Island effects

The urban heat island effect has been one of the most used keywords in thermal regulation services in green roofs, with the period 2010-2019 seeing a rapid increase (Hongqing Liu, 2021). Green roofs promote evapotranspiration process and change surface albedo (T. Susca, 2011) which cause a range of benefits for human health and a reduced requirement for energy use for cooling. Different climates have different potential mitigations with Mediterranean climates offering larger urban heat island mitigation than oceanic climates, while arid climates were limited by evapotranspiration potential (Susca, 2019).

3.1.2 Reducing storm water runoff

A range of studies show the possibilities of reducing storm water runoff, in a variety of climates, due to the ability of plants and substrate to store large quantities of water. The most important factor was the substrate of the green roof (Muhammad Shafique, 2018), with substrate containing biochar showing good water retention, due to higher water holding capacity. While runoff values have been reduced, issues around water quality need further investigation with studies showing increases in the concentration of pollutants such as nitrogen and phosphorous from green roofs runoff, despite lower total pollutant loads.

3.1.3 Air quality

Outdoor air quality is influenced by green roof and walls. Particulate matter is deposited on green walls and captured by stomata, reducing concentrations of particulate matter, though this may impact the growth and development of plant species. Different plant species can have different effects on outdoor air pollution. Small conifers can capture particular matter effectively due to their intricate needle structures, while magnolias can reduce NO_2 pollution, as they use it during metabolism (Gourdji, 2018). This would have addition secondary effects of reducing pollutants such as tropospheric ozone due to chemical dynamics in the air.

3.1.4 Carbon storage

Green roofs can store carbon in vegetation and substrates, which helps remove carbon dioxide from the atmosphere. As well as secondary effects such as reducing energy demands of buildings. Carbon emissions were also released when producing and maintaining green roofs, with one study estimating a payback time of between 5.8-15.9 years (Kuronuma Takanori, 2018). While for the whole life cycle, green roofs will be a net carbon sink, investigation should examine best practices, to improve carbon storage potential. Biochar amendment was shown to be effective at storing carbon (Chen Haoming, 2018a)). *Sedum acre L,* species was shown to have the highest rate of photosynthesis and hence highest about of carbon storage, from the range of taxa studied (Seyedabadi Mohammad Reza, 2022).

3.2 History and types of green infrastructure

Rapid urbanisation has taken place throughout Europe and the developed world, since the start of the industrial revolution, due to the rise of jobs in cities. Nevertheless, this has led to a range of environmental issues. Economists hypothesised the theory of the Kuznets curve, which suggested that environmental degradation would peak during the stage of an industrial society and fall as the economy turned towards the service sector, however many environmental indicators prove this not to be the case, partly due to social changes (Qiang Wang, 2022).

Discussions by residents and policy makers on ideas to solve some of these environmental issues led to the term green infrastructure being used, recognising that along with buildings, roads and railways, there was also a need to recognise the importance of the natural environment and its ecological benefits. In cities, green infrastructure can be used to describe a variety of green spaces, including parks, gardens, allotments, green roofs and living walls.

The term “green infrastructure” was first used in 1994 in a report to the governor of Florida on land conservation strategies (M.Benedict, 2012). With increasing awareness of the issues, a range of projects have taken place globally to investigate if green infrastructure could mitigate environmental degradation, as well as provide ecosystems services (Beatrice L. Gordon, 2018).

3.3 Green infrastructure- special greenery elements (green roofs/walls)

Special greenery elements is a term used to describe green walls and green roofs. They have the possibility of serving a range of ecosystem services and can be designed when constructing a new building. Retrofitting onto existing infrastructure, has also been discussed, however many factors would need to be assessed, such as if a roof can support the weight of the substrate (Stefano Cascone, 2018). The advantage of green roofs and walls over other green infrastructure is the small amount of space required, compared to for example a park or a garden. This benefit is particularly useful in urban spaces where land is an asset. Green roofs can be divided into three types, such as extensive – designed to for minimal maintenance, intensive green roofs, which are designed so people can utilise the space, and semi-intensive, which combine elements off both.

3.4 Environmental concerns of green roofs

While the benefits of green roof systems should be emphasised, a life cycle analysis should be completed to evaluate the total environmental benefits. It was noted that use of plastic in the green roofs sector was becoming more prominent due to the need for low weight materials on green roofs, however analysis of polymer manufacturing should be done (Fabricio Bianchini, 2012). Air pollutants were also released from the degradation of polymers in the air.

Moving large quantities of material to roof spaces often need the use of cranes, which require fossil fuels to operate. Extensive roof systems were shown to have greater environmental benefits, as thinner layers, relative to intensive roof systems need fewer material requirements.

3.5 Optimal locations and adoption of special greenery elements

Green roofs should be designed for the specific climate and to serve an ecological benefit for an urban area, consequently, plant species should be chosen which can survive abiotic conditions in the given urban region – as well as local microclimate factors on green roofs, such as wind and higher exposure to sunlight. In Mediterranean climates, summer heat and drought stress can cause a variety of issues. Drought tolerance species should be selected, with a good ability to resist heat stress in the root system (Savi Tadeja, 2016). Hence shallow substrates may be detrimental, due to rapid heat transfer processes. Non irrigated green roof systems were shown to be effective in temperate monsoon climate like Beijing (Zhang hui, 2021), with greater substrate depths showing better survival and earlier flowering of plants. In oceanic climates like the UK in Birmingham, periods with lower rainfall were most significant on the number of plant species (Adam J. Bates, 2013). Drought disturbances helped the reappearance of annual plants. At some points in time, bare ground was visually seen, however this may not have negative implications due to providing habitats for arthropods and birds. Locations with less nutrient availability performed better in periods of drought since they were smaller and lost less water due to evapotranspiration processes.

A range of social-ecological issues are thought to determine the adoption of green infrastructure in cities, with an increasing number of research papers discussing the topic. (Tayouga, 2016), found education was important to improving adoption of green infrastructure. At present, green roof systems are expensive to implement, however despite this, even in countries with high GDP green roof policies have not been adopted (Semaan, 2016). Reliance on market forces to improve green roof adoption is unlikely to be sufficient (Brudermann Thomas, 2017) due to at present risks being taken on by building owners, consequently public benefits to green roofs tend to not influence individuals. Local policymakers should implement green roofs and walls into urban planning designs, to speed up the adoption process, such as has been demonstrated in cities like Stuttgart. Government subsidies were important to encourage green roof adoption (Claus Klara, 2012) which have a long time for payback and many benefits such as: increased fire protection; decreasing urban temperatures and promoting biodiversity, were not economically quantifiable.

(Joel Lönnqvist, 2021) found in cold weather climates, planted species generally performed poorly and spontaneous colonisation helped improve species richness. Substrate depth was highly correlated with species richness. Moreover, other studies show the importance of substrate depth for water storage capacity– especially on non-irrigated roofs. Southern facing roofs at northern latitude may be snow free for a shorter period and retain more storm water, however in summer months, southern facing slopes may be prone to droughts, hence species should be selected which can withstand droughts.

In this study (Huang Shan, 2020), different substrates were investigated for their hydrological performance. The addition of both biochar and vegetation significantly changes the pore distribution, which improved water retention capacity. Biochar amended soil also increased detention time, with the widest divergence of results seen at light rainfall intensities, while at heavy rainfall intensities, biochar impacts on detention time were small.

3.6 Biochar

3.6.1 History of biochar

Humans have significantly altered the carbon biogeochemical cycle, with carbon being transferred from the geosphere and biosphere to the atmosphere and the hydrosphere. Biochar use has been promoted as a process which can store carbon in soils for a sustained period (Smith, 2016). Moreover, other benefits to soil properties, including increasing water holding capacity and soil fertility for agricultural land have been discussed. Historically, land which has been maintained by the addition of biochar, has also had other inputs, such as pottery and manure (Ferreira T, 2021), hence biochar may not be the only factor that improved soil fertility in historical cases. Biochar has received increasing research in scientific literature, due to its claimed potential benefits. It can be made from a range of feedstock's including crop residues, wood, manure, and sewage and has generally been used for applications to soils, to improve agricultural yield. Nevertheless, a range of applications have being discovered, due to its unique properties (HP Schmidt, 2012).

Biochar has a high pH > 7, which is a factor that influences plant growth, especially in acid soils. In a study on soya bean growth, biochar was found to affect the pH of soils, hence promoted soya bean growth (Yu, 2017). Moreover, biochar that has a high ash content also increased the availability of nutrients, especially in the rhizosphere. Increases in pH and porosity also help with microbial growth, which in turn improve plant nutrient uptake. However, in soils with high pH, due to increased biochar addition, microbial and fungal activity decreased (Huang Danlian, 2017). Biochar also helps to reduce the availability of Al^{3+} ions which are toxic to plants at high levels. A range of mechanisms have been proposed for reduced aluminium uptake, including liming effects, and the absorption and precipitation effects of biochar (Shetty Rajpal, 2021).

Biochar had no significant effect on nitrous oxide emissions at lower soil moisture contents, however at high soil moisture contents, at 27% and 30% by weight water, nitrous oxide emissions fell due to the increase in the abundance ratio of enzymes *nos*Z(Nitrogen oxide reductase) to *nir*S(nitrite reductase), hence enzymes which promote denitrification processes were favoured (Feng Zhengjun, 2018). According to (Liao Jiayuan, 2021) nosZ increased as a function of specific surface area of biochar, causing a reduction in nitrous oxide emission. Biochar pyrolysis at higher temperatures was also more effective at reducing nitrous oxide emissions (Ahmad Zahoor, 2021).

Biochar showed an improved soil carbon storage over a 6-year period when applied to silty clay loam soils (Blanco-Canqui, 2020), however, the exact mechanism for this study is not clear. It is possible biochar decreases the rate of soil organic carbon and crop residue mineralisation. A meta-analysis shows biochar potential to store carbon in soils (Jhon Kenedy Moura Chagas, 2022) with biochar increasing total carbon content by 64%, due to the stability of carbon from pyrolysis. Total carbon was higher with a higher application rate of biochar, as well as higher in finer soils and alkaline soils. Field tests have not been as effective as pot experiments at storing carbon, due to a higher number of disturbances in the field.

3.6.2 Properties of Biochar

The review article written by (Kathrin Weber, 2018) provides an overview of various properties of biochar.

Chemical Properties

Atomic ratios

Van Krevelen diagram for biochar shows that for increasing temperature in the production process, there was an increase in the carbon to oxygen ratio and the carbon to hydrogen ratio for both woody and non woody feedstocks, the temperature range causing the most changes in atomic ratios took place between 250°C and 350°C.

Elemental composition

A decrease in functional groups containing oxygen and hydrogen led to an increased carbon content with increasing temperature of pyrolysis. Temperatures from 200°C to 400°C led to the most significant changes. To reach higher carbon contents >90%, pyrolysis temperatures would need to be above 700°C. Nitrogen typically makes up 0-2% of biochar, with no relationship to temperature of pyrolysis.

Energy content

Energy content increased with temperature, due to higher amounts of carbon, with temperatures between 250°C-350°C, showing the greatest changes in energy content.

Fixed carbon and volatile matter

Volatile matter decreased with increasing temperature of pyrolysis.

Structural composition

Residence time of pyrolysis is the best predictor for structural composition, with longer residence times above 400°C, reducing holocellulose content, and temperatures above 500°C, reducing ligand content.

Functionality

Higher temperature pyrolysis increased the C:H ratio, and hence reduced the number of functional groups. Aromatic structures have high thermodynamic stability, and hence were important for soil amendment, due to long term stability. Grass derived biochar needed lower temperatures than woody feedstocks to create the same proportion of aromatic groups.

pH

Increasing temperature of pyrolysis increased the pH of biochar, due to loss of acidic functional groups. Straw biomass had a higher pH value than woody biomass. Ash content also increases during pyrolysis and is also slightly basic.

Cation exchange Capacity

Highest cation exchange capacities were found at low production temperatures, were surface area has increased relative to the feedstock, but loss of functional groups has not been significant to provide negative charge.

Ash content and composition

Ash content increased with increasing temperature of pyrolysis, however the correlation is weak, and of far more influence is due to feedstock type, with straw like biomass having a higher ash content than woody biomass.

Physical properties

Density and porosity

Higher temperatures of pyrolysis led to higher porosity, with grass derived biochar having a higher porosity than woody biochar. Bulk density falls when drying the feedstock, and the temperature of pyrolysis does not influence bulk density significantly. The bulk density of the feedstock is the best predictor for bulk density of the biochar.

Surface area
Increasing temperature of pyrolysis increased the surface area, though after temperatures exceeding 800°C, surface area falls. Sewage sludge had a lower surface area, relative to other feedstocks.

Pore volume and pore size distribution
Plants need to overcome capillary forces holding water to pores and were not able to access water in pores in the nanometre range. Macropores are defined as 1000-0.05 µm, while micropores are between 0.05-0.001 µm. 80% of biochar is made of micropores, compared to 10% from agricultural residues. The volume of micropores was correlated with surface area.

Hydrophobicity and water holding capacity
Loss in functional groups and increases in porosity act in opposite ways, hence it is tricky to distinguish between these two phenomena in the literature. Increasing temperature of pyrolysis, increased loss of function groups which were polar and hence hydrophilic, hence less water can enter the porous char structure. However, some studies show that at high temperatures, the effect of porosity is more significant, and increases in water holding capacity have been observed.

Mechanical stability
Mechanical stability generally correlates inversely with porosity

Grindability
Pyrolysis decreased mechanical stability and hence increased grindability. Feedstock higher in hemicellulose than ligand content would be more easily grindable after pyrolysis.

Thermal conductivity and heat capacity
Higher densities were associated with higher thermal conductivities; hence the development of a porous structure led to a decrease in thermal conductivity of biochar, relative to initial feedstocks.

3.6.3 Use of biochar in special greenery elements

Several studies have been conducted investigating the use of biochar for application on green walls and roofs. One particular concern with green roofs is the deterioration of water quality, caused by storm water runoff from green roofs, with high concentrations of phosphorus and nitrogen. Research has shown biochar can absorb both organic (L. Beesley, 2010) and inorganic (X. Cao, 2009) pollutants and increase the water holding capacity in plants. In this study (Kirsi Kuoppamäki, 2016), it was shown that biochar treatment significantly reduced storm water runoff, and hence total nutrient loads from runoff were lower over the study period. However, there was no difference in the concentration of nutrients in the runoff, between biochar and control soils. Biochar was more effective during periods of drought in the summer, as the water retention capacity was improved by biochar especially during the summer with infrequent precipitation events. There was a mitigated effect during the autumn with frequent precipitation. During the autumn, the substrate remained continuously moist due to the increased frequency of precipitation and declining temperatures, consequently, which led to lower transpiration by plants and lower evaporation of water from the substrate, resulting in a much lower water retention capacity in biochar compared to the summer season.

In this study (Cao, 2014), the addition of biochar was shown to reduce the bulk density in the growing substrate, hence greater depths of substrate could be applied to roofs, without impacting on the roof structure. Biochar also delayed the onset of permeant wilting by 2 days, thus possibly reducing irrigation frequency, and creating the possibility of a wider range of plants which could be grown on green roofs. Studies have also taken place on the forms of biochar used. Granulated biochar was shown to have the best impacts for plant growth on green roofs (Wenxi Liao, 2022), particularly due to the lower pH values of granulated biochar.

3.7 Plant physiology

Plant growth and development is dependent on a range of physical, chemical, and biological processes, which need energy and material inputs to drive these processes. Liebig's law of the minimum states that plant growth is not dictated by total resource availability but by the scarcest resource, hence growth is not a linear function against nutrient concentration and can be thought of more as a stepwise function, with a critical point, between deficiency and non-deficiency of a given nutrient. It should also be noted that too much of a given nutrient can be toxic to a plant. In higher plants, 95% of the dry weight is made up of three elements, hydrogen, carbon, and oxygen, which are taken up by plants as minerals through the roots, and gaseous compounds through the stomata. Photosynthesis is the most important process and makes most plants autotrophic.

3.7.1 Essential macro and micronutrients

3.7.2 Nitrogen

Often a limiting nutrient for plant growth, nitrogen is a component of many essential compounds, including amino acids and chlorophyll. Plants in excess nitrogen have a higher root to shoot ratio and darker leaves. Nitrogen is usually taken up through the nitrate ion, and occasionally the ammonium ion, and trace amounts from dissolved organic nitrogen.

3.7.3 Phosphorous

Is absorbed by the monovalent phosphate ion and less rapidly the divalent phosphate ion. It is essential for a range of metabolic processes, including energy metabolism. In excess phosphorus conditions, root growth is increased, relative to shoot growth.

3.7.4 Potassium

Taken up in the form K^+, potassium is an activator for many enzymes that are essential for photosynthesis and respiration.

3.7.5 Sulphur

Absorbed as the disulphate ion, it is present in amino acids, vitamins, and coenzyme A, an essential compound for respiration and the synthesis and break down of fatty acids.

3.7.6 Magnesium

Absorbed as Mg^{2+} ion, it is present in chlorophyll. Its combines with ATP and activates many enzymes, needed for photosynthesis, respiration, and formation of DNA and RNA.

3.7.7 Calcium

Up taken through Ca^{2+}, Calcium activates several plant growth regulation enzyme systems, responsible for plant cell division and converts nitrate ions into forms needed for protein formation.

3.7.8 Iron

It forms parts of certain enzymes and various proteins that carry electrons during photosynthesis and respiration.

3.7.9 Chlorine

Up taken as Cl^- ion, it is essential as a catalyst to split H_2O during photosynthesis, and for roots and cell division in leaves.

3.7.10 Manganese

Exists as Mn^{2+}, Mn^{3+}, Mn^{4+}, activates many enzymes and involved in splitting water. It plays an important role in the structure of the chloroplast membrane.

3.7.11 Boron

Used by plants during cell division.

3.7.12 Zinc

Taken up as Zn^{2+}. It's important for production of the hormone auxin and present in many enzymes.

3.7.13 Copper

Present in several enzymes or proteins involved in oxidation or reduction.

3.7.14 Molybdenum

Present in enzymes which reduce nitrate ions to nitrite ions.

3.7.15 Nickel

Part of enzymes urease, which metabolizes urea nitrogen into useable ammonia within the plant.

3.7.16 Others

Other essential nutrients have been speculated for plants, sodium is required for C4 plants as it is involved with the regeneration of phosphoenolpyruvate in C4 and CAM plants. Selenium is required by some accumulator plants, however, is generally toxic to most plants. It is speculated that iodine may have an essential role in plants (Kiferle Claudia, 2021), due to promoting earlier flowering and biomass accumulation, activating multiple pathways involved in defence response and iodinated proteins were synthesised by both roots and shoots in phylogenetically distance species.

3.8 Assimilation of nitrogen and Sulphur

3.8.1 Assimilation of Nitrogen:

The process by which N_2 is reduced to NH_4+ is called nitrogen fixation, which is only carried out by prokaryotic microorganisms. Bacteria and other microbes form symbiotic relationships with the roots of legumes. Most legumes have been found to fix nitrogen, while some pioneer species also were able to fix nitrogen in nitrogen deficient soils.

For plants that cannot fix N_2, the most important nitrogen sources were NO_3^- and NH_4^+. Roots of some species can synthesize all the organic nitrogen they need, whereas roots of other species rely on shoots for organic nitrogen. The relative amounts of NO_3^- and organic nitrogen in the xylem depend upon environmental conditions. Under excessive amounts of NO_3^- in the soil, reduction of NO_3^- cannot keep pace with transport to the shoots, hence reduction occurs in leaves and stems.

Processes of Nitrate reduction:

$$\mathrm{NO_3^-} + 8\mathrm{e}^- + 10\mathrm{H}^+ -> \mathrm{NH_4} + 3\mathrm{H_20}$$

The oxidation number of nitrogen changes from +5 to -3. Since more protons are needed than electrons, this causes the cells pH to rise.

Mineralisation: Organic nitrogen from dead plants and animals is converted to NH_4^+, with a range of bacteria, then nitrification takes place which convert NH_4^+ to NO_2^- and then NO_3^-

NO_3^- is converted to glutamate via two reactive compounds, the reaction is driven by the concentration of NO_3^- in plant cells, to ensure the reactions take place quickly, and plants are not exposed to toxic intermediates. In the cytoplasm, nitrate is converted to nitrite, catalysed by nitrate reductase. In the chloroplasts and plastids, nitrite is converted to NH_4^+, again catalysed by nitrite reductase. The amount of nitrate found in the plant xylem is dependent on plant species, with a lot of variation. NH_4^+ is converted to glutamine in a range of metabolic pathways. One example is the GOGAT cycle, where NH_4^+ reacts with glutamate, using the catalyst glutamine synthetase and energy from ATP, to form glutamine. Glutamine can react with 2 oxoglutarates, from the Krebs cycle to form 2 molecules of glutamine, one can be used again in the cycle, while the other is the product of the reaction, which can be converted to chlorophyll, proteins, and nucleic acids.

3.8.2 Assimilation of sulphate:

Most SO_4^{2-} is absorbed by roots, providing the sulphur needs for plant growth. Reduction of sulphate to sulphite is energy dependant and most sulphur transported in the xylem is non reduced SO_4^{2-}. The reduction in the leaves takes place entirety in the chloroplast, while in roots, most of the reduction takes place in proplastids.
In the first step of the assimilation, SO_4^{2-} reacts with ATP, producing, adenosine-5”-phosphosulfate (APS) and pyrophosphate (PP_i), catalysed by ATP sulfurylase, the PP_i is rapidly and irreversibly hydrolysed into two P_i, by a pyrophosphate enzyme and then the P_i can be used, in mitochondria or chloroplast to regenerate ATP. The sulphur of APS is reduced in chloroplast by electrons donated from a reduced ferredoxin. The oxidation number of sulphur changes from +6 to -2. One potential mechanism for this reduction is the sulphate group of APS is transferred to the sulphur atom of an acceptor molecule by an enzyme called APS sulphotransferase.

The acceptor molecule is unknown, and after it accepts the sulphate from APS denoted X-S-SO_3^-. The SO_3^- is then reduced by ferredoxin, producing X—S--H and free sulphide. The sulphide does not accumulate but is rapidly converted into organic sulphur compounds such as cysteine and methionine.

3.9 Growth and development

Growth in plants is defined as the mean increase in size, due to increases in volume, weight, cell numbers, amount of protoplasm and complexity. To quantify this, measurements of height or diameter can be used to approximate volume, while destructive testing can be used to measure the total biomass, after drying a sample. Growth in plants is restricted to certain zones containing cells recently produced by cell division in a meristem, 3 processes in the plant at the cellular level drive growth and development.

Cell division - one mature cell divides into two separate cells
Cell enlargement - one or both daughter cells increase in volume
Cellular differentiation - a cell becomes specialised

Plant cells grow in volume, primarily through the uptake of water. Water pressure is thought to cause cells to grow and expand. The rate at which water moves into the cells is governed by two factors, the water potential gradient (osmotic pressure) and the permeability of the cell membrane to water. It has been shown that solute concentrations inside many growing cells remain constant, hence growth is driven by a decrease in turgor pressure. Pressure in the cell is caused by the mechanical resistance of the cell wall to stretch, if the cell wall relaxes, the pressure falls which lowers the cell water potential, leading to a larger water potential gradient, and movement of water into the cell. It has been shown that cell walls can stretch plastically and elastically, with plastic stretching increased by auxin a plant growth regulator.

Plant growth can be divided into three stages. The first is the logarithmic phase, where the height of the plant grows exponentially with time, then the linear phase, were increases in size occur at a constant rate, the senescence phase is characterised by a decrease in growth rate. This is an idealised model, as some plants can have longer phases than others for example the pea plant has a long linear phase.

3.10 Abiotic factors on growth and development

A range of environmental factors influence plant growth and development. Abiotic factors, refer to non-living factors, such as temperature and soil pH.

Water availability is the most important factor, as it serves a range of functions in plants. Water is a solvent hence nutrients and solutes can be carried from roots to shoots. Water helps to regulate a plants temperature, through the process of evaporation. Many biochemical reactions need water to function, including photosynthesis. However, if water is limited, often in arid climates, or during afternoon heat, plants will prioritise retaining water, and consequently close their stomata’s, to limit water losses, despite this action stopping the photosynthetic process.

Sunlight is another factor that effects plant growth. It catalyses photosynthesis, and hence plants optimise growth direction and surface area, to reach as much sunlight as possible. This is called phototropism and is driven by the plant hormone auxin. In higher plants, pigments chlorophyll A and chlorophyll B, absorb sunlight in the blue and red parts of the spectrum and use the energy to drive chemical reactions. Light receptors, such as phytochrome also respond to light stimuli, absorbing light in the red and far-red spectrum, and control many aspects of plant development.

A range of properties in soil affect plant growth and development, including:

Soil moisture

Is important to dissolve nutrients and can hence be up taken by plants.

Soil Ph

Soil Ph can affect the availability of nutrients in the soil. At low pH, phosphorous uptake is of most concern, while at high pH, nitrogen uptake is of most concern. Optimal pH ranges are usually between 6.5 - 8 for plant growth, though some plants have adapted to extreme pH levels.

Soil Air

In poorly aerated soils, roots will not be able to take up space and hence will have less ability to absorb water and nutrients. Soil microbes also need air to function and carry out biological processes, such as the decomposition of organic matter and fixation of nitrogen, in poorly aerated soils, anaerobic respiration can take place, leading to the production of more toxic substances.

Deeper soils allow plants to have deeper roots and a greater surface area to uptake water and nutrients, as well as provide stability for the plant. Nutrient concentrations also vary with plant depth, with phosphorus and potassium more abundant in topsoil, while sodium and chlorine were more abundant at greater depths (Esteban G. Jobbagy, 2001).

Soil organic matter

Soil organic matter contains vital nutrients such as sulphur, phosphorus, and nitrogen essential for plant growth, as well as helping microbial diversity and activity.

Relative Humidity

Transpiration processes drive the uptake of nutrients from soils, hence in high relative humidity environments, plants can't transpire and take nutrients from the soil.

3.11 Stress physiology

Due to abiotic or biotic factors causing stress, plants can acclimatise, due to prior exposure to a particular stress, while plants can adapt over a long period of time to mitigate a given stress, caused by selection over many generations. An example of an adaptation is the use of C4 or CAM photosynthetic pathways which is used by plants in arid conditions. Plants which experience water deficiency, limit leaf surface area expansion, as water lost through transpiration processes is proportional to the leaves surface area. Water stressed plants also lose leaves, and grow new leaves when rainfall returns. Plants also focus on root development instead of shoot development, so that root surface area increases and is more likely to find water. In the short term, stomata can open and close, in respond to changing daytime temperatures and relative humidity.

3.11.1 Heat stress

With the rise in global temperatures due to climate change – particularly on land, how plants deal with heat stress is an important topic. Plants use evaporative cooling, hence temperatures on leaves can be several degrees lower than air temperatures. Leaves have several physical adaptions, to help tolerate heat such as reflective leave hairs, waxes and vertical leaf orientation.

3.11.2 Cold stress

Low temperatures limit the distribution of plant species. Herbaceous species were damaged or killed at temperatures between -1 and -5, however some plants have acclimatised to survive temperatures as low as -25. Many plants have meristems covered by soil or snow, avoiding extreme cold temperatures. Most species that survive cold temperatures can tolerated some ice formation in their tissues.

3 .12 Grey water

3.12.1 History of grey water use and water use trends

Grey water is a term used to describe all wastewater household streams, except those from toilet flushing. Being able to live with a plentiful supply of clean water has always been seen as necessary and essential for human development and is goal 6 on the United Nations sustainable development goals. In a household, clean water has a range of uses, such as drinking, cooking, cleaning, and toilet flushing. Moreover, clean water is also used in a variety of industries, especially in agriculture and manufacturing (Hannah Ritchie, 2022). However, producing clean water is an energy intensive process, as well as needing lots of infrastructure to transport water from reservoirs to urban areas.

Developed societies have also invested heavily in treating wastewater. Wastewater is generated from a variety of activities, and needs lots of energy to process, as well as material infrastructure. In many countries, treating wastewater is a huge environmental issue, with many companies dumping raw sewage into rivers and seas (Brown, 2022), despite the detrimental environmental effects. As human civilisations and urbanisation has grown, demand for clean water has grown with rising population and affluence (Hannah Ritchie, 2022), with many regions depleting groundwater (Konikow, 2005) or desalinating sea water, in search for clean water, despite both these methods being energy intensive (Khaled Elsaid, 2020) and detrimental to aquatic ecosystems.

The idea to use grey water for a variety of applications has been suggested, which would reduce demand for clean water and reduce the amount of sewage that needs to be treated. Energy and water would also be saved in the process (Knutsson, 2021). To date, worldwide adoption of grey water has been sporadic, mostly adopted by countries with arid climates, where water resources are limited. Since

2013, there has been an exponential growth of the term grey water in academic research (Gustavo Oliveira Pinto, 2021). Wastewater use has been common in less developed countries, for example in irrigating agricultural land, however developed countries often associate wastewater with disease and malodours, hence ideas such as grey water reuse has been taken up slowly, due to these cultural influences.

3.12.2 Chemical analysis of grey water

Grey waters chemical composition depends on the source it comes from. Grey water can come from sinks, wash basins, showers, and dishwashers, while there is debate whether kitchen wastewater should be included as grey water (Fangyue Li, 2009).

	Bathroom	Laundry	Kitchen	Mixed
pH (−)	6.4–8.1	7.1–10	5.9–7.4	6.3–8.1,
TSS (mg/l)	7–505	68 – 465	134–1300	25–183
Turbidity (NTU)	44–375	50 – 444	298.0	29–375
COD (mg/l)	100–633	231 – 2950	26–2050	100–700
BOD (mg/l)	50–300	48 – 472	536–1460	47–466
TN (mg/l)	3.6–19.4	1.1 – 40.3	11.4–74	1.7–34.3
TP (mg/l)	0.11– >48.8	ND – >171	2.9– >74	0.11–22.8
Total coliforms (CFU/100 ml)	$10–2.4\times10^7$	$200.5–7\times10^5$	$>2.4\times10^8$	$56–8.03\times10^7$
Faecal coliforms (CFU/ 100 ml)	$0–3.4\times10^5$	$50–1.4\times10^3$	–	$0.1–1.5\times10^8$

Table 1: chemical analysis of Grey Water

The table (Table. 1) shows that generally, grey water has a higher pH, which can impact a plants ability to absorb nutrients. A range of different chemical properties were measured in grey water such as: Ph, electrical conductivity, Turbidity, Total Solid, Total dissolved solids, Suspended solids, Total phosphorous, Total nitrogen, biological oxygen demand, chemical oxygen demand, Total organic carbon, Total coliforms, Faecal coliforms, E. coli and Chlorine. A variety of metals and organic compounds can also be found in untreated grey water. Grey water can be treated biologically, chemically or physically.

Grey water has been investigated for being treated using nature-based solutions, such as constructed wetlands and green roof and walls, with growing interest in green roofs and walls over the period 2015-2020. Grey water irrigation on facades can be safely used to irrigate plants (Chung Pei-Wen, 2021), though changes were seen in soil electrical conductivity relative to normal water. It was not significant to cause changes in salinity of the soil substrate. pH values were not affected by grey water, though the grey water used in the experiment was only slightly basic, while grey water waste streams from laundry can have high pH values.

Grey water was collected from a variety of sites (Radingoana Makgalake P., 2020) in sub–Saharan Africa. pH was slightly basic, however was generally in the range 6.4-8.5 recommend for watering food crops. Only 44% of samples were within the recommended salinity limit of 540mg/L, hence grey water should be applied to food crops which can withstand moderate salinity.

Measuring turbidity could be used as a catch all measurement, as decreases in turbidity could be used to assess grey water quality, and hence make it easier to communicate to operational staff or the public how well grey water has been treated (Hyde, 2021).

3.12.3 Use of grey water in special greenery elements

Water systems can be redesigned to use light grey water, for use on green walls and roofs. In this study (Harsha S. Fowdar, 2017) investigated if green walls could act as bio filters for grey water, and hence water could be reused for toilets or in agriculture, after flowing through the bio filters. It showed that the system was effective at removing total suspended solid, biological oxygen demand and total nitrogen demand, though this may be due to effect of plant biomass increasing and longer studies would be need to investigative if nitrogen removal would be effective over time. The advantage of such a system is it could be implemented on a household level, moreover addition of soil medium such as biochar can improve wastewater treatment (Fida Hussain Lakho, 2021). In this study (Har'el Agra, 2018), it was found that grey water had no negative effects on plant growth, and grey water is a useful resource is semi-arid and dry climates, were water demands of green roofs are high during summer months. Grey water is thought to influence electrical conductivity of water and in the experiment, due to dry summer months, reduced rainfall caused accumulation of salts on the surface and limited leaching. Electrical conductivity values were elevated, especially in grey water until the first rainfall event. Therefore, if effluent water is to be used for agricultural purposes, it should be diluted with tap water. In this study (V. Thomaidi, 2022), recirculation of grey water was also tested, and showed enhance pollutant removal in intensive roof systems filled with 20 cm of vermiculite and 5 cm of lightweight expanded clay aggregates. Enhanced denitrification of the effluent and reduced turbidity, organic matter and suspended solids was found upon recirculation.

3.13 Green roof microbiome

Since green roofs have been disconnected from the surface, they would also have been disconnected from the networks of fungi and bacteria living around the soil surface. These interactions may be important for plants as they form a symbiotic relationship, exchanging sugars for nutrients such as nitrogen and phosphorus. A range of benefits could be obtained, if microbial diversity is assimilated into green roof systems (Fulthorpe Roberta, 2018). Nitrogen is often the limiting nutrient in plant growth, hence improving nitrogen retention in green roofs is critical, and using nitrification inhibitors could help increase nitrogen retention and avoid the loss of nitrogen to effluent streams, or gaseous loss of nitrous oxide, a powerful greenhouse gas. It has also been suggested that adding earthworms to a green roof can improve soil properties, including increasing organic matter, organic carbon, and total nitrogen. This phenomenon is hypothesised to be due to earthworms enhancing microbial activity in soils (My Dung Jusselme, 2019).

Lichens can also improve green roofs functions, (Heim A, 2014) investigated lichens impacts on green roofs. Lichens helped reduce water losses, and substrate temperatures during the summer, and increased substrate temperatures in autumn months relative to the control, hence lichen mats could be a viable application for green roofs.

(Chen Haoming, 2018b) investigated biochar impacts on green roof and ground microbial communities. The green roof was in a subtropical monsoon climate, and biochar derived from sewage was applied at volumes of 5%, 10%, 15%, and 20%, mixed with local soil. Biochar significantly increased plant available water and number of days to permeant wilting point. Microbial biomass increased most under 15% biochar, with the greatest effect on fungi, eukaryotes, and anaerobes. Biochar significantly increased richness and diversity of total microbes in the green roof and ground ecosystem. Sludge biochar reduced bulk density and increased air-filled porosity; hence green roof substrates could be designed with less weight.

Most plants form a symbiotic relationship with mycorrihizal fungi, causing a range of benefits for both plant and host. This review (John, 2017) suggests there is a lack of clear evidence for mycorrihizal fungi benefit to green roof systems, due to low soil moisture and lack of nutrient availability. However, limiting plants to those which do not form mycorrihizal relationships, would lower plant biodiversity on green roofs, hence further investigation should be made to integrate mycorrihizal fungi into green roof systems to maximise ecosystem services.

3.14 Why was the experiment done?

Use of grey water and biochar has not been investigated for use in green wall and living roof systems, however these materials have shown from the literature to be widely used in agricultural systems and investigated in green roofs and green walls. However, the interaction between grey water and biochar, and its impacts on plant growth and plant development is at present not been discussed. Therefore, this experiment will investigate, if biochar and grey water have potential for use in green roof and living wall systems.

4. Material and Methods

4.1 Experiment description

4.1.1 Experimental practice

The first part of the experiment took place in a greenhouse located at the campus of the Czech University of Life Sciences. The experiment started on 02/11/21 and ended on 19/01/2022. Plants had already been seeded prior to the start of the experiment. The plants were grown in normal soil and biochar in individual pots of depths of ~20 cm and cross-sectional area of ~10cm^2. Plants were watered on a 3-day basis with either tap water or grey water. Fertiliser was also supplied to plants.

At the end of the first experiment. Plants were destroyed and separated into leaves, flowers and rhizomes.

In the second experiment, the biomass collected from the first experiment was then dried at 60°C for 24 hours and the weight before and after was recorded.

The study I took part in was part of a larger study in the faculty of applied ecology at the department for environmental sciences at Czech University of Life Sciences.

Soil Treatments Used:
Biochar (see appendix (1,2) for physical/chemical properties)
Normal soil

Watering treatment:
Tap water, from greenhouse (TW)
Grey water, from university (CZU) collection system (GW) (see picture below)

Other Equipment:
Trolley to transport grey water barrels (see picture below)
Fertiliser (see appendix (3) for fertiliser properties)
Water barrels
Plant watering device – with micro drips
Plant pots
Measuring devices
Tape measure - used to measure height of the plant, which is assumed to be a proportional to volume of the plant
Lights on a 12-hour cycle.
Electricity from the grid
Marker pen – to label samples
Paper bags – to collect biomass
Pruning shears – to separate plant biomass

40 Plants were planted and placed in different positions in the greenhouse, which all received similar light intensity. Each plant was given a unique number, with 20 receiving grey water treatment and 19 receiving tap water treatment, 20 were grown in a biochar medium and 19 were grown in a normal soil medium.

Grey water was collected every fortnight in 40L barrels, and transported to the greenhouse, with a trolley. Tap water was available in the greenhouse. Water was given to plants on a 3-day cycle, using a plant watering device. Barrels were cleaned regularly to avoid build-up of bacteria and algae. Observations were made weekly on the plant watering device, to check there were no faults with the micro drips and water volumes distributed to plants were consistent and regular. If faults were discovered on a micro drip, then the micro drip were changed manually as spare drips were available in the greenhouse.

Figure 1 left to right:1. Collection point for grey water in CZU basement 2. Container with "SEDA VODA"(grey water). 3.Trolley used to transport grey water from basement to greenhouse

Measurements started on 02/11/2021 and ended on 19/01/2022, afterwards plants were removed from containers and separated into leaves, flowers, and rhizomes. The biomass was dried in an oven at 60°C for 1 day, to remove water contents.

Measurements were recorded twice a week of plant vertical growth by a tape measure, as well as visual measurements of the number of leaves and the number of flowers on a given plant.

The temperature of the greenhouse was maintained at 20°C by an air conditioner and heater. Lights were on a cyclical 12-hour cycle and plants were watered with a plant watering device.

4.1.2 Experimental setup in greenhouse

Plant Id 343: *S. byzantina*

Plant Id 360 – 379: *G. macrorrhizum*

Plant Id 380 – 399: *A. maritima*

392	366	360	343	395	369	372
386	390	365	393	367	378	389
374	361	396	383	388	394	385
387	382	371	368	394	363	398
397	376	364	377	362	399	379
381	370	375				
No Plants	391	No Plants				

(red)	GW and biochar
(orange)	GW
(green)	TP and Biochar
(blue)	TP

Table 2: Arrangement of plants in greenhouse

4.1.3 Measuring days

measuring days	Measuring Point
02/11/2021	1
05/11/2021	2
09/11/2021	3
13/11/2021	4
17/11/2022	5
21/11/2021	6
25/12/2021	7
29/12/2021	8
03/12/2021	9
07/12/2021	10
10/12/2021	11
14/12/2021	12
18/12/2021	13
23/11/2021	14
29/12/2021	15
02/01/2022	16
05/01/2022	17
08/01/2022	18
14/01/2022	19
16/01/2022	20

19/01/2022	21

Table 3: Measuring days for experiment

4.2 Botanical description of plant species used in experiment

Armeria maritima

Is an angiosperm in the family Plumbaginaceae and is common in coastal habitats in the northern hemisphere, around northern Europe, and Greenland.

Geranium macrorrhizum

Is an angiosperm in the family Geraniaceae. It is native to European alpine regions and south-eastern Europe.

4.3 Experimental work, experimental practice and statistical analysis

The experiment took place in a greenhouse. Data was collected on a paper spreadsheet and then transferred to excel. Recorded data included: height of plant, number of leaves on plant and number of flowers on plant. At the end of the experiment in the greenhouse, the plants were separated into rhizome, leaves and flowers, and the weight of the types of biomasses were recorded. The biomass was then dried in an oven at 60°C for 24 hours, and the weights measured, and data entered to an excel spreadsheet. Statistical analysis was then undertaken. Growth rates were calculated as a change in height per measurement. Mean growth rates were calculated as the sum of all growth rates over the total number of measuring points – more statistical parameters on growth rates, including standard deviation and skew were also calculated and can be seen in the appendix (appendix 8). 3-way ANOVA (analysis of variance) testing on how factors such as: plant species, biochar and grey water effected mean growth rates, with a significance level of $p<0.05$ – followed by Tukey's HSD test for significant interactions was then completed. For the number of leaves and flowers recorded, a general linear model was used with a Poisson distribution. For the biomass weights, correlation testing was completed as well as 2-way ANOVA testing with effects of biochar and grey water on total dry biomass and water accumulation, with a significance level of $p < 0.05$. The analysis was completed to determine the response of specific plant species to biochar soil and grey water treatment.

Statistical analysis was computed using R (https://www.r-project.org/).

5. Results

5.1 Description of Results

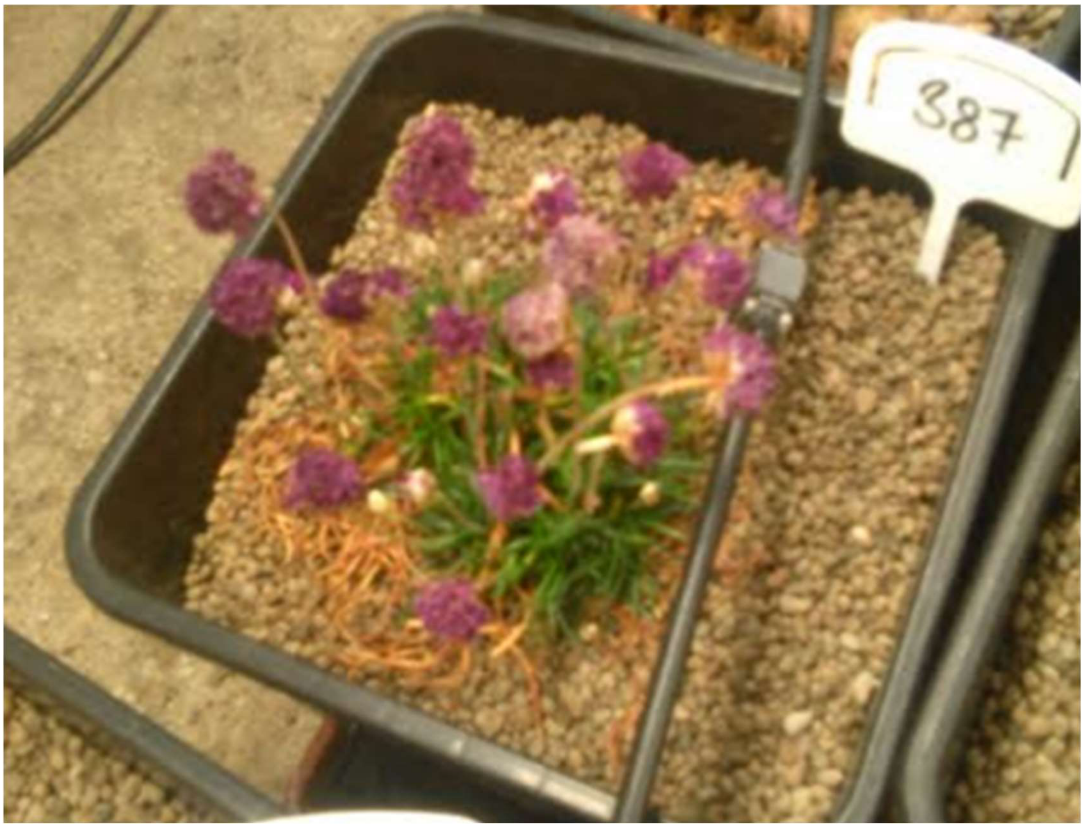

Figure 2: Plant species (A. maritima) with most flowers at the end of experiment 1

Plant species survived well in the experiment, with most species of *A. maritima* developing flowers – and the most successful plant (ID = 387) developed 20 flowers and shoots (Fig. 2). Only 8 of the 40 plants exhibited negative growth rates (see appendix 5) – with 6 of the 8 species with negative growth rates being *G. macrorrhizum*. Factors such as leaf and flower senescence, or lack of any development influenced growth rates negatively. Leaf senescence was observed more frequently in *G. macrorrhizum,* with leaves turning red and then brown before falling off, however this was generally offset by the development of new leaves. Only one species of *S. byzantina,* was observed during the experiment and it suffered from leaf senescence, hence did not grow and was excluded from further analysis. Only 2 plants of *G. macrorrhizum* developed flowers during the experiment. The number of leaves of *A. maritima* was stable during the experiment, though changes in colour from green to red and brown, on the edge of the leaves base was observed in some species – as can be seen in (Fig. 2).

5.2 Sum of all Growth rates:

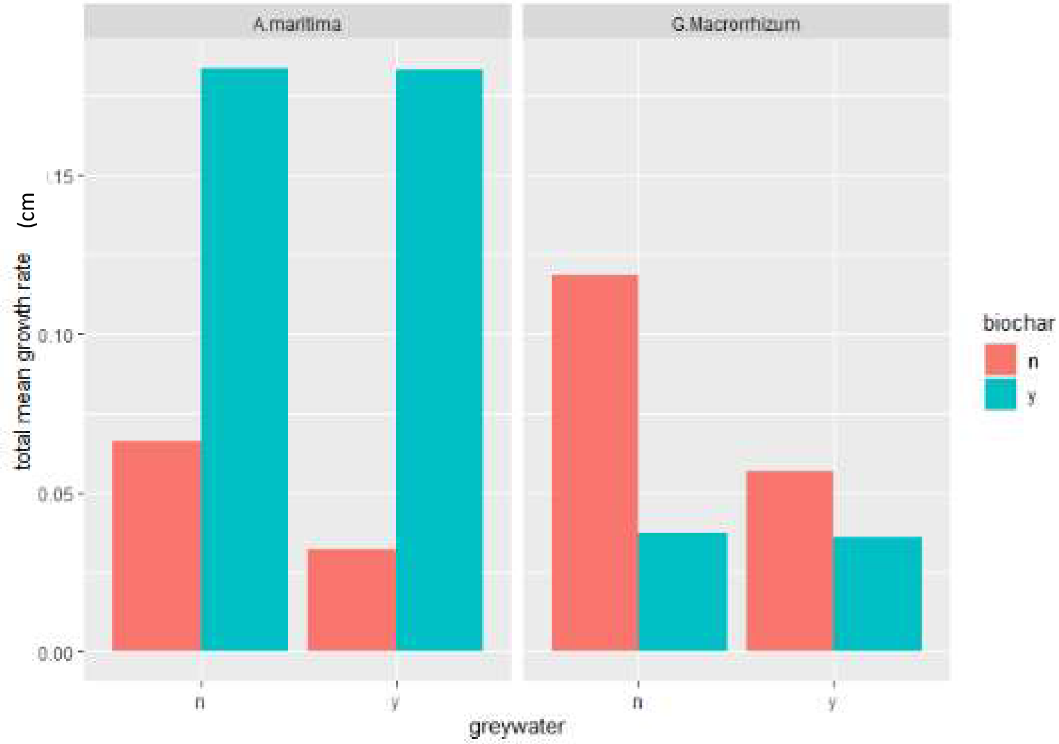

Figure 3: Total growth rate of both plant species studied during experiment:

(Fig. 3) shows the total mean growth rate (change in height) from all the sample. It can be observed that *A. maritima* grew well in biochar, due to the development of flowers, increasing the height of the plant.

5.3 Growth Rate distribution

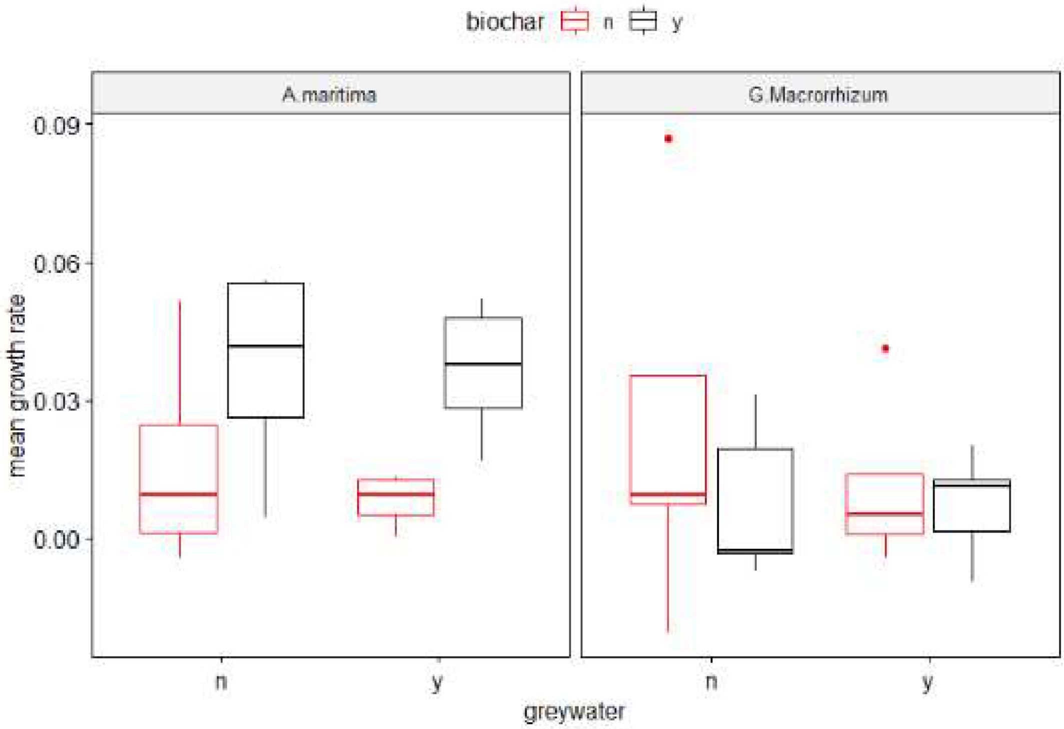

Figure 4: Boxplot of mean growth rates, separated by 3 factors (Biochar, Grey water and Species)

From the 3-way ANOVA analysis, the interaction between species and biochar was significant ($F_{1, 31} = 6.111$, $p = 0.0191$), as can be seen from (Fig. 4), Biochar influenced the growth rate of *A. maritima* – in grey water and tap water. All mean growth rates were greater in biochar in *A. maritima*. The species main effect also had a marginally significant result ($F_{1, 31} = 3.699$, $p = 0.0637$), with *A. maritima* growing faster than *G. macrorrhizum*.

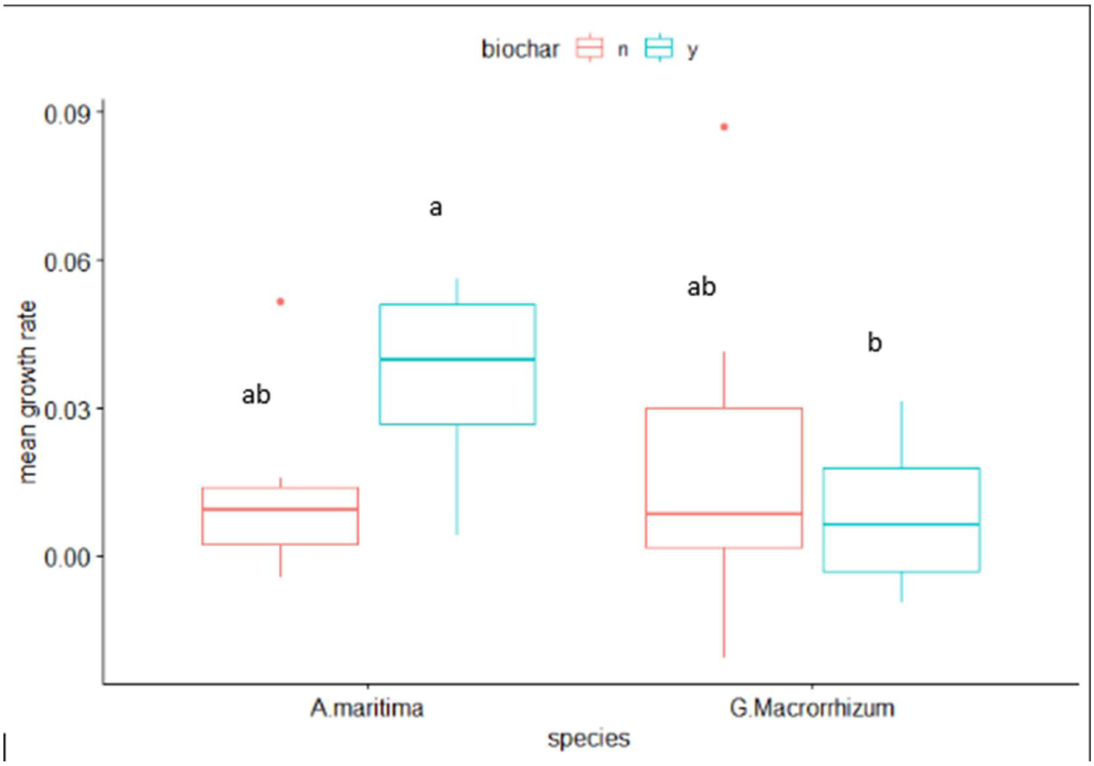

Figure 5: Results of Tukey's HSD test significant levels for mean growth rate, α = 0.05

A. maritima growth rate was most significant in biochar: (mean growth rate = 0.0367 $\frac{cm}{measure}$, Standard error = 0.0111 $\frac{cm}{measure}$) as seen in (Fig. 5), which suggests floral development takes place at an earlier point of time in *A. maritima* due to biochar.

5.4 Growth rate dynamics

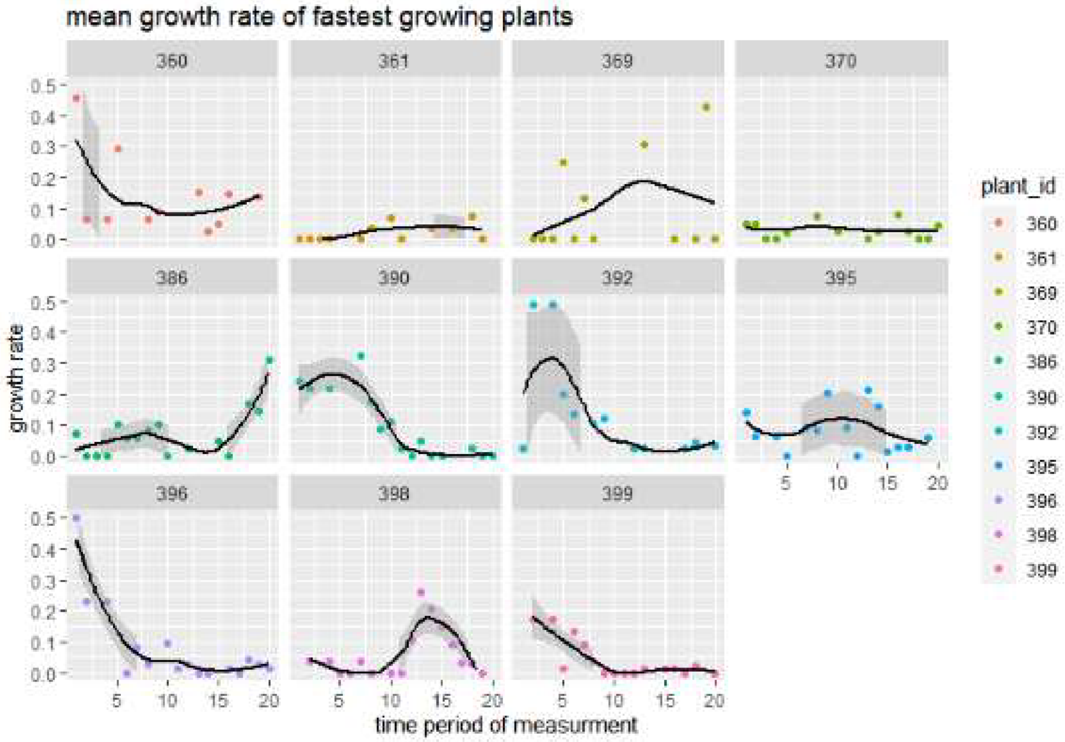

Figure 6: Growth rate dynamics for fastest growing species

The growth pattern of the fastest group of plants over the experiment, shows *A. maritima* (plant_id values > 379), had generally a non-linear growth rate, though for most of the time of the experiment growth rates were linear. The non-linearity can be attributed to the start of flower development (Fig. 6). In the appendix (appendix 6,7), the non-linear growth rates were less pronounced in plants with lower growth rates, especially in *A. maritima*.

5.5 Number of Leaves:

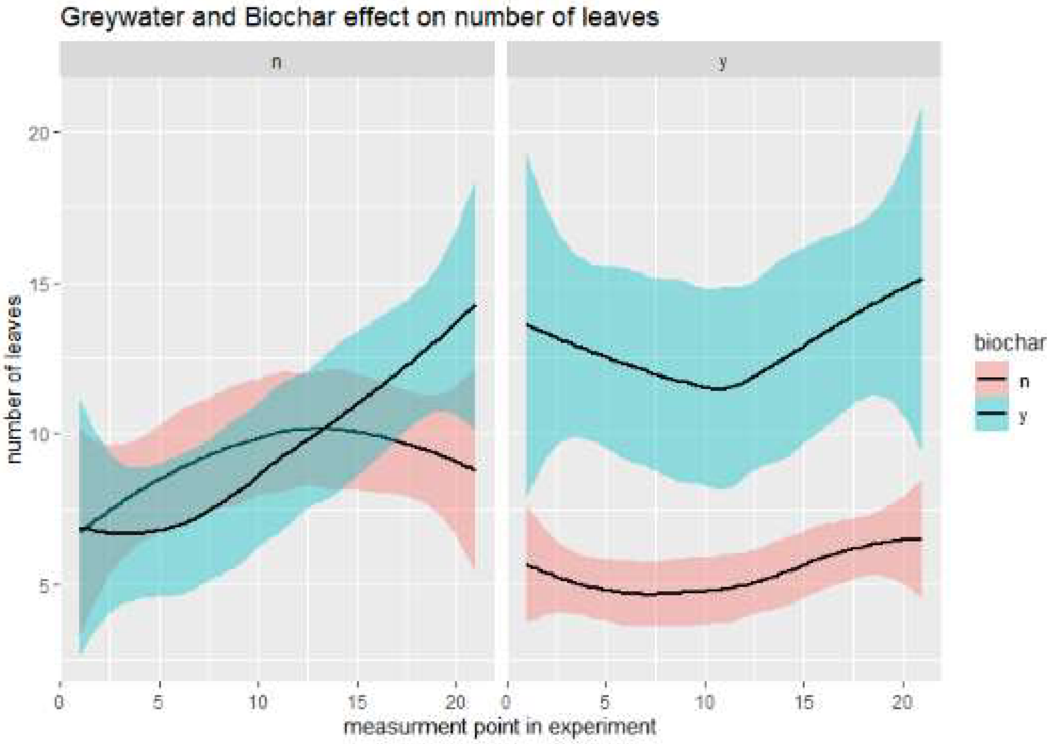

Figure 7: Effects Of grey water and biochar on number of leaves of G. macrorrhizum

There was a significant interaction found for biochar and grey water ($F = 12.385$, $p < 2*10^{-16}$) on the number of leaves, and in grey water ($F = -9.914$, $p < 2*10^{-16}$) on the effect of the number of leaves. As can be seen from (Fig. 7). Biochar produced a greater number of leaves. When *G. macrorrhizum* was grown in grey water treatment, the influence of biochar was most distinct, particularly towards the end of the experiment.

5.6 Number of Flowers

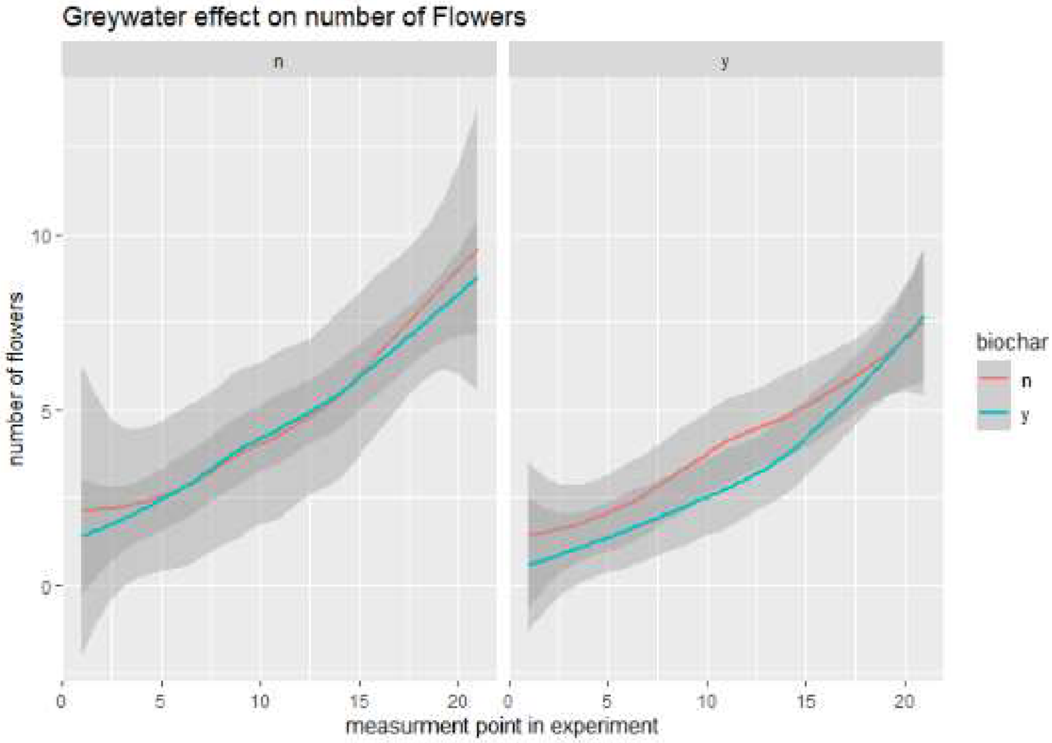

Figure 8: Effects of number of flowers due to grey water and biochar on A. maritima

Grey water had an effect (F = -2.629, p = 0.00858) on the number of flowers that *A. maritima* produced, with tap water treatments gaining a larger number of flowers than grey water treatments over the study period. There is a lag of about 5 measuring points (about 2-3 weeks) in greywater treatments (Fig. 8). Growth rates in the number of flowers were linear over the study period.

5.7 Weight of Dry Biomass

5.8 Dry biomass

5.8.1 Dry mass above ground for *A. maritima*

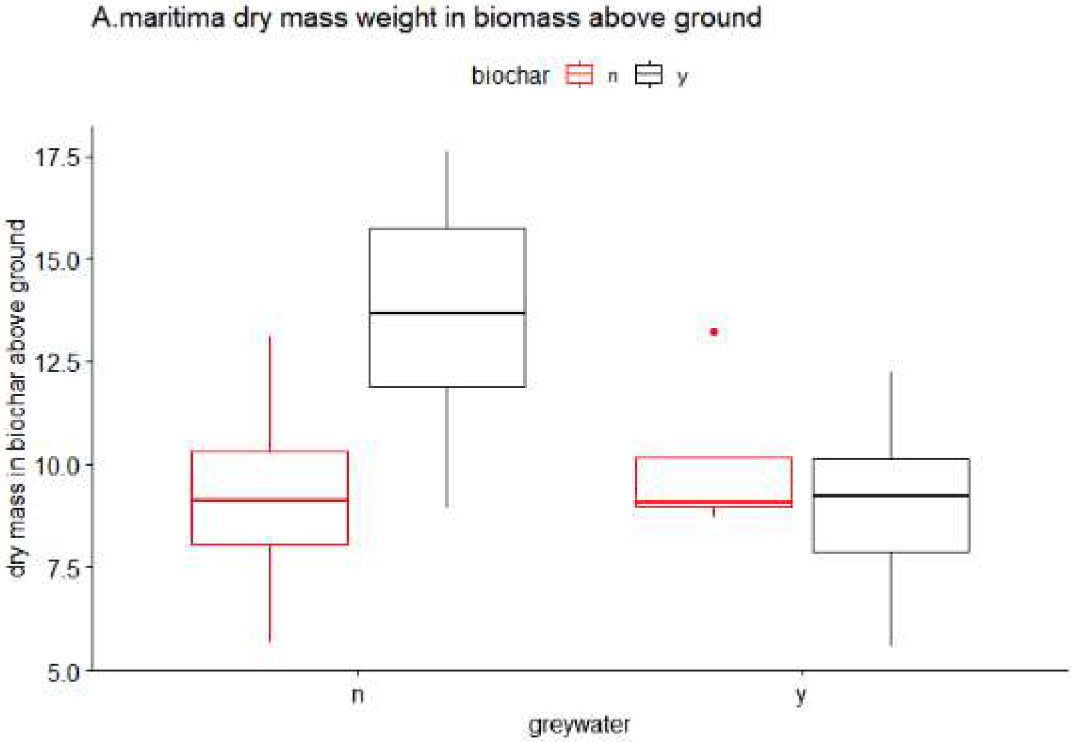

Figure 9: Dry biomass (in grams) above ground in A. maritima

Only a marginally significant result was found for the dry biomass of *A. maritima* ($F_{1,14}$ = 3.947, p = 0.0669) (Fig. 9) in the 2-way ANOVA test. Between the interaction of grey water and biochar. This suggest the biomass accumulation is improved by biochar when treated with tap water in species *A. maritima*.

5.8.2 Dry mass above ground for *G. macrorrhizum*

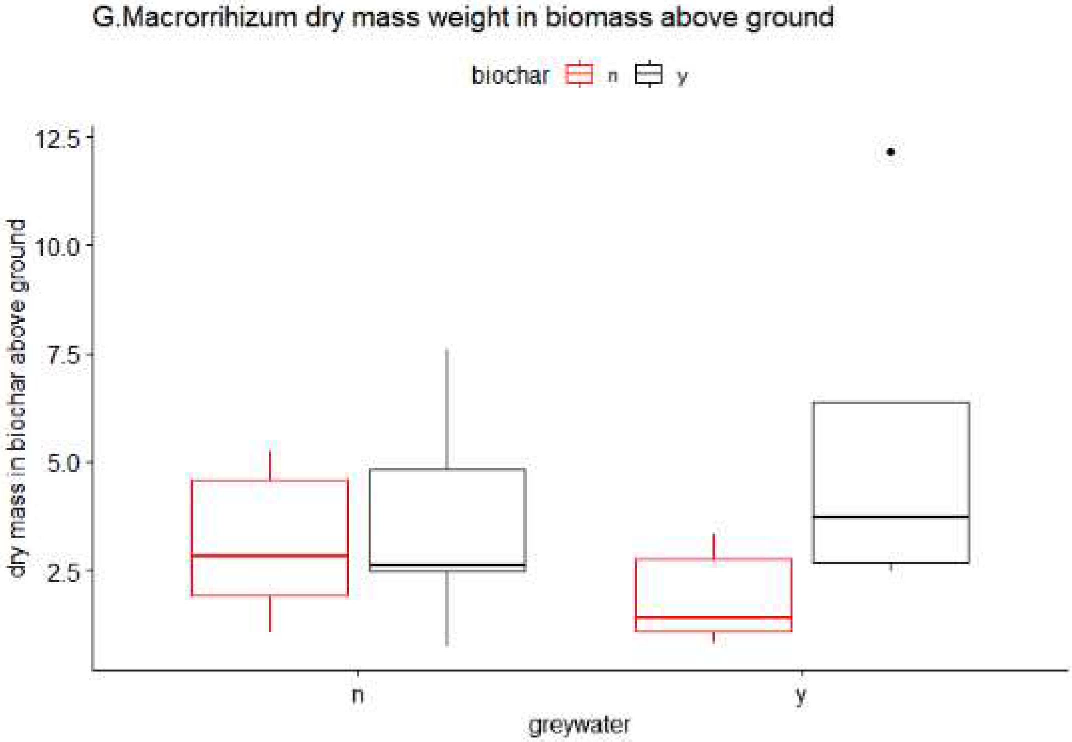

Figure 10: Dry biomass above ground (in grams) in G. macrorrhizum

Only a partially significant result was found for the dry biomass of *G. macrorrhizum* ($F_{1,16}$ = 3.082, p = 0.0983) (Fig. 10) in the 2-way ANOVA test, due to differences in biochar treatment. Hence it can be inferred that biochar improves the accumulation of biomass in *G. macrorrhizum*.

5. 9 Water Mass:

5.9.1 *A. maritima* water mass above ground

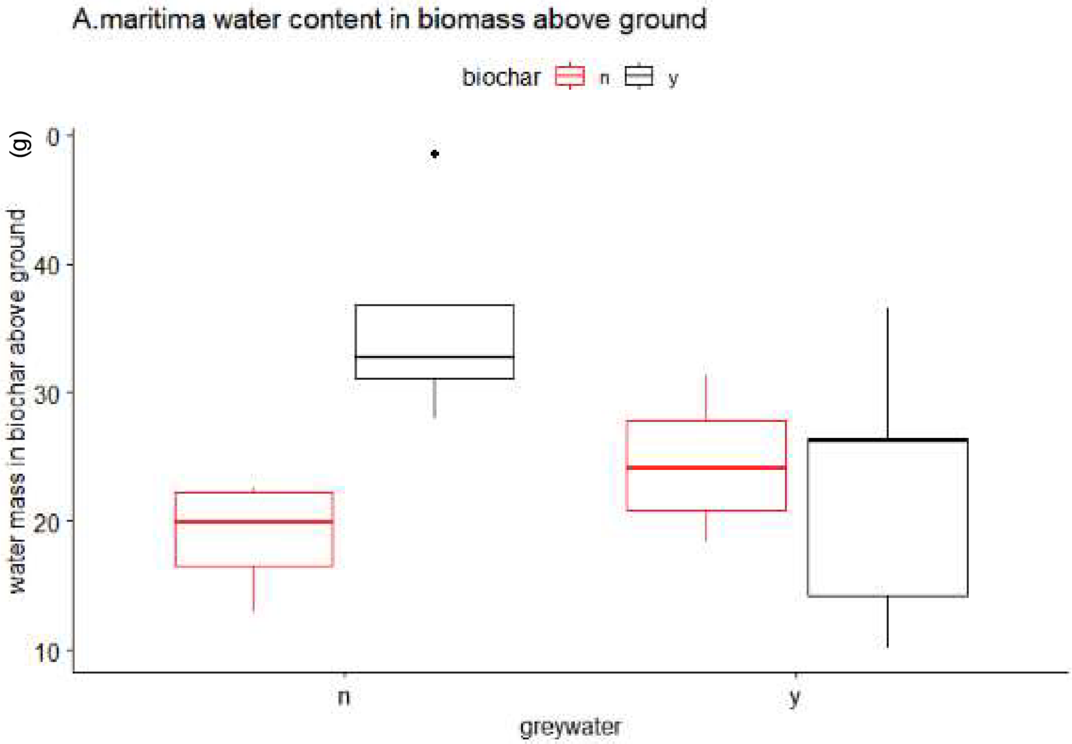

Figure 11: Water content of A. maritima above ground (in grams)

The interaction between biochar and grey water, was significant in the 2-way ANOVA ($F_{1,14}$ = 6.103, p = 0.027) (Fig. 11), in *A. maritima*, on the total water content. There was also a marginally significant result ($F_{1,14}$ = 3.952, p = 0.0667), due to biochar main effects. It can be concluded biochar with tap water treatments was optimal for water retention.

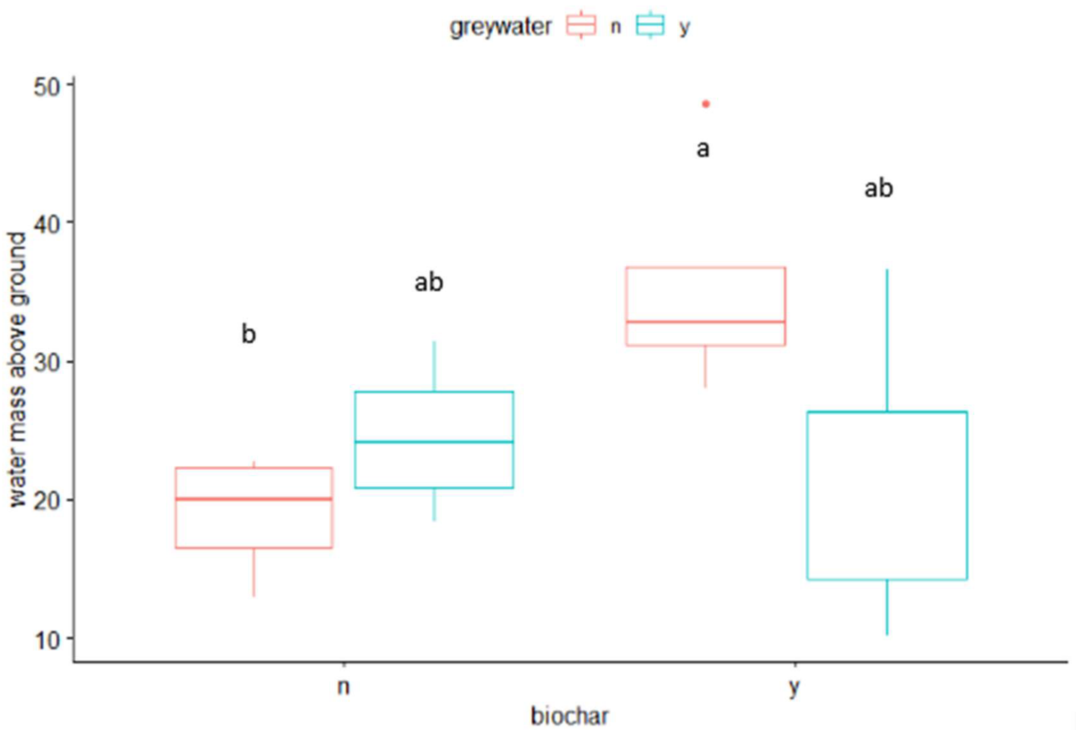

Figure 12: Results of Tukey's HSD significant levels, for water mass (in grams) above ground in A. maritima, α = 0.05

This suggests that *A. maritima* would be more drought tolerant in treatments of biochar and tap water, due to the ability to hold more water inside the plant (Fig. 12), though larger plants may also lose more water in drought conditions, moreover water content in plants can vary significantly from day to day.

5.9.2 *G. macrorrhizum* water mass above ground

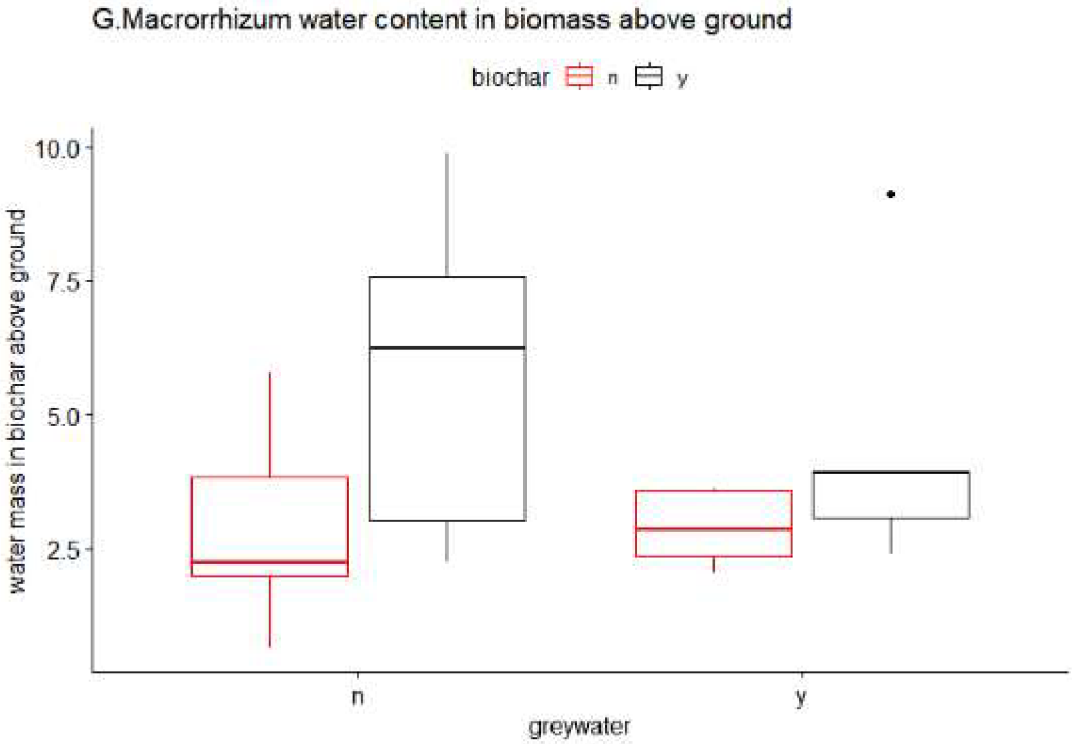

Figure 13: Water content above ground in G. macrorrhizum (in grams)

The biochar treatment was significant in the 2-way ANOVA test in *G. macrorrhizum*, on the total water content. ($F_{1,16}$ = 4.696, p = 0.0457) seen in (Fig. 13). This correlates well with the marginally significant result for the dry biomass weight off *G. macrorrhizum.*

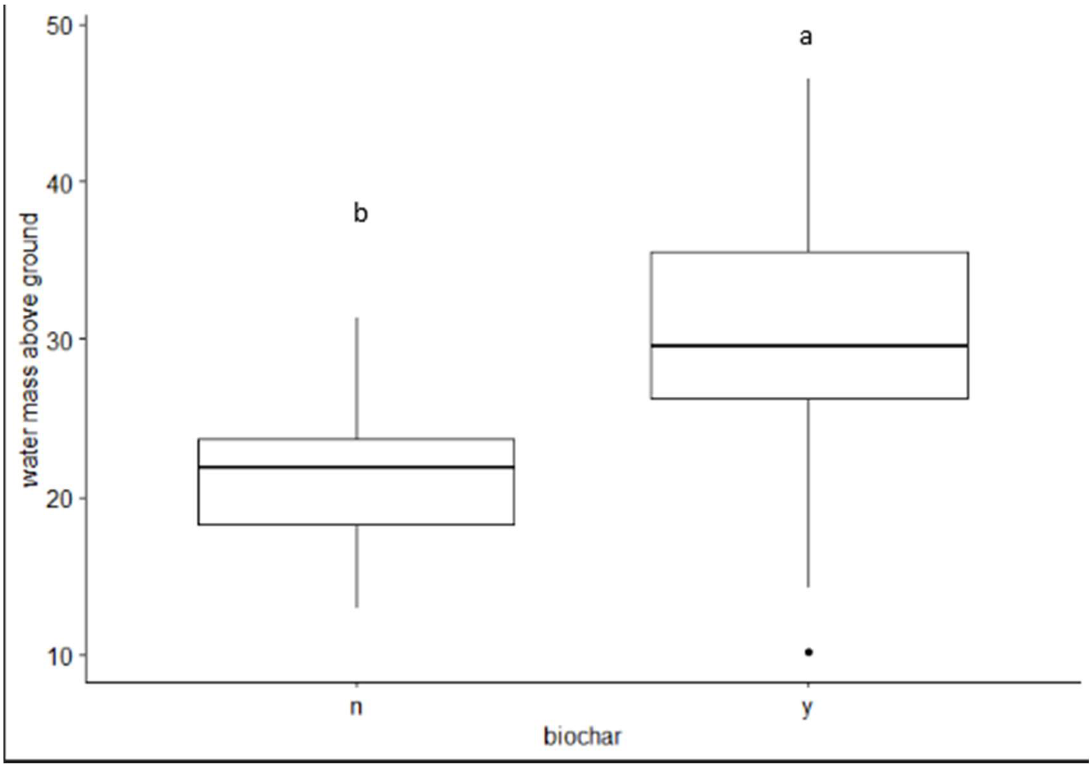

Figure 14: Results of Tukey's HSD test significant levels, for water mass (in grams) above ground in G. macrorrhizum, α = 0.05

Similarly, to *A. maritima, G. macrorrhizum* water content was affected by biochar (Fig. 14), which suggests *G. macrorrhizum* would be suited to drought conditions in biochar. though larger plants may also lose more water in drought conditions. The grey water effect was not significant in *G. macrorrhizum.*

6. Discussion:

A. maritima was successful at flowering, with the number of flowers reaching over 20 in the most successful plant. The dry mass and water mass of *A. maritima* above the soil was affected by grey water (Fig. 11, Fig. 9), though growth rates were not affected (Fig. 4). Grey water often limits plant growth due to high salinity levels, though *A. maritima* is tolerant to high salt levels (Eisikowitch Dan, 1975), hence further investigation may be needed to assess why *A. maritima* took longer to develop flowers in grey water treatments. Major concerns for grey water use in plants include salinity, pH, boron content and levels of surfactants, hence one of these factors likely impacted flower development. *G. macrorrhizum* growth rate was not affected by biochar or grey water (Fig. 4) – suggesting it can tolerate grey water treatment well, though a significant effect was found for the interaction between grey water and biochar on the number of leaves (Fig. 7). This suggests that if treated with greywater, the number of leaves that develop on *G. macrorrhizum* is significantly influenced by biochar. Only 2 *G. macrorrhizum* of the 19 samples produced flowers, compared to a majority of *A. maritima* plant species, which suggests *G. macrorrhizum* would need a longer time to flower or, is affected by factors such as grey water and biochar. Overall grey water did not affect plant growth which agrees with the findings from (Har'el Agra, 2018).

In the literature, biochar's positive effects on plant growth, due to increases water holding capacity; improving nutrient availability and changing pH has been discussed. Biochar also reduces the leaching of nitrogen fertiliser. In the experiment, biochar as a single factor had positive results on the growth rates of *A. maritima* (Fig 4), as well as dry and water mass (Fig. 9 & Fig. 11). Nonetheless, biochar in combination with greywater had little effect on dry mass and water mass of plants, which may be due to the fact the grey water has a high pH. High pH levels in soils effect nitrogen uptake, which would affect the rate of growth and development of a plant. Biochar amended soils affects the local pH at root nodules which can differ from the overall pH of the soil – with significantly elevated pH levels within the biochar particle (Johannes Lehmann, 2015).

To this date, there is little knowledge on the mechanisms driving plant growth and development in greywater and biochar. Measuring abiotic factors such as soil pH and soil moisture would be useful to understand effects of grey water and biochar. Changes in the use of fertilisers could also be made, to investigate interaction between biochar and fertiliser, which were significant in several studies on plant growth: (O.T. Faloye, 2019) and (Regmi, 2022). So far, there has been few studies in the literature on interactions between grey water and biochar and its effects on plant growth, however the present study shows plant species *A. maritima and G. macrorrhizum* have significant potential to be used on green roofs with biochar, and though plant development is slowed down by grey water, it does not affect growth rate of the plant. These results align well with (Ayako Nagase, 2010), which suggest *A. maritima* would be suitable for temperate marine climates and have good levels of drought tolerance in living wall systems (Emilia Danuta Lausen, 2020). Therefore, irrigation may only be needed at certain times during the year in green roofs and living walls. Results found from this experiment correlated well with results found in previous experiments in the study group (Kateřina Berchová, III. 2022, in litt.).

Chemical analysis could also be undertaken on dry biomass, to determine if the plants were deficient in any nutrients, and how biochar and grey water influence nutrient uptake in different types of treatments. Biochar also has a range of feedstocks and using biochar made from different feedstocks could also be studied to ascertain which biochar properties would be most important for plant growth and plant development as discussed in this review (Huang, 2019).

The results showed no significant changes in root biomass, due to grey water and biochar - which may be since the volume of soil that plants grew in was limited, or root structures may have reached their greatest extent and more clarity would have been found if root systems were observed over the whole study period instead of at the end of the experiment. In the literature, greywater has shown no effect on root biomass (U. Pinto, 2010), though biochar has been shown to influence root biomass (Xiang, 2017).

The experiment took place over a short period, and a longer study period may have shown greater plant development in flowers and leaves. Plants were also grown, without competition from other species, which may have applications for living wall systems, but not for green roof systems, especially if biodiversity of species is trying to be promoted. Only two species of plants were investigated in the experiment and further knowledge may be gained from investigation of a range of plant species. The experiment had several controlled factors which would not be applicable to green roofs and living walls. Factors including solar radiation, temperatures, wind, and air pollution would need to be investigated as to how they affect plant growth and development. *A. maritima and G. macrorrhizum* are native to mountainous areas and would therefore be adapted to strong winds and large temperature ranges.

No data was collected on leaf and flower senescence, or time taken for individual flowers and leaves to wilt, though these phenomena were observed during the experiment and collecting data on this may have been informative.

Watering of plants was done with a plant watering device. There was no possibility of measuring total water volume given to plants – and total water loss from plant pots, beyond visual inspection. For further experiments, measuring total water volume delivered to plants may also help when analysing material balances.

There was a good correlation between dry biomass and number of leaves/flowers, indicating these plant traits were good estimates for a plants dry weight – and counting the number of leaves was a good approximation for the total leave surface area. Plants horizonal size and diameter was not measured, however this may have been informative due the morphological growth pattern of *G. macrorrhizum,* which over the course of the experiment increased in width as well as height from observations.

7. Conclusion:

The aim of this experiment was to determine the effects of grey water and biochar on plant growth and plant development and discuss further applications on green roof and living wall systems. The results of the experiment show species *A. maritima* was the fastest growing species and grew fastest in biochar treatment – while floral development was affected by grey water treatment. *G. macrorrhizum* growth rate was not affected by grey water or biochar; however, biochar had a positive effect on the number of leaves. *G. macrorrhizum* did not develop floral displays, except for two plants, and no explanation for this effect can be found within the parameters of the experiments.

Biochar had a significant impact on water mass and dry mass of *A. maritima* and the water content in *G. macrorrhizum,* though the effect was not significant in grey water treatment, which suggests the interaction between biochar and grey water has a detrimental impact to biomass accumulation.

The interaction between biochar and greywater had no positive effect on the growth rates of the studied plant species, though it had a positive effect on the number of leaves of *G. macrorrhizum.* Further experiments should be carried out with biochar and grey water in pot plants to widen the knowledge on how these factors interact and effect plant growth and development.

It can be concluded that use of grey water and biochar in green roofs and green wall systems has potential, and further work could investigate how plant growth rates and plant development vary due to changes in species, biochar feedstocks and type of greywater used.

8. References

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9. Images/Tables

Table 1:

taken from
<https://www.sciencedirect.com/science/article/pii/S0048969709001594,> ,

The characteristics of grey water by different categories.

Figure 1&2: Pictures taken on smartphone belonging to Martin Roe

For raw data visit https://github.com/mr2214/bacerlor_thesis.git