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Assessing the effectiveness of digestate enriched biochar as an alternative fertilizer

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Abstract

Background: Alternatives to conventional inorganic fertilizers are needed if we are to curb CO₂ emissions, reduce the stress on natural resources and develop an efficient, yet sustainable agricultural practice. Biochar and digestate are two promising materials whose combined properties can be harnessed for plant nutrition. Although literature exists on the use of biochar and digestate aimed at nutrient recovery and agriculture, relatively little is known on the effectiveness of enriched biochar compared to conventional fertilization.

Aim: This thesis purports to compare the effects of digestate enriched biochar on plant productivity and nutrient availability with respect to conventional inorganic fertilizers.

Method: A pot trial with ryegrass was conducted with five different treatments over a period of six weeks to study the effects of digestate enriched biochar on plant productivity, nutrient availability, and nitrogen mineralization. Shoot biomass yield, macronutrient concentration in biomass and mineral nitrogen (N) concentrations in soil were measured. In addition, a 24-hour sorption batch test was conducted to study the ammonium and orthophosphate sorption capacity of biochar.

Results: The enriched biochar treatment displayed the best performance in shoot biomass yield, 54.7% higher than conventional fertilization. Biochar was also found to have an effect on increased yields across several treatments containing the same source of N. This observation can potentially be explained by the interaction between microbial activity and biochar. The study could not verify N mineralization, but nitrification (a part of N mineralization processes) was observed in all treatments, with the highest final concentration of nitrate in the enriched biochar treatment. Biochar was also found to adsorb ammonium (NH₄⁺) effectively with an estimated capacity of 5.45 mg NH₄⁺ g⁻¹. Inversely, a release of orthophosphate (H₂PO₄⁻) by more than 4 mg H₂PO₄⁻ g⁻¹ was observed.

Conclusion: This study indicates that enriched biochar is a promising alternative to inorganic fertilizers in terms of shoot biomass yield and N availability. However, more studies are needed to understand its long-term fertilization effects. Desorption of orthophosphate from biochar should also be examined more in detail. Finally, studies on the synergies between microbial activity and biochar are needed to understand/explain the higher productivity levels displayed by the biochar treatment.

1 INTRODUCTION

Humankind is currently facing an unprecedented climate emergency, the destruction of natural environments, depletion of natural resources, and a rapidly growing world population. Innovation is needed to make our agricultural practices and food production systems sustainable if we are to overcome the pressing challenges in this sector and to mitigate and adapt to climate change.

Conventional agriculture is by far the prevailing method for food production in the world (Reganold, 2016). Conventional agriculture provides with increased yields and a higher productivity, which benefited the world particularly during the decades of fast population growth following the 1950's. However, it is also undeniable that it has come at a high environmental cost, with both direct and indirect negative effects (Średnicka-Tober et al., 2016). Conventional farming relies on the production of synthetic fertilizers, which have major negative effects on the environment. Conventional food production systems at a global scale utilize mainly inorganic synthetic fertilizers, also known as mineral fertilizers, whose production is based on extractive operations (i.e., mining) with high energy requirements and significant associated greenhouse gas (GHG) emissions (Bellarby et al., 2008; Yara International ASA, 2021b).

The impacts and challenges associated with the production of synthetic fertilizers can be illustrated with the case of phosphorus (P) and nitrogen (N), two of the main macronutrients needed for plant nutrition along with potassium. Phosphorus (P) fertilizer is made from mined *phosphate rock*, a mineral whose deposits are scarce and under depletion; in fact, the world production peak is estimated to happen within the next few decades, period after which the global production will decline in a sustained way, meaning that eventually there will be no sources left. Some assessments indicate that this decline could begin as soon as 2033 (Neset & Cordell, 2012). Similarly, the production of nitrogen (N) fertilizers has a large environmental footprint. It requires mining operations since natural gas is needed to produce ammonia, the main component of most N fertilizers. It also requires a very high energy input, in the order of 69,000 kJ kg⁻¹ of final nutrient product (Gellings, 2009). In addition, there are significant emissions associated to its production, not only of CO₂ but also N₂O (nitrous oxide) a very

potent GHG that is released both in the factories and on the fields. Emissions in the range of 3.6 ton CO₂ per ton of N nutrient are released even with the best available technologies and highly efficient systems in Europe (Yara International ASA, 2021a).

Within this context, it is imperative to find new solutions to shift towards a circular economy paradigm in both agriculture and food production, by rethinking the sourcing of fertilizers. In these regards, reclaiming nutrients from waste sources or from by-products of other industrial processes can be a very promising alternative (Yang et al., 2020).

Biochar, a porous carbon rich material made from pyrolyzed biomass, offers several advantages in relation to conventional fertilization. An example being an increased cation exchange capacity that improves nutrient retention and reduces its leaching potential (Ok et al., 2018). Biochar can also be used as a carrier to sorb nutrients and render them available in soil. Equally, digestate is another good alternative to conventional fertilizers due to its high content of both macro and micronutrients, and its use would reincorporate waste stream into agricultural production.

Studies have been conducted focusing on the characterization of nutrients and the potential use of digestate as a fertilizer (Akhiar et al., 2017; Albuquerque et al., 2012; M. E. Lee et al., 2021; Panuccio et al., 2019). Similarly, biochar has been assessed as a potential plant nutrient carrier (Liu et al., 2016; Shang et al., 2018; Takaya et al., 2016; Yang et al., 2020).

However, no studies were found on the usage of biochar enriched with digestate in a pot trial with soil substrate, with the purpose of comparing its effectiveness against conventional inorganic fertilization. Limited studies exist involving biochar on the recovery of nutrients from digestate, the availability of nutrients to plants, and nitrogen mineralization in soil. This limits the strategic usage of biochar and digestate for large scale applications. Therefore, the current thesis aims at answering the following question:

What are the effects of digestate enriched biochar on plant productivity and nutrient availability with respect to conventional fertilizers?

To answer the research question, the following sub-questions are formulated:

- A. How does digestate enriched biochar compare with synthetic fertilizers in terms of their shoot biomass and macronutrient content in biomass?
- B. Does digestate enriched biochar inhibit the mineralization of nitrogen?
- C. What is the nutrient sorption capacity of biochar for ammonium and phosphate?

The study found amongst other results that the digestate enriched biochar in combination with digestate was able to effectively fertilize ryegrass achieving the highest biomass yield, above conventional inorganic fertilization, representing a promising fertilizer substitute. Biochar was also found to have an effect on increased yields across several treatments having the same source of N. Biochar was also found to adsorb ammonium (NH_4^+) effectively with an estimated capacity of $5.45 \text{ mg NH}_4^+ \text{ g}^{-1}$. Inversely, a release of orthophosphate (H_2PO_4^-) by more than $4 \text{ mg H}_2\text{PO}_4^- \text{ g}^{-1}$ was observed.

The remainder of this thesis is as follows: Section 2 includes the theoretical background and related work. Section 3 presents the methodology. Section 4 presents the results from the different activities as foundation to attempt answering the research questions. Section 5 discusses the results in terms of their contribution to the body of knowledge in the field of valorisation of waste for soil fertilization, their implications in practice, as well as the limitations of the study. Section 6 presents the conclusions and suggestions for future research.

2 THEORETICAL BACKGROUND

This chapter first introduces the main concepts to be studied, describing in detail the characteristics of both digestate and biochar as the main materials that were assessed as an alternative fertilizer. Secondly, it describes the related work in the field of valorisation of waste for soil fertilization that has investigated biochar and digestate.

2.1 Main concepts

Circular economy: The concept of circular economy started in the early 1960's under the discipline of environmental economics; and it has become a widely used term in the context of sustainable development. Although several definitions have been proposed by different governmental bodies and organizations (Sillanpää & Ncibi, 2019), the European Parliament defines it in a very precise way as “*a production and consumption model which involves reusing, repairing, refurbishing and recycling existing materials and products to keep materials within the economy; wherever possible waste itself will become a resource, consequently minimizing the actual amount of waste. It is generally opposed to a traditional linear economic model*”.

According to the Ellen Macarthur Foundation (2015) circular economy also encourages to cycle nutrients safely back to the biosphere in what it defines as *biological cycles*. This is in essence a mimicking of the multiple transformations that happen in nature, where there is no such thing as *waste*, but all materials are cycled repeatedly through what it is also known as biogeochemical cycles (Schlesinger & Bernhardt, 2020).

Digestate: Digestate is the effluent or by-product of anaerobic digestion processes. Anaerobic reactors, or biodigesters are considered one of the best available technologies to treat organic waste streams given the flexibility of the process to different feedstocks such as the organic fraction of municipal solid waste, manure, garden waste and energy crops. In addition, anaerobic digestion requires low energy input and allows the recovery of valuable products like biogas. Digestate consists of a liquid-solid suspension high in solids content, weakly alkaline (pH ~8.0) containing high amounts of macronutrients (N, P, K, Ca, S, Mg), micronutrients (B, Cl, Mg, Fe, Zn, Cu) and other non-labile organic materials that have remained undigested (poorly broken down into simpler forms), like lignin and non-hydrolysable lipids (Logan & Visvanathan, 2019). However, despite its high content of nutrients and several successful experimental results, the effect of using digestate as a potential substitute for

synthetic fertilizer, alone or in combination with other amendments such as biochar, is still poorly understood (Alburquerque et al., 2012; M. E. Lee et al., 2021; Panuccio et al., 2019).

Digestate in general represents a very rich source of mineral nitrogen (i.e., ammonium, NH_4^+) despite the large variations found between reactors, which is caused mainly due to the varying composition of feedstocks according to Akhbar et al. (2017). In their study, digestate from eleven reactors was characterized finding concentrations of total nitrogen (TKN) in the liquid fraction ranging from 1500 to 6500 mg L^{-1} , with ammonium accounting for between 62-98% of the total nitrogen in the dissolved fraction. Given the nature of anaerobic digestion where highly reducing conditions predominate, ammonium (constituting almost the totality of the mineral N), is the main form of nitrogen that comes out of the reactors (Logan & Visvanathan, 2019). Total concentrations of N can even be slightly higher than in the feedstock because of biochemical changes that enhance the nutrient availability of organic compounds to crops (Lukehurst et al., 2010). Regarding the distribution of ammonium between phases, it is estimated that 70-80% is in the liquid fraction and the remaining fraction (20-30%) is distributed on the solid fraction (Logan & Visvanathan, 2019). Nonetheless, further research is needed to understand the plant availability of N in digestate, and its possible use as a nutrient recovery source using sorbents like biochar.

Along with nitrogen, digestate also contains high amounts of other macronutrients like phosphorus (P) and substances that can amend soil and promote plant growth including carbon compounds (organic matter) and plant hormones. Again, variations in the feedstock and the process determine the composition of the digestate. For example, in an extensive study conducted by Tambone et al. (2017) on digestate from anaerobic reactors treating mostly cow manure, pig manure and energy crops, concentrations of total P (in liquid unseparated digestate) were reported in the range of 23.2 to 56.7 g kg^{-1} (dry matter) with the liquid fraction having a higher content of P than the solid fraction, 71.6% on average. Other authors have reported a greater share of the total P in the solid fraction, in the order of 55-65% (Peng & Pivato, 2017, cited by Logan & Visvanathan, 2019). When it comes to plant hormones, Li et al. (2016) reported that gibberellic acid (GA), indoleacetic acid (IAA) and abscisic acid (ABA), all present in digestate, have beneficial effects in characteristics like germination power and biomass accumulation as well as in stress responses such as water deficit, freezing or salt stress. Lastly, the high content of organic matter and non-labile compounds remaining in the digestate can provide an energy source for microorganisms and, for example, improve soil structure and water holding capacities (Logan & Visvanathan, 2019; Li et al., 2016).

Uses of digestate: Given the high content of plant nutrients, digestate has been used traditionally for application on soil. However, there are technical, environmental, economic, and legal considerations to bear in mind. One is related to the emissions of ammonia and odour nuisances, although this can be mitigated, by e.g., using equipment that pressure-injects digestate in the soil (Orzi et al., 2018). Another consideration is the quantity and the point in time when digestate can be applied to fields. Regulatory bodies often allow only for seasonal usage and restrict to a certain dosage per unit area to avoid pollution of both groundwater and surface water. For example, the European Commission establishes a maximum application of 150-250 kg N Ha⁻¹ year⁻¹ (Akhlar et al., 2017). The problem with this limitation is that it creates the need for storage infrastructure or to transport the digestate off-site, both activities involving very high costs. Thus, research has been conducted in recent years to find innovative ways to extract or recover nutrients in such a way that the remaining liquid can be used for irrigation without concerns for excessive nutrient application on the land. One such example is digestate dewatering followed by ammonia stripping and recovery (Li et al., 2016)

Other regulatory issues have to do with the strict controls of both chemical and microbiological properties that are required to be analysed prior to field application. It is important to stress that depending on the feedstock and the quality of it, there can be a higher or lower risk of having certain contaminants in the digestate. For example, if not properly segregated, the organic fraction of municipal solid waste is more likely to carry contaminants like heavy metals, glass, metal, plastic, rubber and organic pollutants (e.g.: pharmaceuticals) that could potentially affect human health through the food chain if incorporated into agricultural systems (Logan & Visvanathan, 2019). Conversely, digestate from manure and other animal by-products have a higher risk of propagating pathogens or propagules (e.g., bovine spongiform encephalopathy) for which they are required by some regulators to be hygienized/sanitized to reduce this risk (*idem*).

A recent review of digestate managing strategies by Cesaro (2021) found that the only alternative apart from application to soil for agricultural purposes that has been implemented extensively is the thermo-chemical treatment for energy recovery purposes. In this method, digestate is converted to synthesis gas (syngas) containing hydrogen and methane through gasification, pyrolysis or hydrothermal carbonization. Although it is a promising method, the main setback is that the energy demand is high, it requires significant technological complexity, and its focus is not on nutrient recovery.

Other novel applications of digestate that have been explored recently include the generation of value-added products like biopesticides, use as culture media for microalgae (e.g., for biorefineries and production of biodiesel) or as growing media for fungal, insect and invertebrate cultivation (e.g., to produce mushrooms or obtain proteins from larvae) and applications in hydroponic cultivation. Most of these solutions imply a separation of the liquid and solid phases of the digestate, and some additional processes such as hygienization and/or fermentation. Despite this promising landscape, none of these technologies have reached a full-scale operation (*idem*). There is a strong pressure to find sound, cost-effective and relatively simple applications to deal with the large quantities of digestate coming from the increasing number of anaerobic reactors all over the world, Europe not being the exception with 17,240 plants in 2014 (Akhiar et al., 2017).

Biochar, a promising multi-purpose material: As precisely defined by Ralebitso-Senior & Orr (2016, p. 2) biochar is a “carbon-rich, solid by-product obtained from the carbonization of biomass, such as wood, manure or leaves, heated to temperatures between 300°C and 1000°C under low (preferably zero) oxygen concentration. The process, known as pyrolysis, can typically give three products: a liquid (bio-oil), a solid (biochar) and a gas (syngas) with yields depending on the pyrolysis process (slow, fast, flash) as well as in the specific conditions (feedstock, temperature, pressure, time, heating, and rate)”.

Biochar can be made through different thermochemical processes including pyrolysis, torrefaction (dry or wet), gasification and hydrothermal processing. Even though all the mentioned processes produce biochar or a ‘biochar-like’ solid, they pursue different objectives. For example, torrefaction is often used as a pre-treatment for biomass before combustion in thermoelectric power stations and gasification is targeted at recovering synthesis gas (a mixture of H₂, CO and CO₂) (Ok et al., 2018). The different processes result in different biochar yields from less than 10% under gasification to around 80% following dry torrefaction (Ok et al. 2018). Also, their use and applications differ between the different production technologies. For instance, for agricultural purposes slow pyrolysis with low temperatures (450-550°C) is normally used; conversely, for fuel recovery (e.g. ethane, methane) gasification under high temperatures (600-1200°C) and fast heating rates are usually used (Ralebitso-Senior & Orr, 2016).

The process conditions and the feedstock will strongly determine the properties of the biochar and consequently its efficacy or suitability for a certain application, therefore they should be assessed depending on the intended use of the biochar. The feedstock determines the chemical composition of the final product, influencing properties like the total organic carbon content, the mineral

concentration, the ash content, or the carbon sequestration capacity, whereas the temperature of the process influences other properties such as pH, surface area and the sorption capacities of the biochar (Ok et al., 2018; Ralebitso-Senior & Orr, 2016). For example, if the intended use of the biochar is as a sorbent agent to recover cations, evidence has been found that at lower pyrolysis temperatures (400-500°C) there are more functional groups containing oxygen, both associated with basicity and ion exchange/interactions that facilitate sorption processes (e.g., ammonium) (Mukome et al., 2013; Zhang et al., 2020). Conversely, softwood feedstocks have been shown to have higher C:N ratios, which if applied in soil can have an effect on N immobilization (Mukome et al., 2013).

Uses of biochar: The most widespread application of biochar is in agriculture. Biochar has shown to increase the cation exchange capacity (CEC) of soil, making fertilization more efficient as more nutrients are held adsorbed on exchange sites and less nutrients can leach down the soil profile (Liang et al., 2006, cited by Ok et al., 2018). However, due to a great variability in CEC of different biochar and the specific soil conditions, the effect of biochar for increased nutrient retention is yet inconclusive. For example, Cornelissen et al. (2013) found strong positive effects of up to four-fold increased yields on maize with a dosage of 4 ton Ha⁻¹ (4%), whereas Spokas & Reicosky (2009) reported no significant improvements in a range of soil-biochar combinations. Also, given its highly porous structure, biochar increases the water holding capacity of soil (Jeffery et al., 2011), benefiting particularly soils that lack finer fractions (e.g.: sandy soils), which in turn reduces the burden on irrigation (Basso et al., 2013; Yu et al., 2013). In addition, depending on its alkalinity and acid neutralizing capacity biochar can be used to raise the pH of acidic soils (Chintala et al., 2014; Jeffery et al., 2011).

Furthermore, a study by Bruun et al. (2014) showed that biochar can facilitate root penetration and increase its density in soils with high compactness in addition to promoting mycorrhizal fungi, which is associated with reduced incidence of plant disease (Ralebitso-Senior & Orr, 2016). Still, evidence has been found that an immediate fertility increase due to the enhanced cation retention by the biochar is likely to have a greater impact in sandy soils or with critically low soil organic matter, rather than in highly productive agricultural areas (Singh et al., 2017).

It is important to stress that the characteristics and response of biochar with soil can be vary considerably even between batches using similar feedstocks and pyrolytic conditions and thus the result of a given study cannot be extrapolated universally to all biochar materials (Chintala et al., 2014). However, research has been conducted to investigate trends between feedstocks and a given property and help practitioners making trade-offs, for example Mukome et al. (2013) carried out an extensive

characterization of different feedstocks and provided guidelines for selecting them based on the need of a higher or lower C:N ratio, ash content, and surface area.

Other uses of biochar consist mostly of carbon sequestration and environmental applications. The principle behind carbon sequestration lies on the fact that biochar is a recalcitrant material, estimated to remain stable even for thousands of years in the soil, and thus keeping the carbon out of the cycle (Harvey et al., 2012). As explained above, process conditions are important depending on the intended use, for instance, choosing a high temperature pyrolysis (>550°C) will produce biochar with increased aromaticity (higher content of polycyclic aromatic hydrocarbons -PAHs-) and therefore a higher recalcitrance, which is more suitable for carbon sequestration.

Regarding environmental applications, some of the most common are remediation of abandoned mine lands (sites prone to acid mine drainage) and decontamination of soil polluted with heavy metals/metalloids (Ok et al., 2018). These applications make use of the sorption capabilities and the liming effect of biochar, which in general terms can reduce the mobility of the pollutants, for instance by precipitation of metals at a higher pH, or also by improving the general conditions of the soil and stimulating the growth of plants used for phytoremediation.

2.2 Related work

Use of biochar for nutrient recovery: Studies have been conducted to determine the suitability of biochar to reclaim nutrients like ammonium, nitrate and phosphate, often consisting on batch sorption experiments using synthetic nutrient solutions (Hu et al., 2020; Liu et al., 2016; Shang et al., 2018; Takaya et al., 2016). These studies compared the sorption capacities of different types of biochar made from different feedstocks, under different conditions or modified after pyrolysis (e.g., with an acid wash), and in general they have found that it feasible to use biochar as a sorbent for plant nutrients.

When it comes to specific literature on the interaction of biochar and digestate, little was found and in most of the cases, it was related to the recovery of nutrients from the liquid fraction of digestate (Kizito et al., 2017; Kocatürk-Schumacher et al., 2017; Tuszyńska et al., 2020). Plaimart et al. (2021) studied the effects of applying digestate from pig manure digestion in a biochar amended soil, finding that biochar slowed down nitrification, retained nutrients for a longer period, and reduced the risk of groundwater pollution by nitrate leaching. In addition, they concluded that biochar did not show an effect on ammonia volatilization, however this seemed to be related to the fact that digestate was

applied on the surface of the testing containers and the interaction with biochar was minimal. This study did not investigate the availability of nutrients to plants.

Ding et al. (2020) assessed different biochar made from agricultural waste and sawdust, modified with basic, acidic, and ferric chloride solutions, running static and dynamic column tests with unseparated digestate from pig manure. Their findings suggest that temperatures between 550°C and 600°C with further caustic solution treatment would yield a predominance of -OH (hydroxyl) and -COOH (carboxyl) functional groups, which have affinity and thus a good capacity to sorb NH_4^+ . Although they used unseparated digestate, as in the present research, their approach was focused more on the sorption behaviour and the microbiological activity, not addressing the desorption and eventual availability to plants.

Previous research with pot trials: Carey et al. (2015) performed an extensive study that included the production of biochar from dewatered biosolids, an assessment of its sorption capacities and a pot trial to determine its effect on plant growth. Although the nutrient source was filtrate from biosolids dewatering of a wastewater treatment plant and not digestate, it has similarities to the liquid fraction of a digestate, as both have dissolved ammonium as their main fraction of mineral N. Their main finding was that NH_4^+ and K^+ was successfully sorbed from the waste nutrient source and the enriched biochar positively affected turfgrass, particularly in relation to higher growth rates, as compared to other fertilization treatments like conventional mineral fertilizer.

A two stage study by Kocatürk-Schumacher et al. (2017, 2019), with similarities to the present work, initially investigated the nutrient sorption of NH_4^+ , potassium (K^+) and phosphate (PO_4^{3-}) from the liquid fraction of a digestate (from pig and cattle manure digestion with various food wastes as the co-substrate) by using biochar made from holm oak (hardwood, slow pyrolysis 650°C, atmospheric pressure) and a biochar-clinoptilolite¹ mix in a column experiment. It was found that the biochar effectively sorbed/removed NH_4^+ , orthophosphate and dissolved organic carbon (DOC). However, K^+ was released from the biochar and thus not removed from the digestate likely due to the high K content in the biochar and the fact that it might not have been pre-washed to remove ash and soluble salts (this was not reported in the methodology). The biochar was able to remove up to 8.61 mg NH_4 -

¹ A mineral (natural zeolite) with high sorption properties (Kocatürk-Schumacher et al., 2017).

N g⁻¹, 1.95 mg P g⁻¹ and 13.01 mg DOC g⁻¹ at high loading ratios (that is the rate at which digestate was filtered through the columns).

In the second stage of the research, Kocatürk-Schumacher et al. (2019) conducted a pot trial with rye grass grown on quartz sand as the substrate comparing the effect of two sorbents, biochar and clinoptilolite, a mineral with high sorption properties, enriched with the liquid fraction of a digestate. Micronutrients, P and K were added in the same dose for all treatments while nitrogen was applied at varying loads (required dose and excess dose). The main findings were that both enriched sorbents, biochar and clinoptilolite, were able to provide the nutrients to the plants as the biomass yield and N uptake in the plants was significantly higher than in the controls (non-enriched) and that initial loading ratios (the amount of digestate filtered by mass of sorbent) influenced not only the nutrient removal efficiency but also the availability of N to plants, and thus the efficiency of the enriched material. However, it is worth noting that the pot trial was not conducted on actual soil, but instead in a double compartment pot system over which the grass grew.

As a summary, digestate has a high potential for nutrient recovery aimed at agricultural production, and its combination with biochar has promising applications, given the feasibility of using the latter as a sorbent or nutrient carrier, and given its positive effects in soil. Biochar can greatly vary, depending on the feedstock and process conditions, for which specific studies are necessary each time, for example to assess the nutrient sorption potential, which in some cases has been found to be poor for anions but mostly good for cations such as ammonium. Few studies have been conducted on the recovery of nutrients from digestate using biochar, and the availability of nutrients to plants has only been assessed to a little extent. As far as it is known, this is the first study where biochar enriched with unseparated digestate was used in a soil pot trial, to evaluate possible synergies between biochar and digestate, against conventional mineral fertilization.

3 METHODOLOGY

This chapter contains the methodology of the study. Section 3.1 describes the overall study design, Section 3.2 describes the pot trial and Section 3.3 describes the sorption batch test.

3.1 Overall study description

Two methods were used in this study: *pot trial* and *sorption batch test*.

Pot trial was used because it is an empirical approach that allows comparing responses to different treatments under controlled conditions, which in turn enables reproducibility and comparability (Kawaletz et al., 2014; Passioura, 2006). Three metrics were measured in the pot trial:

- A. **Shoot biomass:** This parameter is a generally recognized indicator of fertilization efficacy of a treatment in *supplying nutrients* and *promoting plant growth* (i.e., nutrient availability).
- B. **Nutrient concentration:** Total Nitrogen (N), phosphorus (P) and potassium (K) in biomass was chosen as additional indicators of nutrient availability.
- C. **Mineral nitrogen concentrations:** Nitrogen mineralization plays an essential role to ensure soil fertility and availability of N for plant use (V. C. Pandey, 2020). Initial and final concentrations of plant available/mineral N (ammonium nitrogen, $\text{NH}_4^+\text{-N}$ and nitrate nitrogen, $\text{NO}_3^-\text{-N}$) were measured to evaluate whether the enriched biochar interferes with this process or not.

Given that biochar was used as a sorbent material for nutrient recovery from digestate, it is worth examining the nutrient sorption capacity for ammonium (NH_4^+) and orthophosphate (H_2PO_4^-). To assess the sorption capacity, a **sorption batch test** was carried out.

3.2 Pot trial

The general approach of the pot trial was to compare the fertilizing effect of **digestate enriched biochar** against different treatments (including conventional inorganic fertilization), all treatments having equal starting concentrations of plant available macronutrients ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, P and K). As

mentioned previously, the compared metrics were yield (shoot biomass) and nutrient concentration in shoot biomass.

Rye grass (*Lolium perenne*) was selected as a testing plant and a soil substrate was used in all treatments. The pot trial was conducted within a six-week period under controlled conditions at the Soil Research Group building, NMBU, Ås.

Light was supplied 18 hours a day (ca. 8000 lux), the temperature was kept at ~23°C with air conditioning. Throughout the trial period (from February 22nd to April 5th 2021) each pot was weighed, and distilled water added every 1-2 days in order to adjust the soil water content back to 60% field capacity. The shoot biomass was harvested only one time, at the end of the trial and further processed for chemical analyses.

In addition, a parallel trial was conducted only with the amended substrates (without sowing) to assess whether digestate enriched biochar interferes or inhibits nitrogen mineralization (Section 3.2.4). The pots were smaller in volume but subject to the same conditions (light, temperature, and soil water content). Soil samples were collected at the beginning and at the end of the trial and kept frozen until analysis.

3.2.1 Characterization of treatments materials

Nine different materials were analyzed for plant available nitrogen (NO₃-N and NH₄-N), phosphorus (P) and potassium (K), as required to carry out the pot trial. The materials are listed in Table 3.1. Some were prepared only for preliminary purposes.

Table 3.1. List of materials characterized for plant available N, P and K.

Material		As described in	
<i>Dry unwashed biochar (DUB)</i>		Section 3.4.2	
Dry washed biochar	<i>For Preliminary analyses (DWB1)</i>		
	<i>For Pot trials and Sorption batch test (DWB2)</i>		
<i>Liquid digestate (LD)</i>		Section 3.4.3	
Enriched biochar	For Preliminary analyses	<i>Rinsed (EB1R)</i>	Section 3.4.4
		<i>Unrinsed (EB1U)</i>	
	For Pot trial and Sorption batch test	<i>Dry rinsed (EB2R)</i>	
<i>Compost mix (CM)</i>		Section 3.4.5	
<i>Mineral soil (MS)</i>		Section 3.4.6	

The materials were tested for plant available P and K by ammonium lactate (AL) extraction followed by inductively coupled plasma (ICP) analysis. Plant available or mineral nitrogen, i.e., ammonium and nitrate, was tested by potassium chloride (KCl) extraction and subsequent flow injection analysis (FIA). Both methods are described in Section 3.5.2. and 3.5.3 respectively.

Table 3.2 summarizes the analytical data for the characterization of the materials, which was done to prepare the pot trial substrates.

Table 3.2. Macronutrient content as plant available N, P and K for biochar/enriched biochar, digestate, compost mix and mineral soil.

			<i>Plant available nutrients (mg kg⁻¹)^a</i>								
			<i>Dry matter</i>	<i>NO₃⁻-N</i>	<i>s.d</i>	<i>NH₄⁺-N</i>	<i>s.d</i>	<i>P</i>	<i>s.d</i>	<i>K</i>	<i>s.d</i>
<i>Dry unwashed biochar (DUB)</i>			59.2%	n.a.	n.a.	n.a.	n.a.	2426	169	9594	662
Dry washed biochar	<i>For Preliminary analyses (DWB1)</i>		98.0%	0.32	0.33	0.79	0.32	1395	155	4085	560
	<i>For Sorption tests and Pot trial (DWB2)</i>		97.0%	0.24	0.17	6.48	0.95	1466	79	4764	286
<i>Liquid digestate (LD)</i>			4.1%	28.86	3.52	56911	1863	6341	0	44715	1408
Enriched biochar	For Preliminary analyses	<i>Rinsed (EB1R)</i>	45.2%	0.22	0.02	2545	108	1049	86	4209	625
		<i>Unrinsed (EB1U)</i>	35.2%	0.29	0.14	4308	161	1235	180	6267	809
	For Pot trial	<i>Dry rinsed (EB2R)</i>	65.1%	0.29	0	589	117	4096	205	3755	118
<i>Compost mix (CM)</i>			55.7%	0.13	0	53.5	1	885	16	2635	184
<i>Mineral soil (MS)</i>			95.5%	36.12	0.46	1.27	0.08	59.3	1.8	84.8	1.3
a. Dry basis; s.d.: standard deviation; n.a.: not available.											

3.2.2 Final composition of the substrates

The bulk of the substrate for all treatments consisted of a mixture of sandy loam (Section 3.4.6) and compost mix (Section 3.4.5). In order to foster a rich microbiological activity necessary for nitrogen mineralization, it was decided to use a 30% v/v compost mix content in all treatments, assuming that similar results could be attained to those reported by Pérez-Piqueres et al. (2006) and Fuchs & Larbi (2004), who studied the influence of compost content on soil microbiology and its impacts on microbial density.

The pH of the base substrate, consisting of 30% v/v compost mix and 70% v/v sandy loam, was verified in order to assure an adequate value in the pots, finding a value of 6.51, considered suitable for ryegrass development (Pennsylvania State University, 1996).

Five treatments, including the control, were defined for the pot trial, as shown in Table 3.3. EBC constitutes the treatment to evaluate to answer the research questions. For the treatments with biochar (EBC and BCNPK) and to facilitate comparability, a 12% v/v application rate was chosen. The fraction of mineral soil was reduced in EBC and NPK to keep the same compost content (30% v/v) in all treatments.

Table 3.3. Substrate relative composition of materials (v/v) in pot trial treatments (density indicated under the material). Detailed information on the materials can be found in Section 3.4.

Acronym	Treatment	Composition of the substrate (v/v%)			
		Mineral soil (MS)	Compost mix (CM)	Biochar (DWB2)	Enriched biochar (EB2R)
		1.24 g cm ⁻³	0.98 g cm ⁻³	0.31 g cm ⁻³	0.38 g cm ⁻³
Control	Control (Base substrate)	70%	30%	0%	0%
NPK	Base substrate + inorganic fertilizer	70%	30%	0%	0%
BCNPK	Base substrate + biochar + inorganic fertilizer	58%	30%	12%	0%
DI	Base substrate + digestate	70%	30%	0%	0%
EBC	Base substrate + enriched biochar + digestate	58%	30%	0%	12%

Based on the characterization of treatments materials (Table 3.2) and the relative composition of each substrate (Table 3.3), total content of plant available of N, P and K was calculated for each treatment pot (Equation 1). Then base nutrient dosages were calculated in kilograms per decare (kg daa⁻¹) with the area of the pot (0.0235 m²) (Equation 2) to determine if they would fulfill the recommended dosages, defined for this study as 15 kg daa⁻¹ for both N and K, and 5 kg daa⁻¹ for P (Roy et al., 2006).

$$\text{Total nutrient content (mg)} = \frac{B_D \cdot R \cdot V \cdot N_c}{1000} \quad (\text{Equation 1})$$

Where:

B_D = Bulk density (g cm⁻³)

R = Relative composition (%)

V = Volume of the pot (cm³); Pot trial=3000 cm³; Parallel trial=500 cm³

N_c = Mean nutrient concentration (mg kg⁻¹ on a wet basis)

$$\text{Nutrient dosage (kg} \cdot \text{daa}^{-1}\text{)} = \frac{\text{Total nutrient content (mg)}}{(0.0235 \text{ m}^2) \cdot (1000)} \quad (\text{Equation 2})$$

To have approximately equal nutrient dosages at the beginning of the pot trial, treatments were amended with inorganic fertilizers as shown in Table 3.5. It should be noted that P and K were already supplied above the requirements by the base substrates (Figure 3.1) and therefore, addition of P and K to some treatments was done only to equalize the dosage between the treatments.

An important detail from the characterization of treatments materials was that the enriched biochar used in the pot trial (EB2R) showed an ammonium concentration of 589 mg kg⁻¹ which resulted 77% lower than the one prepared for preliminary analyses (EB1R). This reduction could be explained by ammonia volatilization when EB2R was left air-drying overnight. Although pH was not measured during the enriching process, the pH of the biochar used in the enriching process (DUB) was high (9.63), thus it is likely that this caused the ammonia volatilization. This reduction on ammonium concentration required an adjustment for the enriched biochar treatment (EBC) by adding liquid digestate (LD). This was done because the calculated N base dosage (6.6 kg daa⁻¹) would not fulfill the minimum of 15 kg daa⁻¹. As a result, the fraction of plant available nitrogen supplied directly by the enriched biochar (EB2R) was approximately 15%, in the EBC treatment (Table 3.4).

Table 3.4. Percentage of plant available N supplied by each material in the substrate of treatments DI and EBC

<i>Treatment</i>	<i>Substrate component</i>		
	<i>Mineral soil and Compost Mix</i>	<i>Digestate</i>	<i>EB2R</i>
DI	34%	66%	-
EBC	29%	56%	15%

Table 3.5. Nutrient dosage in the pot trial treatments, before and after the addition of amendment.

Acronym	Treatment	Base dosage (kg daa ⁻¹)			Added amendment								Final dosage (kg daa ⁻¹)		
		K	P	N	K (mL)		P (mL)		N (mL)		Digestate (g)		K	P	N
					3.0 L pot	0.5 L pot	3.0 L pot	0.5 L pot	3.0 L pot	0.5 L pot	3.0 L pot	0.5 L pot			
Control	Base substrate	64.1	24.8	5.1	0	0	0	0	0	0	0	0	64.1	24.8	5.1
NPK	Base substrate + inorganic fertilizer	64.1	24.8	5.1	38	6.3	50	8.3	19	3.2	0	0	83.2	31.1	14.9
BCNPK	Base substrate + dry washed biochar ^b + inorganic fertilizer	84.5	30.5	4.4	0	0	9	1.5	21	3.5	0	0	84.5 ^a	31.6	15.3
DI	Base substrate + digestate ^c	64.1	24.8	5.1	23	3.8	41	6.8	0	0	98.8	16.5	83.4	31.1	14.9
EBC	Base substrate + enriched biochar ^d + digestate ^c	76.8	39.2	6.6	16	2.6	0	0	0	0	87.7	14.6	91.5	40.2 ^a	15.3 ^a

a. Dosage used as reference to amend the other treatments; b. DWB2; c. LD; d. EB2R.

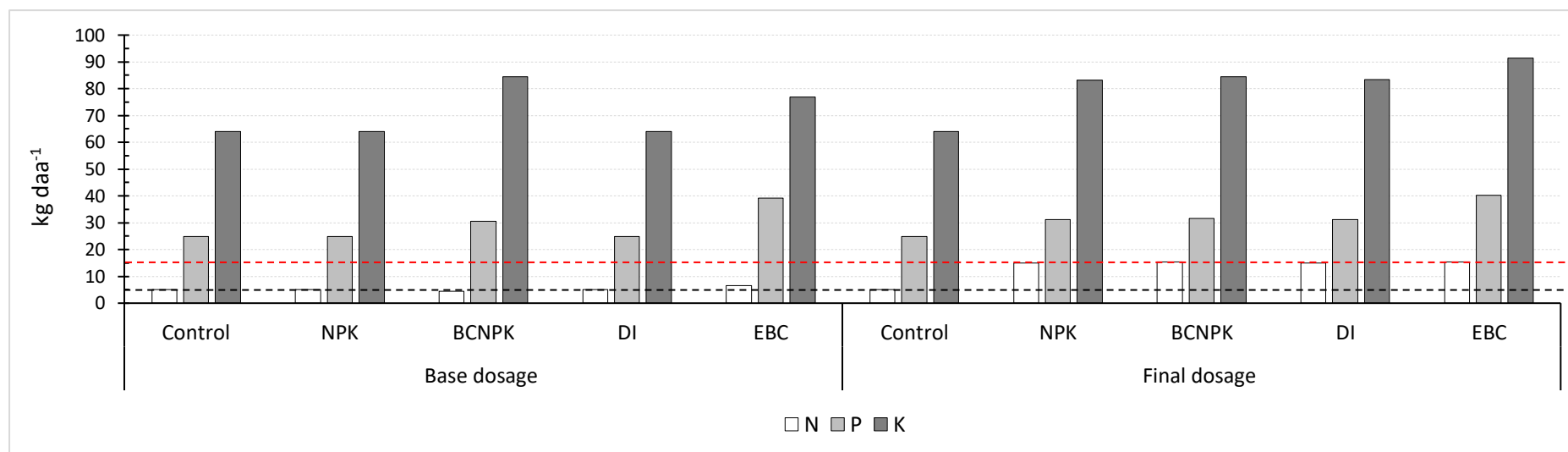


Figure 3.1. Base and final nutrient dosages (kg daa⁻¹) in the pot trial treatments. Dashed lines indicate the minimum recommended dosages. The red and black dashed lines indicate the minimum N or K dosage (15 kg daa⁻¹) and minimum P dosage (5 kg daa⁻¹) respectively

3.2.3 Preparation of the pots

From the bulk density and volume percentage of each component (Table 3.3), pots were filled weighing each component. The materials were homogeneously mixed in a stainless-steel mixing basin and placed in 3.0 L and 0.5 L cylindric polypropylene containers, for the sowed and unsowed pots respectively. Five replicates were used in the sowed pot trial and three replicates for the unsowed parallel trial. The pots had no perforations at the bottom, consequently, water losses were due to evapotranspiration.

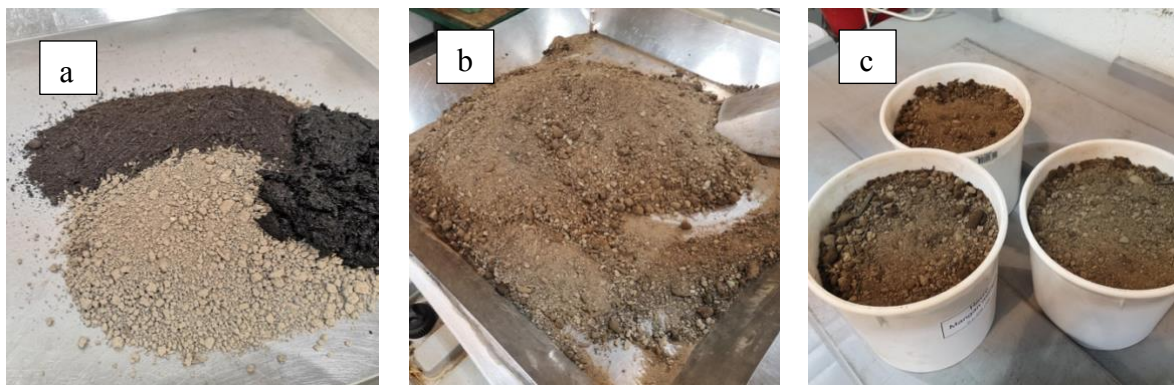


Figure 3.2. (a) Weighed materials in mixing basin (base substrate); (b) homogeneously mixed components; prepared pots before addition of amendment and sowing.

The digestate, enriched biochar and the inorganic fertilizers were added after filling up all the pots, in the following way: **NPK**, inorganic fertilizers were added homogeneously on top of the substrate; **BCNPK**, inorganic fertilizers were mixed with the biochar and then mixed with the base substrate; **DI**, the pots were filled gradually alternating layers of base substrate and digestate (3-4 layers of each, the last one being substrate); **EBC**, enriched biochar was mixed with the base substrate.

Pots were sowed with 2.5 g of homogeneously scattered seeds, covered with approximately 1 cm of substrate. Then, all the pots (sowed and unsowed) were watered to 60% of the field capacity and left capped for two days to maintain the soil moisture and promote germination.

3.2.4 Assessment of shoot biomass yield, macronutrient content and nitrogen mineralization

Shoot biomass yield, a generally recognized indicator of fertilization efficacy and nutrient availability, was compared between treatments to identify potential advantages of using

digestate enriched biochar combined with digestate. The nutrient concentration in biomass was also compared between treatments as an additional indicator of the nutrient availability. The analyzed parameters were total N, P, K and in addition total C, to calculate C:N ratios. Finally, mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) was compared before and after the trial to determine whether a positive net ammonification and nitrification had taken place (i.e., sum of mineral N higher at end of trial) as indicators of N mineralization. The intention was to assess whether enriched biochar could negatively affect mineralization, given the importance of this process in soil to ensure fertility and availability of N for plant use (V. C. Pandey, 2020).

As an additional analysis, a N mass balance was carried out to compare the total N taken up by the plants with the mineral N at the beginning of the trial. It was assumed that if total N in biomass was higher than the initially available mineral N, then mineralization of organic N had occurred. The balance was calculated using the analytical results from the unsowed pots ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) assuming the same concentrations in the sowed pots. Then it was compared with the total nitrogen in the shoot biomass.

Given that in the present study, root was not harvested, N allocated in root biomass was estimated based on the findings by Redin et al. (2018) who conducted growing experiments with ryegrass. A shoot to root ratio of 4.8 (dry matter), and a shoot to root ratio in N content of 2.8 were assumed.

3.2.5 Sampling of Biomass and Soil

At the end of the pot trial, all sowed pots were harvested cutting the grass at the height of the pot (approx. 3 cm from the soil surface). Three sub-samples out of the five replicates were taken to determine dry matter content. All samples were stored in paper bags and dried at 60°C for three days, then analyzed for total C and N (Section 3.5.4); and total P and K (Section 3.5.5).

Between 15.0 - 20.0 g of soil were sampled from the unsowed pots using a small hand auger (1 cm diameter) in 3-4 places, homogeneously distributed on the surface of the soil. The auger was washed between sampling of the different treatments. Samples were taken at the beginning and at the end of the pot trial and were stored in 45 mL polypropylene vials in a freezer (~-18°C) until analysis. All soil samples were analyzed for plant available or mineral N

(Section 3.5.3). In addition, sub-samples from the beginning of the pot trial were analyzed for total C and N (Section 3.5.4); and total P and K (Section 3.5.5).

3.3 Sorption batch test

3.3.1 *Batch-test principle*

The Standard Test Method ASTM D4646-16 “24-h Batch-Type Measurement of Contaminant Sorption by Soils and Sediments” was used as reference (ASTM International, 2016). Briefly, biochar samples of known mass are mixed with nutrient solutions at different concentrations. After a 24-h contact time, the nutrient of interest is measured in the remaining solutions, called the equilibrium solution, and the difference between this and the initial concentration is assumed to have been sorbed in the biochar.

Sorption batch tests were performed only for ammonium and orthophosphate. Potassium was not tested since its behavior is expected to be very similar to that of ammonium (Stuanes et al., 1984) given that both cations have the same net positive charge and similar ionic radius.

3.3.2 *Batch-test general procedure*

Dry washed biochar (DWB, Section 3.4.2) was sieved to 2 mm and 1-gram sub-samples were mixed with 20 mL of aqueous solutions containing either ammonium chloride (NH_4Cl) or potassium dihydrogen phosphate (KH_2PO_4) at various concentrations in acid washed borosilicate glass flasks. The samples were placed on a horizontal shaker for 24 hours at 140 rpm. The remaining equilibrium solution was filtered with a 0.45 μm pore size membrane syringe filters (polyethersulfone) and stored cold ($\sim 4^\circ\text{C}$) in 45 mL polypropylene vials until analysis. All batch-tests were conducted in triplicate and concentrations of ammonium (NH_4^+) or orthophosphate (H_2PO_4^-) in the initial and equilibrium solutions were analyzed spectrophotometrically (Section 3.5.1).

3.3.3 *Sorption of ammonium (NH_4^+)*

Based on preliminary sorption tests to determine the range of concentrations to be used (see Appendix B, Table B1) a set of biochar samples were prepared for batch-tests with NH_4Cl solutions in the following concentrations: 0 (distilled water), 20, 100, 200, 400, 600, 800 and

1000 mg NH₄⁺ L⁻¹. In addition, and to verify whether there had been any ammonia (NH₃) volatilization due to an increased pH in the solution caused by the biochar, parallel batch tests with solutions of 20, 400 and 1000 mg NH₄⁺ L⁻¹ were prepared using washed biochar adjusted to pH 7 with 0.01 M HCl (Munera-Echeverri et al. 2018).

Analysis of sorption data and estimation of maximum sorption capacity

The sorbed concentrations were calculated with the following equation:

$$C_s = \frac{(C_o - C_w) \cdot V_l}{M_s} \quad (\text{Equation 3})$$

Where:

C_s = Nutrient concentration in the solid (sorbed concentration) (mg kg⁻¹)

C_o = Nutrient concentration in the initial solution (mg L⁻¹)

C_w = Nutrient equilibrium concentration in solution (mg L⁻¹)

V_l = Volume of the nutrient solution (L)

M_s = Mass of biochar (kg)

Equilibrium and sorbed concentrations were plotted to determine whether the sorption behavior fitted better a linear or linearly transformed Freundlich/Langmuir isotherms (Table 3.6). The best coefficient of determination (r^2) was used as basis to determine the best fit, and Langmuir's slope (Q_{max} parameter) was used to estimate a sorption maximum.

Table 3.6. Equations and arguments used to determine the best isotherm fit.

<i>Isotherm</i>	<i>Dependent variable</i>	<i>Independent variable</i>	<i>Equation/Linearization</i>
Linear	C_s	C_w	$C_s = K_d \cdot C_w$
Freundlich	$\log C_s$	$\log C_w$	$\log C_s = \log K_f + n \cdot \log C_w$
Langmuir	C_w / C_s	C_w	$\frac{C_w}{C_s} = \frac{1}{Q_{max} \cdot K_L} + \frac{1}{Q_{max}} \cdot C_w$

K_d (partition coefficient), indicates the distribution of a solute between a solid and a liquid phase; the units are L kg⁻¹. The higher the value, the higher the quantity of solute sorbed in the solid phase.

K_f (Freundlich partition coefficient), the higher its value, the higher the adsorbent loading that can be achieved.

n is related to the energetic heterogeneity of the adsorbent surface and determines the curvature of the isotherm. The lower the n value, the more concave the isotherm (with respect to the equilibrium concentration axis). $n=1$ is a linear isotherm; $n < 1$, are most commonly found values, indicates high adsorbent loading at low concentrations.

K_L = Langmuir partition coefficient; similarly to K_d and K_f , it expresses the relative affinity of a solute between a solid and liquid phase, however the Langmuir describes better a system reaching saturation or maximum sorption capacity; units are L kg⁻¹.

Q_{max} gives an estimate of the maximum sorption capacity.

Verification of steady state equilibrium

A steady state is reached when no major differences in the equilibrium solution at intermediate times (before 24 h) are observed. It provides more certainty that the sorption has reached a maximum at each tested concentration.

As described in the standard (ASTM D4646-16) and to verify whether a steady state had been reached, two additional sample sets with initial concentrations of 20 and 600 mg NH₄⁺ L⁻¹ were prepared and batch-tested with 15 and 19 hours of duration respectively.

Determination of cation exchange capacity (CEC)

To quantify the NH₄⁺ that was associated with exchange sites in the biochar, and thus effectively adsorbed, CEC was determined. Biochar samples from the batch-test with 1000 mg NH₄⁺ L⁻¹ of initial concentration and previously adjusted pH were chosen for this, adapting a procedure by Munera-Echeverri et al. (2018). Briefly, after saturating biochar samples with 1000 mg NH₄⁺ L⁻¹, they were transferred wet into acid washed borosilicate glass flasks prior to addition of 20 mL of ethanol. Samples were left two hours in a horizontal shaker and ethanol was decanted. The procedure was repeated three times. The remaining biochar was left to air-dry at room temperature inside a fume hood for 10 days, then it was weighed and extracted with KCl (Section 3.5.3) for NH₄⁺ determination (Section 3.5.1).

3.3.4 Sorption of orthophosphate (H₂PO₄⁻)

Preliminary sorption tests using concentrations of 2, 20, 100, 200 and 400 mg H₂PO₄⁻ L⁻¹ added in the form of KH₂PO₄ showed that instead of orthophosphate sorption, equilibrium concentrations were higher than in the initial solution indicating a desorption of orthophosphate (59, 121, 198, 288 and 508 mg H₂PO₄⁻ L⁻¹, see Appendix B, Table D1).

A sorption batch test was performed with initial concentrations of 2, 20, 200 and 400 mg H₂PO₄⁻ L⁻¹ to verify the preliminarily observed desorption behavior. To further investigate the desorption and potential utilization of the released orthophosphate (as plant available phosphorus), biochar samples from the batch tests at 2 and 20 mg H₂PO₄⁻ L⁻¹ were filtrated and processed in additional *desorption* batch tests. For it, 20 mL of distilled water was added to the remaining biochar, then the flasks were placed on a horizontal shaker for another 24 h and the equilibrium solution was sampled. This process was carried out three times in total. To

calculate a total released orthophosphate mass, biochar was assumed to lose 0.03 g between each desorption batch test².

3.4 Materials

3.4.1 Biochar

The biochar was produced at Lindum's facilities in Drammen, Norway. The feedstock was soft-wood pellets from pine and spruce (49.3% C, 6.6% H, 0.11% N, 43.4% O) pyrolyzed at 600°C for three hours in a microwave assisted pyrolysis unit (MAP). The composition of the biochar, in dry basis, was 92.4% C, 1.5% H, 4.1% O, 0.22% N, 0.05% S and 0.03% P. It was received unwashed, with a moisture content of 40.8% and a pH of 9.63. The biochar was kept refrigerated (~4°C) in a plastic box until use.

3.4.2 Biochar pretreatments: washing with distilled water

The biochar was washed with distilled water to remove soluble ashes and salts not associated to exchange sites. The washing procedure was adapted from Munera-Echeverri et al. (2018), using distilled water in a 1:20 ratio (g to mL). Two sample sets were prepared, one for preliminary analyses/digestate enriching tests, and another one for the pot trial:

- a) **Preliminary analyses/digestate enriching test:** 0.10 kg of biochar was mixed with distilled water in polypropylene containers and left in a horizontal shaker (Edmund Bühler GmbH, model SM-30) for 4.75 h at 125 rpm. Then, the washed biochar was recovered with a 1.0 mm diameter sieve (steel mesh) and left to air-dry at room temperature (~22°C) for 4 days.
- b) **Pot trials:** 2.50 kg of biochar (DUB) was mixed with distilled water in an 80 L high-density polyethylene container using a bench drill with a twin propeller paint mixer bit (Figure 3.3). The speed was set to 500 rpm for 1.5 h. A reduced time was chosen not to excessively break down the biochar into smaller particles. Then, the biochar was separated using 1.0 and 0.22 mm pore diameter sieves, therefore all biochar larger than

² Based on the recovered biochar mass from the CEC determination (Section 3.3.3), whose procedure was homologous.

0.22 mm was used (the maximum size of biochar was ~ 3.15 mm). Lastly, it was left scattered on a table to air-dry at $\sim 22^{\circ}\text{C}$.



Figure 3.3. Mixing equipment used for the washing and enriching of biochar

3.4.3 Digestate

Digestate was generated in anaerobic reactors treating cow manure, pig manure and source separated food waste mostly from households, at Lindum AS facilities in Tønsberg, Norway. The digestate was sampled at the outlet of a screening unit, which consists of a rotary drum screen removing particles larger than 0.2 mm. However, it is considered as ‘unseparated digestate’ because it was not subject to a conventional sludge dewatering process (e.g., polymer addition and centrifuge decanting) which produces a solid and a liquid fraction. The sample was placed in plastic polypropylene buckets and sent to NMBU, where it was stored in a cold room ($\sim 4^{\circ}\text{C}$) until use.



Figure 3.4. Digestate, the consistence is of a liquid suspension with high viscosity.

3.4.4 Digestate-enriched biochar

Washed biochars (Section 3.4.2) and digestate were mixed in a solid to liquid ratio of 1:30 (g to mL) and a contact time of 66 h using as reference the work by Hale et al. (2013). Two sample sets were prepared differently, as first, a preliminary small-scale test was performed before scaling up to prepare the enriched biochar for the pot trials:

- a) **Preliminary digestate enriching test:** washed biochar and digestate were mixed in 1 L polypropylene containers in a horizontal shaker. After 66 h, the mixtures were filtered using 1.0 mm pore diameter sieves. Given that unseparated digestate was used, the biochar grains remained covered with considerable amounts of digestate, so it was decided to divide the enriched biochar into two sub-samples, rinsing one with water to compare the variation in plant available nutrients with and without the 'excess digestate', to verify the amount of nutrients that were sorbed. One sub-sample (20 g biochar) was rinsed with 1500 mL of tap water to remove the excess digestate. The other sub-sample was left unrinsed. The sieves containing each sub-sample were gently shaken and the excess liquid underneath them was wiped with paper towel; then both sieves were placed inside a fume hood at full suction for one hour. The two sets of biochar samples (i.e., size fractions >1 mm with rinsed and unrinsed material) were stored cold (~4°C) in 200 mL polypropylene containers prior to chemical analysis.
- b) **Enriched biochar for pot trial:** Biochar was mixed with digestate and further separated using the same equipment as described in Section 3.4.2.b. Due to the prolonged contact time and given that the mixing propeller could potentially break down the biochar into

too fine pieces if let mixing continuously for 66 h, the drill was set with a timer, mixing for 30 minutes every 4 hours at 450 rpm. The propeller was placed close to the wall of the bucket for tangential mixing force.

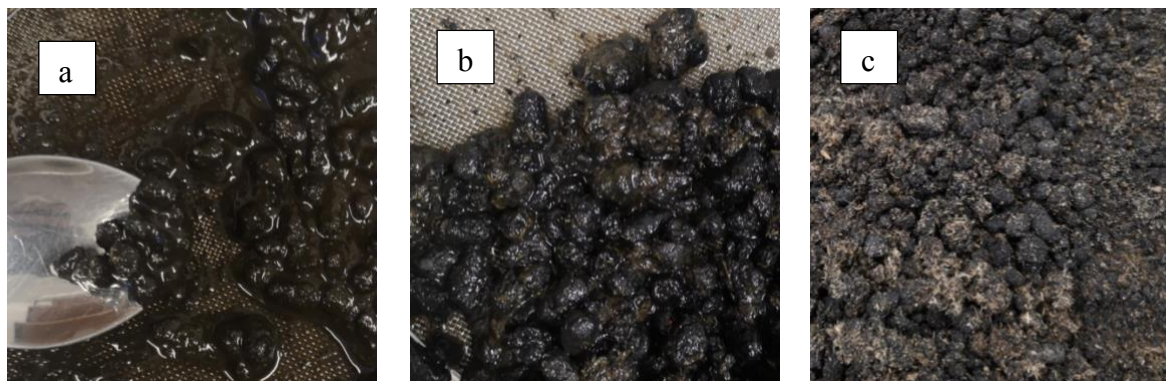


Figure 3.5. Unrinsed enriched biochar (EB1U) (a); rinsed enriched biochar (EB1R) (b); rinsed enriched biochar (EB2R) for pot trials (dry).

3.4.5 Compost mix

The compost was produced from green waste at Lindum's facilities in Drammen, Norway. A mechanically dried (average pH=8.24, 22.5% water content) and a wet, sulfur-treated compost (average pH=5.57, 46.3% water content) were combined in a 1:1 volume ratio, with a resulting pH of 6.51 and water content of 42%.

3.4.6 Mineral soil

Mineral soil collected from NMBU's experimental farmland in Vollebekk, Ås, was used in the present study. The soil is classified as a sandy loam (65% sand, 27% silt and 8% clay) with an average pH of 5.95 and 4.5% water content (Appendix 2, Table A.1).

3.4.7 Inorganic fertilizers (NPK)

Inorganic fertilizers in aqueous solutions were used in the pot trial as potassium, phosphorus, and nitrogen sources respectively (concentration indicated in brackets): potassium sulphate K_2SO_4 [12 g K L⁻¹], calcium dihydrogen phosphate $Ca(H_2PO_4)_2$ [3 g P L⁻¹], calcium nitrate $Ca(NO_3)_2$ [12 g N L⁻¹].

3.5 Analytical Approach

3.5.1 Ammonium (NH_4^+) and orthophosphate (H_2PO_4^-) sorption tests

Ammonium and orthophosphate were measured spectrophotometrically (Gilford Instrument, Stasar II); a detailed description of the method can be found in the Appendix B. This method employs chemical reagents (indicators) that react and give a specific coloration that is proportional to the concentration of the analyte. A lamp emits a light, set at a specific wavelength (655 nm for NH_4^+ and 660 nm for H_2PO_4^-), which goes through the sample and a detector measures the absorbance. Standard solutions are used to generate a calibration curve from which absorbances can be converted to concentrations by linear regression.

Results were corrected for dry matter content, and all analyses were conducted within 21 days after the preparation of the samples. Blanks and control samples were prepared for analytical quality control, both for the batch tests as for the chemical analyses (See Appendix B, Table F1). All samples were analyzed by triplicate.

3.5.2 Plant available Phosphorus (P) and Potassium (K)

Ammonium lactate (AL) extraction: 2.00 g samples were mixed with 40 mL of a 0.1 M ammonium lactate + 0.4 M acetic acid extracting solution in acid washed borosilicate glass bottles. Then placed on a horizontal shaker for 90 min at 150 rpm. Finally, samples were filtered using paper filter (Whatman 'Blue ribbon' quantitative ashless paper filter) previously rinsed with the extracting solution and stored cold ($\sim 4^\circ\text{C}$) in polypropylene vials until analysis. All samples were analyzed in triplicate.

ICP analysis: The instrumental method was *inductively coupled plasma* (ICP), performed on an ICP-OES Agilent 5110 dual view instrument. The principle of the method is that atoms or ions can absorb energy causing electrons to move from a ground state to an 'excited' state. When they transition back to a lower level of energy, light is released at a specific wavelength, for every element. The wavelength and the intensity of the light is detected and a concentration is calculated based on a calibration graph (Agilent Technologies, 2021).

3.5.3 Plant available/mineral nitrogen ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$)

KCl extraction: 5.00 g samples (except for biochar, where 3.00 g was used) were mixed with 25 mL of an extracting solution containing 2 M KCl in acid washed borosilicate glass bottles in a horizontal shaker for 30 min at 150 rpm. Then, samples were filtered using paper filter previously rinsed with the extracting solution and stored cold ($\sim 4^\circ\text{C}$) in polypropylene vials until analysis. All samples were analyzed in triplicate

Flow injection analysis (FIA): Ammonium ($\text{NH}_4^+\text{-N}$) was measured in a Foss FIAStar 5000 *flow injection system*. The instrumental method principle is as follows: sample extract is injected into a carrier stream, mixed with another stream of sodium hydroxide (NaOH). As a result, gaseous ammonia is formed (NH_3) which diffuses through a gas permeable membrane into an indicator stream. The indicator stream comprises a mixture of acid-base indicators which react with the ammonia gas. The resulting color shifts can be measured photometrically, therefore this method requires the preparation of calibrating solutions in the range of $0\text{-}5\text{ mg L}^{-1}$, using ammonium chloride.

Nitrate ($\text{NO}_3^-\text{-N}$) was measured in a Foss FIAStar 5000 *flow injection system*. The chemical principle of the method is reduction of nitrate to nitrite in a cadmium reductor. This provides the sum of nitrates and nitrites in the original sample. However, the original concentration of nitrite is negligible in comparison with the nitrate concentration³. This is also a spectrophotometric method.

3.5.4 Total Carbon and Nitrogen

Total carbon was analyzed as per the *dry combustion method*, proposed by Allison and described in Nelson & Sommers (1983). Samples were initially dried at 55°C and crushed in a mortar. Approximately 200 mg samples were weighed in a tin foil and analyzed in a Leco CHN628 instrument. By complete combustion, all the carbon (CO) is oxidized to CO_2 and the concentration of the latter is measured by infrared light.

³ As per the "Application note 5206: Determination of nitrate in 2M KCl soil extracts by FIAStar 5000" by the manufacturer of the analytical instruments, Foss.

Total nitrogen was analyzed according to the Dumas method, described in Bremner & Mulvaney (1983). The principle is the same as for total carbon, but instead, nitric oxide compounds (NO_x) are reduced by copper to nitrogen gas (N_2). Then the concentration N_2 is measured by thermal conductivity (TC cell) on the same analytical instrument, Leco CHN628.

3.5.5 Total Phosphorus and Potassium

The method consists of an initial nitric acid digestion or decomposition followed by ICP analysis. The preparation of the samples was as follows: 0.25 g were weighed in teflon tubes, adding 2 mL of milliQ water and 5 mL of double distilled HNO_3 , and then decomposed in a Ultraclave equipment (Milestone) for 1 hour. After cooling, samples were diluted up to 50 ml and analyzed by ICP-OES (Section 3.5.2).

3.5.6 Statistical analysis

All chemical analyses were performed on triplicate samples for each treatment ($n=3$). Error bars in figures/plots indicate the standard deviation.

One-way Analysis of Variance (ANOVA), with a significance level $\alpha=0.05$ was used to determine differences in: (1) shoot biomass yield and nutrient concentration (Total N, P and K) between pot trial treatments; (2) sorbed concentration of NH_4^+ (C_s) between biochars with and without adjusted pH.

Following ANOVA, differences between treatments were assessed by means of pairwise comparisons using Student's t-test ($\alpha=0.05$).

4 RESULTS

This chapter contains three sections; one section per each of the research sub-questions described in the introduction: 1. *How does digestate enriched biochar compare with synthetic fertilizers in terms of shoot biomass and macronutrient content in biomass?* 2. *Does digestate enriched biochar inhibit the mineralization of nitrogen?* and 3. *What is the nutrient sorption capacity of biochar for ammonium and phosphate?*

4.1 How does digestate enriched biochar compare with synthetic fertilizers in terms of shoot biomass and macronutrient content?

4.1.1 Shoot biomass

The shoot biomass yield was significantly different between all treatments (Figure 4.1). The greatest yield was found for the EBC treatment (14.9 g pot⁻¹) and the smallest yield was found in the control, without any fertilizer addition (5.6 g pot⁻¹). Yields for the control treatment, with significantly lower levels of N (5.1 kg daa⁻¹) than the recommended doses (15-20 kg daa⁻¹, Roy et al., 2006) were about 42% lower than for conventional fertilization (NPK).

By taking the inorganic fertilizer treatment (NPK) as reference, BCNPK, DI and EBC had an increased yield of 18.5%, 35.3% and 54.7% respectively.

It should be noted that for treatments having the same main source of N, that is inorganic fertilizer in NPK and BCNPK, and digestate in DI and EBC, the treatment containing biochar had a significantly higher yield.

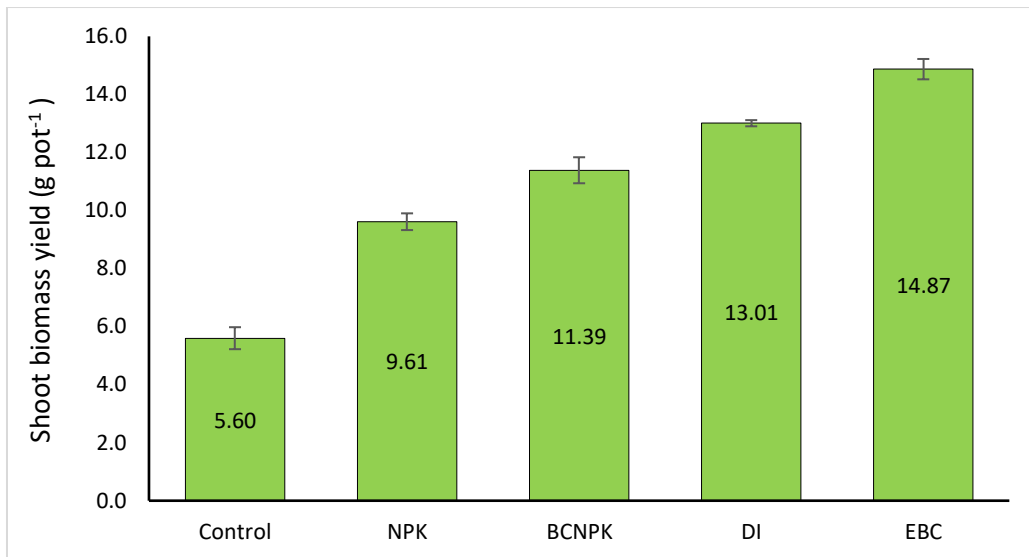


Figure 4.1 Mean shoot biomass yield per pot for the different treatments. Values are shown on a dry basis. Error bars indicate standard deviation ($n=5$). Different letters indicate significant difference ($\alpha=0.05$, $n=25$).

The visual appearance of the treatments at the end of the pot trial is shown in Figure 4.2. Pots for treatments BCNPK, DI and EBC had a voluminous grass growth compared to the control and NPK.

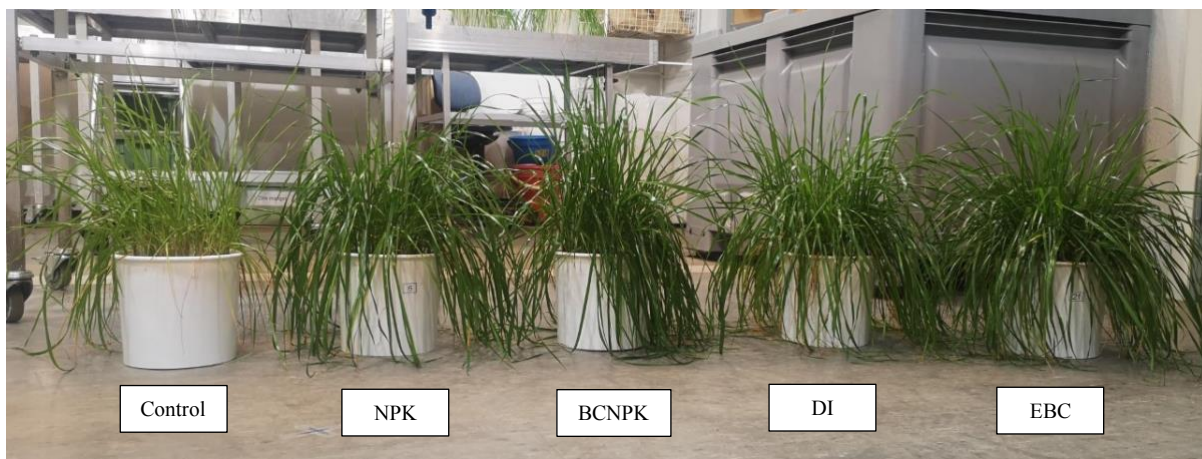


Figure 4.2. A replicate of each treatment at the end of the pot trial.

4.1.2 Macronutrient content

The mean concentration of total N, P and K in shoot biomass are presented in Figure 4.3.

When it comes to K and N, the control treatment, as expected, had the lowest total nitrogen and potassium content, 16.5 g kg^{-1} and 45.2 g kg^{-1} respectively. NPK, DI and EBC showed similar values for both total N and total K, and no significant difference for both nutrients.

Treatment BCNPK had the highest N and K content, with N markedly higher than the other treatments (39.8 g kg⁻¹), although the analytical results also showed the highest variance.

P content between the treatments showed a small variation, ranging from 4.2 g kg⁻¹ (Control) to 5.1 g kg⁻¹ (EBC). NPK, BCNPK and EBC in the upper end of the range with no significant differences. Control and DI in the lower end, also without a significant difference.

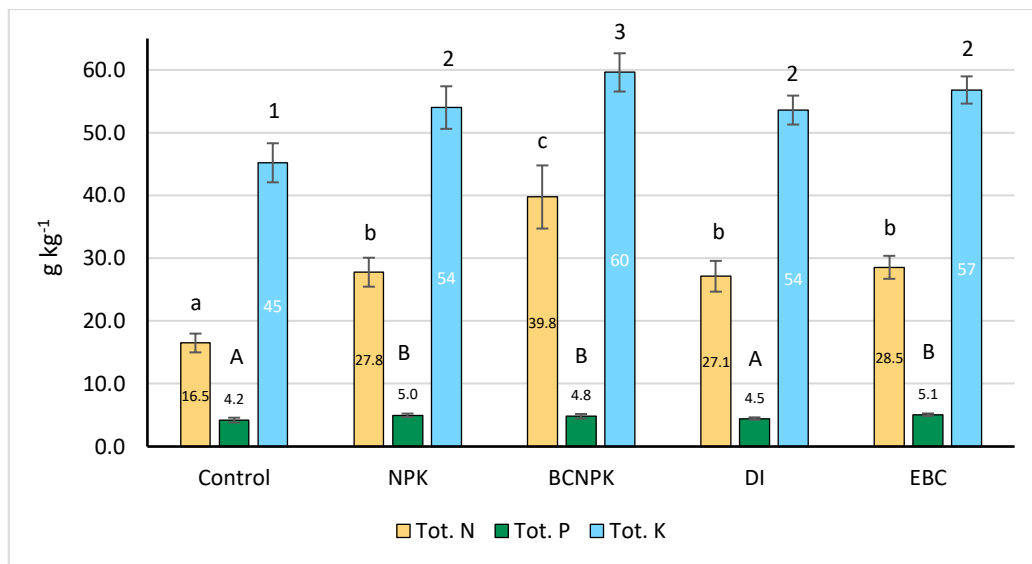


Figure 4.3. Mean concentration of total N, P and K in plant shoot biomass. Error bars indicate standard deviation ($n=3$). Different letters (Lowercase, Total N; Uppercase, Total P) or numbers (Total K) between treatments indicate significant difference ($\alpha=0.05$, $n=15$).

Carbon to nitrogen ratios are shown in Figure 4.4. The data is consistent with the total N concentration in shoot biomass. For example, the Control treatment, nitrogen deficient and lowest in N uptake, has the highest ratio, whereas BCNPK, highest in N uptake showed the lowest C:N ratio.

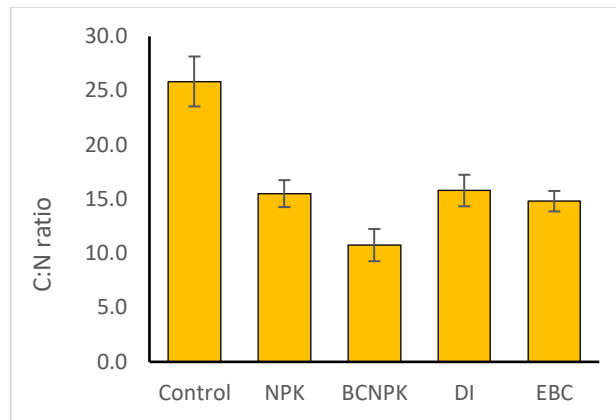


Figure 4.4. Mean carbon to nitrogen ratios (C:N) in plant shoot biomass. Error bars indicate standard deviation ($n=5$)

4.2 Does digestate enriched biochar inhibit the mineralization of nitrogen?

Figure 4.5 shows mean values for plant available mineral N (NO_3^- -N and NH_4^+ -N), at the beginning and end of the parallel trial. The dominant form of mineral N at the start is NO_3^- for Control, NPK and BCNPK, whereas NH_4^+ is the dominant form for DI and EBC. At the end, nearly all NH_4^+ has been converted to NO_3^- , therefore nitrification took place in all treatments.

For the control the sum of mineral N (i.e., $\text{NH}_4^+ + \text{NO}_3^-$) is about the same at the end as at the start suggesting that the fraction of potential plant available N is the same at the start and at the end of the experiment. This suggests no net immobilization or mineralization.

Conversely, for treatments NPK, BCNPK and DI, levels of mineral N are smaller at the end than at the start, indicating a net microbial immobilization of mineral N and therefore potentially lower amounts of plant available N in course of the experiment.

For EBC treatment, the sum of mineral N (i.e., $\text{NH}_4^+ + \text{NO}_3^-$) is about the same at the end as at the start (i.e., the same as observed for the control treatment) suggesting that the fraction of potential plant available N is the same at the start and at the end of the experiment without plants (i.e., no net immobilization or mineralization). However, in contrast to the control treatment, the net nitrification is greater, which indicates that the form of N has changed, with almost a complete conversion of NH_4^+ to NO_3^- .

The N balance, a comparison of the estimated total N content in biomass with the initially available mineral N is included in Figure 4.6. The estimated N content in root biomass accounted for a small increase of 7.4% with respect to shoot biomass. Based on the estimations, it is possible to see that the sum of ammonium and nitrate available at the beginning of the pot trial could have supplied enough to account for the total N biomass, in all treatments.

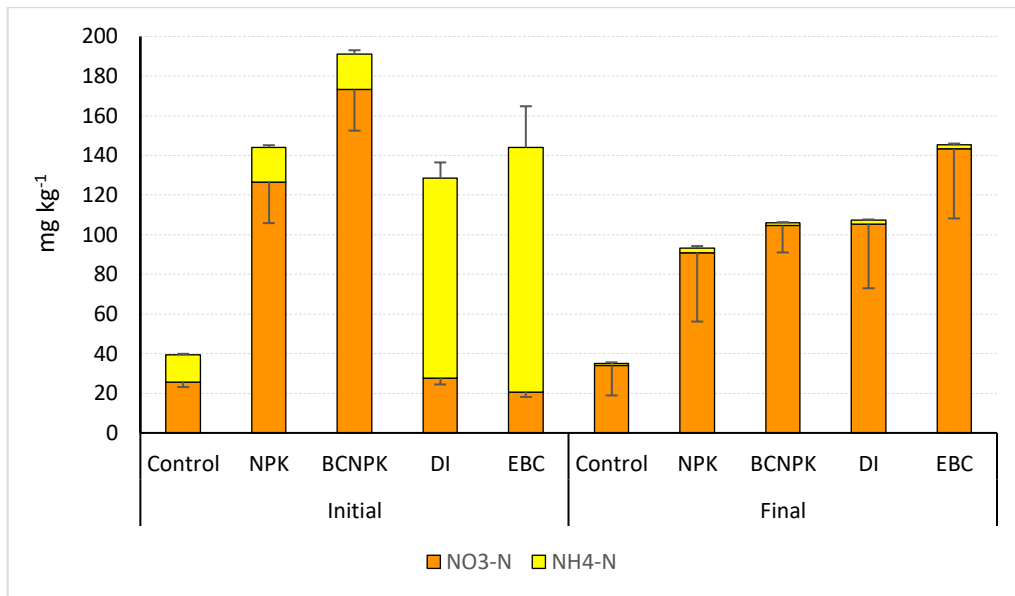


Figure 4.5. Mean NO₃⁻-N and NH₄⁺-N concentrations in unsowed pots at the beginning and end of the pot trial. Error bars indicate standard deviation (n=3)

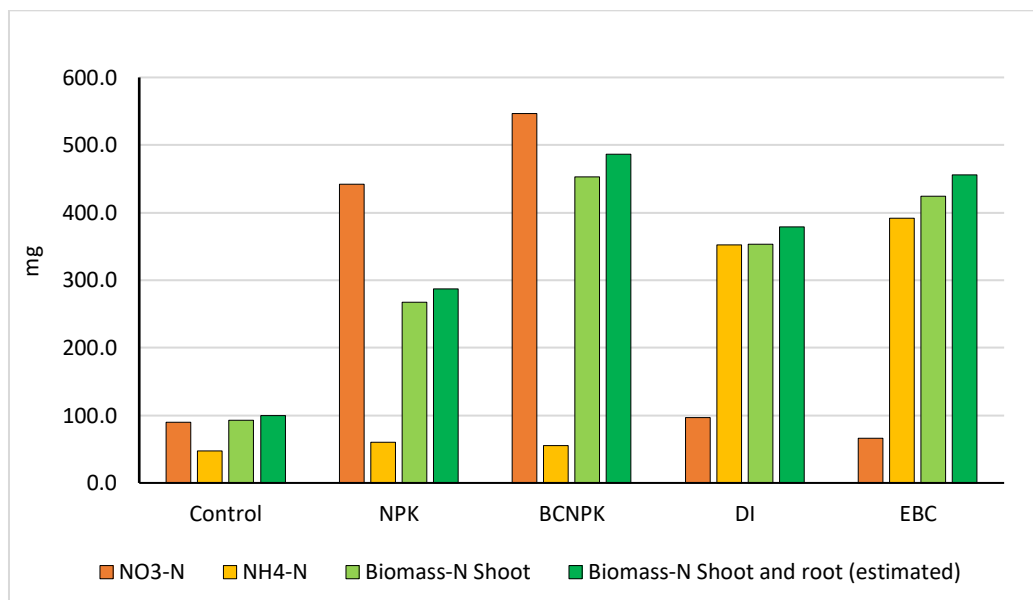


Figure 4.6. Initial mineral N (NO₃⁻-N and NH₄⁺-N) content (mg) in the pot trial treatment substrates. N content in shoot biomass and an estimation of a N in shoot and root biomass are included.

A similar comparison but using the total nutrient content in soil versus content in biomass was conducted for P and K. The data is presented in Table 4.1. EBC treatment had the highest content of P and K as well as the highest fraction of plant available P and K taken up by the plants. Note that although plant available P and K were greater in EBC treatment, the fraction taken up is still highest, and this suggests that the availability was greater in EBC.

In all treatments, even Control, there was a substantially large difference between the amount of nutrient taken up by plants, and the initially available, which is consistent with what was mentioned in Section 3.2.2, on plant available P and K supplied in excess, already by the base substrates.

Table 4.1. Total and plant available P and K content in soil, estimation in shoot and root biomass and fraction of plant available P and K taken up by the plants.

	Total P (mg)	Plant available ¹ P (mg)	Biomass-P Shoot and root (estimated) (mg)	Fraction of plant available taken up (%)	Total K (mg)	Plant available ¹ K (mg)	Biomass-K Shoot and root (estimated) (mg)	Fraction of plant available taken up (%)
Control	3486	582	6.0	1.03%	18476	1505	31.9	2.12%
NPK	4183	731	12.4	1.70%	20451	1956	60.6	3.10%
BCNPK	4097	742	15.9	2.14%	18697	1985	72.6	3.66%
DI	4067	706	16.3	2.31%	21148	1778	84.8	4.77%
EBC	4659	922	23.4	2.54%	21070	1990	106.0	5.33%

1. Plant available values are calculated based on the data for the preparation of the pot trials (Table 3.5)

4.3 What is the nutrient sorption capacity of biochar for ammonium and orthophosphate?

4.3.1 Sorption of NH_4^+

The NH_4^+ sorption batch results in this section correspond to sorbed concentrations at 24-hour equilibrium, in a non-steady state, as detailed in the Appendix B (Section C).

Figure 4.7 summarizes the results showing the distribution of the initial solution concentration (range 20-1000 mg NH_4^+ L⁻¹) into two fractions: the equilibrium concentration and the difference or reduction due to sorption in the biochar. At the lowest range the initial solution concentration decreased by about 72% and 59% respectively for batch tests starting at 20 and 100 mg NH_4^+ L⁻¹ and by about 50% for the batch test at 200 mg NH_4^+ L⁻¹. As the initial concentration increases, in the range of 400-1000 mg NH_4^+ L⁻¹, the trend reverts with most of the ammonium remaining in the equilibrium solution (dark shaded areas). This can be also

observed in the decreasing K_d values, where a higher K_d indicates a greater the affinity to the solid phase, and thus a decreasing K_d indicates a decreasing affinity to the solid phase, or in this case a decreasing capacity of sorption.

Isotherms, plotted as sorbed concentrations against equilibrium concentrations, allow to distinguish if there is a particular trend in the sorption behavior or if a maximum sorption capacity has been reached. Figure 4.8 shows the results from the batch test, with all the analytical data and predictions based on a Freundlich model, explained further below. First, the steeper slope approximately before an equilibrium concentration of $100 \text{ mg NH}_4^+ \text{ L}^{-1}$ indicates that a higher fraction of the NH_4^+ in the initial solution has been sorbed, as commented above. After $100 \text{ mg NH}_4^+ \text{ L}^{-1}$ the concentration in the solid fraction (C_s) continues to increase, but it is not clear where it starts to level off, which is an indication that the maximum sorption capacity has been achieved, and therefore Freundlich and Langmuir transformations are employed to identify if the sorption behavior is accurately represented by one of these models, and then calculate a sorption maximum.

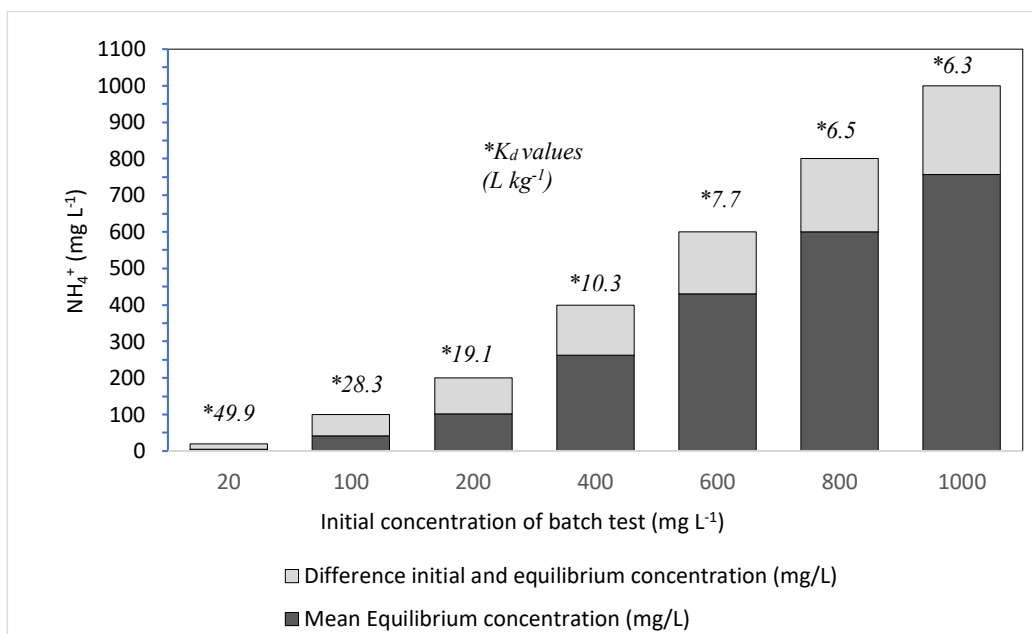


Figure 4.7. Distribution of the initial concentration of NH_4^+ in the batch tests into equilibrium concentration and the difference or decrease due to sorption (mean values). The values on top of the bars indicate the distribution constant K_d (L kg⁻¹)

Both regression curves resulted in a high coefficient of determination (r^2), with 0.94 and 0.98 for Langmuir and Freundlich respectively (See Appendix B, Section G). The highest and thus better representing the sorption behavior was Freundlich, for which empirical parameters

were calculated finding a K_F and n of 0.135 L g^{-1} and 0.548 respectively. Sorbed concentrations were modelled within the range of the experimental data and extrapolating from 800 mg L^{-1} to 1500 mg L^{-1} , as shown in Figure 4.8. The predicted values fit the experimental data (measured C_s) accurately, however, in the extrapolated range, the isotherm does not foreseeably level off, but appears to keep increasing, for which no maximum sorption can accurately be estimated.

Nonetheless, given that the Langmuir linear regression also fitted the data with a high r^2 (0.94), and that it is possible to obtain the parameter $1/Q_{max}$, a maximum sorption capacity was calculated, resulting in $5.45 \text{ mg NH}_4^+ \text{ g}^{-1}$. This value can be taken as a reference.

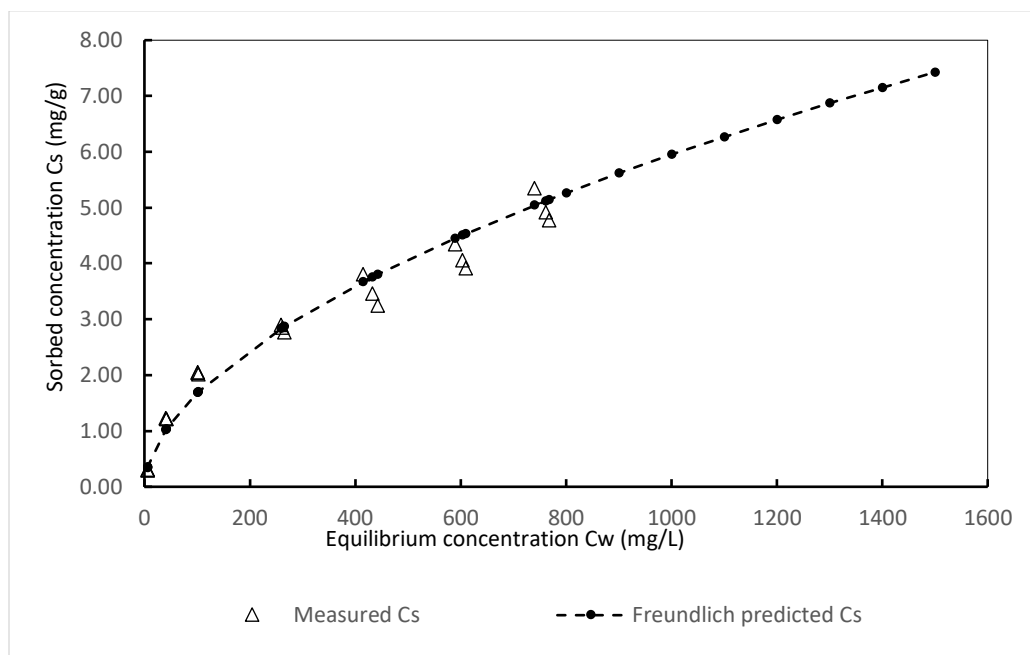


Figure 4.8. Measured and Freundlich predicted sorbed concentration (C_s)

The initial and final pH of the batch tests conducted to identify possible ammonia losses are summarized in Table 4.2. As can be seen, the pH increases as ammonium concentration decreases. The lowest pH, within the range of the sorption batch test was 8.18, for the highest initial ammonium concentration.

Figure 4.9 shows the isotherms for both pH adjusted and non pH adjusted sorption batch tests. From the plot, an apparent higher sorbed concentration on NH_4^+ occurred at the middle ($C_w \cong 230 \text{ mg L}^{-1}$) and high range ($C_w \cong 730 \text{ mg L}^{-1}$) of the equilibrium concentrations, however

the difference between the groups was statistically significant only for the batch tests at 400 mg NH₄⁺ L⁻¹ of initial concentration (Figure 4.10) with a higher mean sorbed concentration of 3.49 mg NH₄⁺ L⁻¹ for the sample with pH adjusted to 7; the mean of the sample without pH adjustment has a sorbed concentration of 2.76 mg NH₄⁺ L⁻¹, about 20% lower.

Table 4.2. Adjusted pH sorption batch tests, Initial and final values

NH ₄ ⁺ Initial concentration (mg/L)	Initial pH ^a	s.d.	Final pH ^a	s.d.
20	9.15	0	6.99	0.015
400	8.51	0.01	6.95	0.08
1000	8.18	0.02	6.98	0.04

a. Measured directly in the equilibrium solution after 24 h (solid to liquid ratio 1:20, with distilled water)
s.d.= standard deviation (n=3)

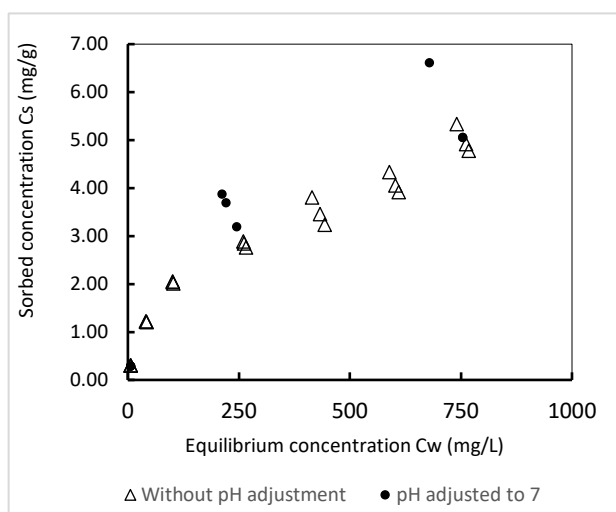


Figure 4.9. Sorption batch test results for samples with and without pH adjustment.

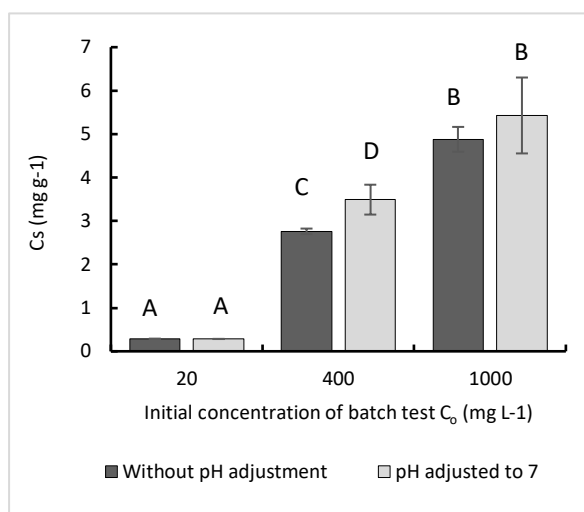


Figure 4.10. Sorbed concentration of NH₄⁺ (C_s) for samples with and without pH adjustment at three different concentrations of initial solution. Different letters between treatments indicate significant difference (α=0.05, n=9)

4.3.2 Determination of cation exchange capacity

The mean cation exchange capacity (CEC) of the biochar was 1.02 ± 0.06 cmol₍₊₎ kg⁻¹, which corresponds with a sorbed concentration (C_s) of 184 ± 11 mg kg⁻¹.

4.3.3 Sorption of Orthophosphate (H₂PO₄⁻)

The equilibrium concentration in the batch tests showed a uniform increase of near 100 mg H₂PO₄⁻ L⁻¹ for the whole range of the batch test (2 – 400 mg H₂PO₄⁻ L⁻¹), in other words,

indistinctly of the initial concentration of the liquid phase in contact with the biochar, phosphate was released in the approximately the same quantity, as can be observed in Figure 4.11 (light-gray shaded areas).

The additional batch tests with distilled water indicate that the desorption continues to occur at a decreasing rate (Figure 4.12) with no marked differences between the two tested groups (biochar from batch tests at 2 and 20 mg $\text{H}_2\text{PO}_4^- \text{L}^{-1}$ of initial concentration).

Based on the increased concentrations in the equilibrium solutions after three additional batch tests with water, a total mass of H_2PO_4^- per gram of biochar was calculated, resulting in $4.08 \pm 0.06 \text{ mg g}^{-1}$ and $4.30 \pm 0.01 \text{ mg g}^{-1}$ for the batch tests at 2 and 20 mg H_2PO_4^- of initial concentration respectively.

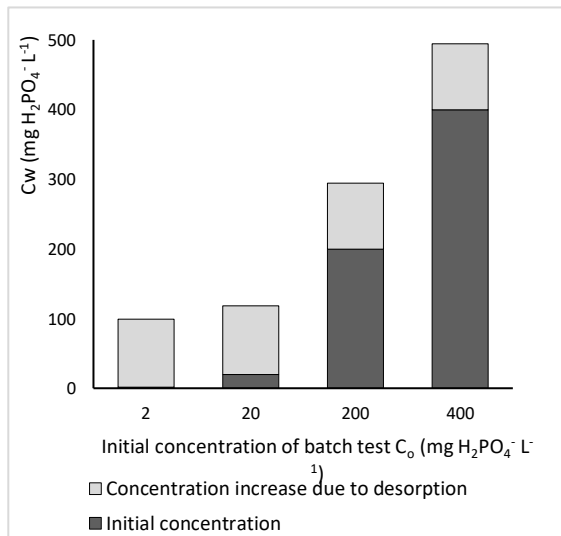


Figure 4.11. Equilibrium concentration (C_w) of phosphate for batch sorption tests at four different initial concentrations (mean values, $n=3$)

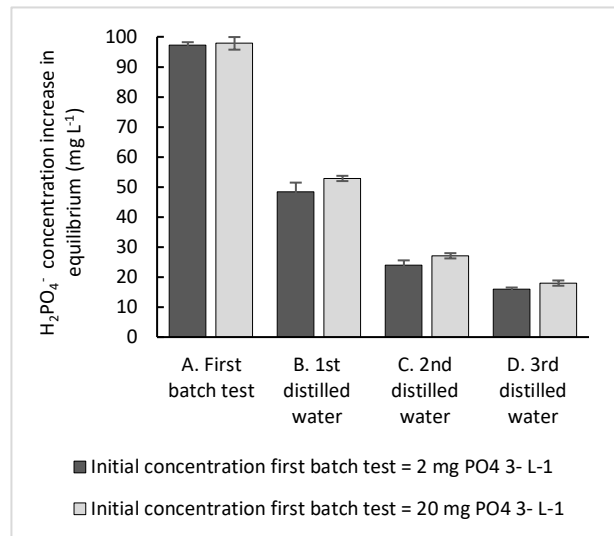


Figure 4.12. Mean values of H_2PO_4^- concentration increase in equilibrium solutions. Group A is the first batch test with phosphate solutions; groups B-D are the consecutive batch tests performed with distilled water (Error bars indicate standard deviation, $n=3$)

5 DISCUSSION

This chapter will discuss the findings in terms of each of the research questions. Then, the impact and applicability of the results is discussed, concluding with a discussion on the limitations of the study.

5.1 How does digestate enriched biochar compare with synthetic fertilizers in terms of shoot biomass and macronutrient content?

5.1.1 *Shoot biomass*

The results of the pot trial showed that EBC, the treatment consisting of enriched biochar and digestate, displayed the best performance in both plant growth and yield of shoot biomass and 54.7% higher than conventional fertilization (NPK) indicating that nutrients in the treatment were available.

For the treatments having the same main source of nitrogen but differing mainly in the presence/absence of biochar, i.e., NPK vs. BCNPK (having inorganic fertilizer) and DI vs. EBC (having digestate), the results indicate that the presence of biochar significantly influenced the yields, with BCNPK having a yield 18.5% higher than NPK, and EBC 14.3% higher than DI.

Both results are in accordance with Glaser et al. (2015) who conducted a field experiment finding that biochar in combination with inorganic fertilizer (urea) increased the yield of maize by 20% with respect to inorganic fertilizer, and digestate combined with biochar (at an application rate of 40 Mg Ha⁻¹) had a yield 42% higher than inorganic fertilizer. The study differed in terms of the N dosages (20 kg N daa⁻¹), the testing plant and the soil (sandy soil); the biochar (green cuttings, 650°C), dosage (in his study the dosage was higher i.e., ~58 Mg Ha⁻¹) and the digestate (maize digestion).

M.S. Lee et al. (2021) observed similar findings on the positive effect of biochar combined with digestate in biomass yield. The study compared the nitrogen fertilization effect of granular urea (conventional N fertilizer), digestate and a combination of biochar and digestate on biomass yields and feedstock quality of switchgrass (a perennial grass used as bioenergy crop) showed that, for the same dosage or N application rate used in this study (15 kg N daa⁻¹) after 8 weeks the treatment with biochar and digestate had a yield 25% higher than the conventional fertilizer and 8.2% higher than digestate alone, although in a longer period (32 week) the

conventional treatment showed a yield 12% higher than the biochar-digestate. In addition, the study found no significant difference in the quality of the grass (hemicellulose, cellulose, and lignin) concluding that both tested treatments could potentially replace chemical fertilizers. The study differed mainly in the type of biochar (corn stover, 600-875 °C), the digestate (from sewage sludge digestion) and the soil (mix of sand and silt loam) which did not include compost.

To summarize, both studies by Glaser et al. (2015) and M.S. Lee et al. (2021), are in accordance with the results of this thesis when it comes to the synergistic effects of biochar in combination with conventional N fertilizer, as well as with digestate, on plant biomass, where in both cases yields are significantly higher. This suggests that, despite differences between the studies, the observed synergistic effects of biochar could be independent of the context and have a wider applicability.

When it comes to the reasons why treatments with digestate and biochar had higher yields, perhaps the answer is in connection with microbial activity. As mentioned in the theoretical background, digestate introduces a rich microbiology to the soil. Biochar has also been connected with functions like supporting microbial growth and reproduction (Pietikäinen et al., 2000). An additional element is the high content of compost in the substrates. Thus, it can be hypothesized that the combined effect of these factors could have increased the microbial activity in such way that nutrient availability was also increased through processes such as nutrient mobilization and microbial biomass turnover, as reported by (Malik et al., 2013).

5.1.2 *Macronutrient content*

The discussion on macronutrients is centered around N levels since both plant available P and K had already been supplied by the substrates (Table 3.5).

According to López-Arredondo et al. (2017) nitrate is the preferential form in which plants use nitrogen. On that basis, treatment NPK can be taken as a reference where nitrogen is expected to be most available. This in turn implies that the N content in the biomass of treatment NPK is expected to be within standard values for ryegrass.

Aasen (1997) reported the following normal concentration values in ryegrass biomass: N (30-42 g kg⁻¹), P (3.5-5 g kg⁻¹) and K (25-35 g kg⁻¹). Based on these reference values, treatment NPK shows consistent values for N and P, with a N concentration slightly lower (27.8 g kg⁻¹), and P in the upper range (5.0 g kg⁻¹). K exceeded the normal range by a significant margin,

about 57% higher, indicating a luxury consumption which was likely to occur given the excess dosage at the beginning of the pot trial.

N concentrations had no significant difference between treatments NPK, DI and EBC. Consequently, it can be assumed that nitrogen in treatments DI and EBC was available to the same degree than NPK, which is in line with the results on shoot biomass yield, where the increased yields indicate an effective nutrient availability.

When it comes to BCNPK, the nitrogen-amended entirely with inorganic fertilizer, it was the treatment with the highest N concentration in biomass, about 43% higher than NPK. The added volume of inorganic fertilizer N did not differ much between NPK and BCNPK, the latter having about 10% more. This suggests that biochar can potentially lead to a higher nitrate uptake, in addition to a higher biomass yield, as discussed previously.

5.2 Does digestate enriched biochar inhibit the mineralization of nitrogen?

The results showed that nitrification, a part of N mineralization processes, was observed in all treatments, but to a greater extent in treatments DI and EBC, given the almost complete conversion of the high initial concentration of ammonium (Figure 4.5).

Furthermore, conversion of NH_4^+ to NO_3^- was the greatest for treatment EBC and given that no net immobilization took place, nitrate concentration was the highest at the end of the trial. Since ryegrass has been reported to have a preference for NO_3^- (Cao et al., 2010) this may have resulted in a greater availability of N which could help explaining the high shoot biomass yield discussed previously. This suggest that enriched biochar did not only inhibit nitrification, but might have increased its rate, thus causing the positive effect observed on the shoot biomass yield. Nevertheless, studies in this topic are divisive and reported biochar to have no effect, reduce or enhance nitrification, respectively (Borchard et al., 2019; Duan et al., 2018; Munera-Echeverri et al., 2018).

The nitrogen balance result (Figure 4.6) provides an additional insight over N mineralization and availability in the enriched biochar. The estimation of total N content in biomass was lower than the initial sum of mineral N (i.e., $\text{NH}_4^+ + \text{NO}_3^-$) for all treatments. Consequently, it can be assumed that nitrogen needs would have been supplied by the initial mineral N, and not

through mineralization or conversion of organic nitrogen, therefore from this perspective, N mineralization could not be verified.

However, it can be hypothesized that if the N (i.e., mineral N) used by the plant would have come entirely from the amendment (enriched biochar EB2R and digestate), then the N in the enriched biochar could have been indeed available. This hypothesis is aligned with the observations of Kocatürk-Schumacher et al. (2019) where ammonium sorbed in enriched biochar (with a liquid fraction of digestate) was effectively released and taken up by plants (ryegrass) in a pot trial (quartz sand as substrate instead of soil).

5.3 What is the nutrient sorption capacity of biochar for ammonium and phosphate?

Sorption batch tests showed that ammonium is effectively sorbed in biochar, although the estimation of sorption maximum could not be estimated with accuracy as an asymptote was not clearly observed in the plotted isotherm (Figure 4.8), therefore it is likely that the curve was representing a medium sorption concentration. This is supported by the fact that the Freundlich regression showed the highest coefficient of determination ($r^2 = 0.98$) and Freundlich isotherms often represent very well medium sorption ranges (Worch, 2012). This implies that a batch test with initial concentrations higher than $1000 \text{ mg NH}_4^+ \text{ L}^{-1}$ are required in order to be able to estimate with more certainty the maximum sorption capacity of ammonium, which is likely to be higher.

Nonetheless, the sorption maximum estimated with the Langmuir regression ($r^2=0.94$) of $5.45 \text{ mg NH}_4^+ \text{ g}^{-1}$ can be used as a proxy to compare with other studies, although it should be clear that the actual value could be higher. Reported values in literature vary greatly, for instance, Hu et al. (2020) found a maximum sorption capacity of 5.60 mg g^{-1} , while Takaya et al. (2016) reported $114.44 \text{ mg NH}_4^+ \text{ g}^{-1}$, however, in both cases there were either different feedstocks/process conditions or batch sorption test methodologies, for which comparability is challenging. A study by Kocatürk-Schumacher et al. (2017), although not a sorption batch test, found sorbed values of $8.61 \text{ mg NH}_4\text{-N g}^{-1}$ in biochar, using filtration columns and the liquid fraction of a digestate as nutrient source. This may be a closer value to what could be expected in the biochar of this study, as feedstock and pyrolysis temperatures were similar (slow pyrolysis of holm oak, 650°C).

In addition, to contextualize the estimated sorption maximum ($5.45 \text{ mg NH}_4^+ \text{ g}^{-1}$) within this study, comparisons can be done with other materials of the pot trial. The estimated sorption maximum is equivalent to a concentration of $5450 \text{ mg NH}_4^+ \text{ kg}^{-1}$. The enriched biochar (EB2R) had a concentration of $589 \text{ mg NH}_4^+ \text{ kg}^{-1}$ (Table 3.2), suggesting that the biochar could have sorbed considerably more NH_4^+ . An additional comparison can be made with the content of plant available N at the beginning of the pot trial, for instance, in treatment BCNPK, which had 286 mg of plant available P mostly as NO_3^- (calculated from the data in Table 3.2 and Table 3.5). This means that if biochar could have been enriched up to the estimated sorption maximum, approximately 108 g of enriched biochar could have provided all the nitrogen requirements in a pot trial treatment. Treatment EBC for example, was amended with about 137 g of enriched biochar (EB2R) and additional digestate to fulfill the nitrogen dosage of the treatment. This suggests that if an effective enriching process can be carried out, the use of enriched biochar can be more efficient.

When it comes to the effect of pH in the sorption of ammonium, there could have been volatilization at pH of 8.51 (where significant difference was found with the pH adjusted sample) (Figure 4.10). This can be explained by the relative distribution of ammonia and ammonium as a function of pH and temperature in aqueous systems (Huang & Shang, 2006). At pH 8.51, a 15% fraction of ammonia can be expected, which might explain the lower sorbed concentration as compared to the same batch test at pH 6.95 (20% less).

The CEC found in the biochar ($1.02 \pm 0.06 \text{ cmol}_{(+) } \text{ kg}^{-1}$) is low as compared to typically reported values. For example, in an extensive characterization of biochar properties from different feedstocks and process temperatures by Ok et al. (2018), cation exchange capacity (CEC) values ranged from 23.6 to $562.0 \text{ cmol}_{(+) } \text{ kg}^{-1}$, the lowest corresponding to pig manure biochar fired at 200°C . Mukome et al. (2013) carried out a study of chemical and physical characteristics to investigate trends in biochar feedstocks, finding the lowest CEC value in softwood (pine, $500\text{-}650^\circ\text{C}$) of $3.2 \text{ cmol}_{(+) } \text{ kg}^{-1}$. The reason behind the low CEC value is most likely due the KCl extraction time of 30 min , which is typically used for soil samples as opposed to longer extraction times (24 h) used in biochar characterization studies (Munera-Echeverri et al., 2018).

No sorption of orthophosphate was observed, on the contrary a release of more than $4 \text{ mg H}_2\text{PO}_4^- \text{ g}^{-1}$ of biochar occurred in a series of consecutive batch tests.

The result is not consistent with the findings of Takaya et al. (2016) where biochars from oak wood and greenhouse waste (600-650°C pyrolysis) sorbed between 3.6 to 15.1 mg g⁻¹ of phosphate, although the conditions of the batch test were very different (1:1000 solid to liquid ratio, pH 7, fraction of biochar <0.85 mm). Conversely, Hale et al. (2013) observed a desorption of phosphate from cacao shell and corn cob biochar finding a release of between 0.17 and 1.48 mg g⁻¹ PO₄-P in a 60 days test.

As commented in Chapter 2, properties of biochar can greatly vary depending on feedstock and process conditions, and different batch sorption test methodologies can lead to different results. For example, grain size (different between Takaya et al. (2016) and this study), has been reported to influence properties such as total surface charge and content of functional groups, which in turn has an effect on sorption of ions. Zhang et al. (2020) concluded that anionic forms like phosphate and nitrate are poorly sorbed in unmodified biochar and suggested the addition of metals like magnesium (Mg) during the pyrolysis stage to increase the sorption of anions.

It should be noted that the biochar that was used for the batch sorption tests (DWB2) had already a content of plant available P of 1.46 g kg⁻¹ (Table 3.4), a much lower value than what was desorbed. This could be explained by the ammonium lactate extraction not exchanging the totality of the orthophosphate in the biochar, given that a mixing time of only 90 minutes, as opposed to several 24-hour batch tests with water. The use of a procedure typical for soil analysis of plant available P was certainly a limitation and a different procedure, especially with a longer extracting time is recommended to be used in future studies.

Finally, an additional discussion can be done regarding the desorbed orthophosphate, as plant available P that could have potentially been supplied, for example, in the pot trial treatments amended with biochar (BCNPK and EBC). The content of biochar in the treatments (12% v/v) was equivalent to 112 g and 136 g respectively for BCNPK and EBC. By assuming a desorbed orthophosphate mass of 4.08 ± 0.06 mg g⁻¹ of biochar (Section 4.3.3) approximately 457 mg and 555 mg of plant available P as orthophosphate could have been desorbed in treatments BCNPK and EBC respectively, over the course of the trial. These quantities are equivalent to about 60% of the plant available P at the beginning of the pot trial in the treatments. This may have had an effect over P availability and concentration in biomass despite no significant differences in P biomass between treatments NPK, BCNPK and EBC

(Figure 4.3). This analysis suggests that the biochar in this study can be considered a source of plant available P by itself given the relatively high desorbed quantities.

5.4 Impact and Applicability

The results of the pot trial suggest that it is feasible to replace inorganic fertilizers with a combination of enriched biochar and digestate with the added benefit of achieving significantly higher yields and no differences in the content of N, P and K in biomass, when comparing to conventional fertilization.

It is however important to contextualize the applicability of the results. A 12% v/v application rate of enriched biochar, approximately equivalent to 60-ton Ha⁻¹ can be costly for direct land application, unless subsidized. However, results may be more suited to other products such as potting soils or horticulture substrates for greenhouse production. This application can be a more sound implementation in the light of global population growth and increased food production needs, since greenhouse production have demonstrated to be highly efficient in terms of land area and water usage, as well as in increased yields (Hemming, 2013). The results of this study can contribute to further research in the field of horticultural production as well as provide reference guidelines for experiments in farmland, perhaps with different enriched biochar application rates (i.e., %v/v or ton Ha⁻¹) and combination ratios with digestate.

The ammonium batch sorption test results provided an approximate ammonium sorption capacity of a biochar with the characteristics of the one used in this thesis (feedstock and pyrolysis temperature). This could be used as preliminary reference data for example, in a pre-design phase of a filtering column aimed at the recovery of ammonium from waste sources (e.g., liquid fraction of digestate, or filtrate from biosolid dewatering), given that it is a conservative value.

5.5 Study Limitations

This study focused on studying the fertilization effects of enriched biochar in combination with digestate, under particular conditions (biochar, tested plant, source of digestate). To increase the external validity of the results, trials in actual soil are needed, also including

different species or plant families. This study did not investigate undesirable indirect effects of digestate such as ammonia and N₂O emissions, nor performed a risk assessment on the potentially harmful substances in the digestate, which as mentioned in Chapter 2, can vary greatly depending on the feedstock of the anaerobic reactors.

The available data did not allow to verify whether a net N mineralization took place. Therefore, in future studies total nitrogen in the substrates should be analysed at the end of the pot trial to ease the verification of N mineralization and to determine with accuracy whether a treatment could inhibit this process or not.

The verification of whether steady state was reached in the sorption batch tests as per detailed in the reference standard could not be confirmed, as detailed in Appendix A, Section C. However, this was not part of the scope of the study, and sorption results were reported as per a 24-h non steady state equilibrium. The implication is that batch sorption tests longer than 24-h could have shown higher sorbed concentrations, thus the ammonium sorption capacity could have been higher.

One limitation of the sorption batch test was that microbial activity was overlooked as a factor leading to conversion of ammonium into nitrate. This might have led to an overestimation of sorbed values (i.e., if significant amounts of ammonium had nitrified). To overcome this limitation in future studies, it is possible to employ bactericide agent, as done by Hale et al. (2013).

Finally, it should be noted that in the present study, sorption batch tests at neutrally adjusted pH were not conducted for the full range of initial concentrations. For future studies, this can be carried out. Additionally, if the focus of the study is on the sorption and stability of the ammonium in the biochar, gas analysis (gas chromatography) could be employed to directly measure and verify any ammonia loss.

6 CONCLUSIONS AND FUTURE WORK

This study attempted to investigate the effects of digestate enriched biochar on plant productivity and nutrient availability by means of pot trial and sorption batch tests.

The results of the pot trial showed that EBC, the treatment consisting of enriched biochar and digestate, had the best performance in shoot biomass yield, 54.7% higher than conventional fertilization (NPK) indicating an effective fertilizing effect and that nutrients supplied by the treatment were available. This was also confirmed by the macronutrient concentration in biomass, statistically the same than in conventional fertilization.

The study could not verify whether enriched biochar inhibited N mineralization due to lack of measurements on total nitrogen at the end of the pot trial. However, it was observed that nitrification (a part of N mineralization processes) took place in all treatments with the highest conversion of ammonium and final concentration of nitrate in EBC, suggesting a greater availability of nitrogen which can be linked to the high shoot biomass yields observed in the treatment.

Sorption batch tests showed that the tested biochar (DWB) could sorb ammonium effectively. Although the maximum sorption capacity could be higher, it was estimated in $5.45 \text{ mg NH}_4^+ \text{ g}^{-1}$ by Langmuir regression. This sorption capacity is considerable, if successfully scaled-up in NH_4^+ recovery processes.

No sorption of orthophosphate was observed. On the contrary, a release of more than $4 \text{ mg H}_2\text{PO}_4^- \text{ g}^{-1}$ of biochar occurred in a series of consecutive batch tests. This was estimated to be a considerable quantity in proportion to the initially plant available P in the treatments with biochar (~60%). The potential P fertilizing effect of the biochar used in this study could be further assessed, e.g., in a pot trial with low P content in the substrate.

The results of the study may be applicable to horticulture substrates for greenhouse production, which is becoming a mainstream practice due to their high efficiency in terms of land area and water use, as well as in increased yields.

Given that for some nitrogen amended treatments the presence of biochar led to increased yields, further research can be carried out to understand the underlying mechanisms, which could be related to microbial activity.

Future studies could replicate the pot trial test for a longer period with several harvests, to understand long term fertilization effects and to verify whether the difference in performance is sustained over time.

Future studies could also explore different dosages of nitrogen and biochar. Of particular interest is to study the synergistic effect of digestate and biochar and verify if increased productivities can still be attained at biochar application rates lower than 12% v/v.

Future studies could consider risk assessments to evaluate negative side effects of the biochar treatment such as ammonia and N₂O emissions, as well as analysis of harmful substances in the digestate. This will provide more information to make trade-offs when using biochar at scale.

Finally, further research and testing are needed to improve the efficiency of enriching processes, e.g., by comparing mixing against filtering columns, performing pH adjustments, or using different feedstocks, with or without modifications (e.g., addition of an iron or magnesium rich material).

References

Aasen, I. (1997) *Mangelsjukdomar og andre ernæringsforstyrrelser hos kulturplanter*. 96s edn. Landbruksforlaget.

Agilent Technologies, I. (2021) *The ICP-OES Principle*.

Akhiar, A. *et al.* (2017) 'Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion', *Waste Management*, 59, pp. 118–128. doi: <https://doi.org/10.1016/j.wasman.2016.11.005>.

Albuquerque, J. A. *et al.* (2012) 'Agricultural use of digestate for horticultural crop production and improvement of soil properties', *European Journal of Agronomy*, 43, pp. 119–128. doi: <https://doi.org/10.1016/j.eja.2012.06.001>.

ASTM International (2016) 'ASTM D4646-16, Standard Test Method for 24-h Batch-Type Measurement of Contaminant Sorption by Soils and Sediments'. West Conshohocken, PA.

Basso, A. S. *et al.* (2013) 'Assessing potential of biochar for increasing water-holding capacity of sandy soils', *GCB Bioenergy*. John Wiley & Sons, Ltd, 5(2), pp. 132–143. doi: <https://doi.org/10.1111/gcbb.12026>.

Bellarby, J. *et al.* (2008) *Cool Farming: Climate Impacts of Agriculture and Mitigation Potential*. Amsterdam: Greenpeace International. Available at: <https://eprints.lancs.ac.uk/id/eprint/68831/1/1111.pdf>.

Borchard, N. *et al.* (2019) 'Biochar, soil and land-use interactions that reduce nitrate leaching and N₂O emissions: A meta-analysis', *Science of The Total Environment*, 651, pp. 2354–2364. doi: <https://doi.org/10.1016/j.scitotenv.2018.10.060>.

Bremner, J. M. and Mulvaney, C. S. (1983) 'Nitrogen—Total', *Methods of Soil Analysis*. (Agronomy Monographs), pp. 595–624. doi: <https://doi.org/10.2134/agronmonogr9.2.2ed.c31>.

Bruun, E. W. *et al.* (2014) 'Biochar amendment to coarse sandy subsoil improves root growth and increases water retention', *Soil Use and Management*. John Wiley & Sons, Ltd, 30(1), pp. 109–118. doi: <https://doi.org/10.1111/sum.12102>.

Cao, H. *et al.* (2010) 'Nitrate/ammonium ratios affect ryegrass growth and nitrogen accumulation in a hydroponic system', *Journal of Plant Nutrition*. Taylor & Francis, 34(2), pp. 206–216. doi: 10.1080/01904167.2011.533322.

Carey, D. E., McNamara, P. J. and Zitomer, D. H. (2015) 'Biochar from Pyrolysis of Biosolids for Nutrient Adsorption and Turfgrass Cultivation', *Water Environment Research*. John Wiley & Sons, Ltd, 87(12), pp. 2098–2106. doi: 10.2175/106143015X14362865227391.

Cesaro, A. (2021) 'The valorization of the anaerobic digestate from the organic fractions of municipal solid waste: Challenges and perspectives', *Journal of Environmental Management*,

280, p. 111742. doi: <https://doi.org/10.1016/j.jenvman.2020.111742>.

Chintala, R. *et al.* (2014) 'Effect of biochar on chemical properties of acidic soil', *Archives of Agronomy and Soil Science*. Taylor & Francis, 60(3), pp. 393–404. doi: 10.1080/03650340.2013.789870.

Cornelissen, G. *et al.* (2013) 'Biochar Effect on Maize Yield and Soil Characteristics in Five Conservation Farming Sites in Zambia', *Agronomy*, 3(2), pp. 256–274. doi: 10.3390/agronomy3020256.

Ding, J. *et al.* (2020) 'Study on dynamic sorption characteristics of modified biochars for ammonium in biogas slurry', *International Journal of Agricultural and Biological Engineering*, 13(1), pp. 234–240. doi: DOI: 10.25165/j.ijabe.20201301.4697.

Duan, P. *et al.* (2018) 'Field-aged biochar stimulated N₂O production from greenhouse vegetable production soils by nitrification and denitrification', *Science of The Total Environment*, 642, pp. 1303–1310. doi: <https://doi.org/10.1016/j.scitotenv.2018.06.166>.

Ellen Macarthur Foundation (2015) *Towards a Circular Economy: Business rationale for an accelerated transition*. Available at: https://www.ellenmacarthurfoundation.org/assets/downloads/TCE_Ellen-MacArthur-Foundation_9-Dec-2015.pdf.

Fuchs, J. G. and Larbi, M. (2004) 'Disease control with quality compost in pot and field trials', in *I International Conference Soil and Compost Eco-Biology*. León, España. Available at: https://orgprints.org/4887/1/Fuchs-2005-Quality_Compost.pdf.

Gellings, C. W. (2009) *Efficient Use and Conservation of Energy - Volume II*. Eolss Publishers (Efficient Use and Conservation of Energy). Available at: <https://books.google.no/books?id=9nCWCwAAQBAJ>.

Glaser, B. *et al.* (2015) 'Biochar organic fertilizers from natural resources as substitute for mineral fertilizers', *Agronomy for Sustainable Development*, 35(2), pp. 667–678. doi: 10.1007/s13593-014-0251-4.

Hale, S. E. *et al.* (2013) 'The sorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob biochars', *Chemosphere*, 91(11), pp. 1612–1619. doi: <https://doi.org/10.1016/j.chemosphere.2012.12.057>.

Harvey, O. R. *et al.* (2012) 'An Index-Based Approach to Assessing Recalcitrance and Soil Carbon Sequestration Potential of Engineered Black Carbons (Biochars)', *Environmental Science & Technology*. American Chemical Society, 46(3), pp. 1415–1421. doi: 10.1021/es2040398.

Hemming, L. F. M. M. and S. (2013) 'Greenhouse Production in The Netherlands', *Resource Magazine*. St. Joseph, MI: ASABE, 20(2), p. 14. Available at: <https://elibrary.asabe.org/abstract.asp?aid=42554&t=11>.

Hu, X. *et al.* (2020) 'Comparison study on the ammonium adsorption of the biochars derived

from different kinds of fruit peel', *Science of The Total Environment*, 707, p. 135544. doi: <https://doi.org/10.1016/j.scitotenv.2019.135544>.

Huang, J.-C. and Shang, C. (2006) 'Air Stripping', in *Advanced Physicochemical Treatment Processes*. Totowa, NJ: Humana Press, pp. 47–79. doi: 10.1007/978-1-59745-029-4_2.

Jeffery, S. *et al.* (2011) 'A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis', *Agriculture, Ecosystems & Environment*, 144(1), pp. 175–187. doi: <https://doi.org/10.1016/j.agee.2011.08.015>.

Kawaletz, H. *et al.* (2014) 'Pot experiments with woody species – a review', *Forestry: An International Journal of Forest Research*, 87(4), pp. 482–491. doi: 10.1093/forestry/cpu017.

Kizito, S. *et al.* (2017) 'Phosphate recovery from liquid fraction of anaerobic digestate using four slow pyrolyzed biochars: Dynamics of adsorption, desorption and regeneration', *Journal of Environmental Management*, 201, pp. 260–267. doi: <https://doi.org/10.1016/j.jenvman.2017.06.057>.

Kocatürk-Schumacher, N. P. *et al.* (2017) 'Does the combination of biochar and clinoptilolite enhance nutrient recovery from the liquid fraction of biogas digestate?', *Environmental Technology*. Taylor & Francis, 38(10), pp. 1313–1323. doi: 10.1080/09593330.2016.1226959.

Kocatürk-Schumacher, N. P. *et al.* (2019) 'Recovery of nutrients from the liquid fraction of digestate: Use of enriched zeolite and biochar as nitrogen fertilizers', *Journal of Plant Nutrition and Soil Science*. John Wiley & Sons, Ltd, 182(2), pp. 187–195. doi: <https://doi.org/10.1002/jpln.201800271>.

Lee, M.-S. *et al.* (2021) 'Effect of digestate and digestate supplemented with biochar on switchgrass growth and chemical composition', *Biomass and Bioenergy*, 144, p. 105928. doi: <https://doi.org/10.1016/j.biombioe.2020.105928>.

Lee, M. E., Steiman, M. W. and St. Angelo, S. K. (2021) 'Biogas digestate as a renewable fertilizer: effects of digestate application on crop growth and nutrient composition', *Renewable Agriculture and Food Systems*. 2020/06/29. Cambridge University Press, 36(2), pp. 173–181. doi: DOI: 10.1017/S1742170520000186.

Li, X. *et al.* (2016) 'Properties of plant nutrient: Comparison of two nutrient recovery techniques using liquid fraction of digestate from anaerobic digester treating pig manure', *Science of The Total Environment*, 544, pp. 774–781. doi: <https://doi.org/10.1016/j.scitotenv.2015.11.172>.

Liu, Z. *et al.* (2016) 'Removal of ammonium from aqueous solutions using alkali-modified biochars', *Chemical Speciation & Bioavailability*. Taylor & Francis, 28(1–4), pp. 26–32. doi: 10.1080/09542299.2016.1142833.

Logan, M. and Visvanathan, C. (2019) 'Management strategies for anaerobic digestate of organic fraction of municipal solid waste: Current status and future prospects', *Waste Management & Research*. SAGE Publications Ltd STM, 37(1_suppl), pp. 27–39. doi: 10.1177/0734242X18816793.

- López-Arredondo, D. L., Sánchez-Calderón, L. and Yong-Villalobos, L. (2017) 'Chapter 1 - Molecular and genetic basis of plant macronutrient use efficiency: concepts, opportunities, and challenges', in Hossain, M. A. et al. (eds). Academic Press, pp. 1–29. doi: <https://doi.org/10.1016/B978-0-12-811308-0.00001-6>.
- Lukehurst, C. T., Frost, P. and Seadi, T. AL (2010) *Utilisation of digestate from biogas plants as biofertiliser*. Available at: https://www.researchgate.net/profile/Peter-Frost-7/publication/228367700_Utilisation_of_digestate_from_biogas_plants_as_biofertiliser/links/00b7d52ca7ca1f381a000000/Utilisation-of-digestate-from-biogas-plants-as-biofertiliser.pdf.
- Malik, M. A. *et al.* (2013) 'Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments', *Journal of soil science and plant nutrition*, 13(ahead), pp. 0–0. doi: 10.4067/S0718-95162013005000075.
- Mukome, F. N. D. *et al.* (2013) 'Use of Chemical and Physical Characteristics To Investigate Trends in Biochar Feedstocks', *Journal of Agricultural and Food Chemistry*. American Chemical Society, 61(9), pp. 2196–2204. doi: 10.1021/jf3049142.
- Munera-Echeverri, J. L. *et al.* (2018) 'Cation exchange capacity of biochar: An urgent method modification', *Science of The Total Environment*, 642, pp. 190–197. doi: <https://doi.org/10.1016/j.scitotenv.2018.06.017>.
- Nelson, D. W. and Sommers, L. E. (1983) 'Total Carbon, Organic Carbon, and Organic Matter', *Methods of Soil Analysis*. (Agronomy Monographs), pp. 539–579. doi: <https://doi.org/10.2134/agronmonogr9.2.2ed.c29>.
- Neset, T.-S. S. and Cordell, D. (2012) 'Global phosphorus scarcity: identifying synergies for a sustainable future', *Journal of the Science of Food and Agriculture*. John Wiley & Sons, Ltd, 92(1), pp. 2–6. doi: <https://doi.org/10.1002/jsfa.4650>.
- Ok, Y. S. *et al.* (2018) *Biochar from Biomass and Waste : Fundamentals and Applications*. San Diego, UNITED STATES: Elsevier. Available at: <http://ebookcentral.proquest.com/lib/itcr-ebooks/detail.action?docID=5582328>.
- Orzi, V. *et al.* (2018) 'Anaerobic digestion coupled with digestate injection reduced odour emissions from soil during manure distribution', *Science of The Total Environment*, 621, pp. 168–176. doi: <https://doi.org/10.1016/j.scitotenv.2017.11.249>.
- Pandey, V. C. (2020) 'Chapter 5 - Microbial responses to fly ash-aided soil', in Pandey, V. C. B. T.-P. of F. A. (ed.). Elsevier, pp. 141–165. doi: <https://doi.org/10.1016/B978-0-12-818544-5.00005-5>.
- Panuccio, M. R. *et al.* (2019) 'Use of digestate as an alternative to mineral fertilizer: effects on growth and crop quality', *Archives of Agronomy and Soil Science*. Taylor & Francis, 65(5), pp. 700–711. doi: 10.1080/03650340.2018.1520980.
- Passioura, J. B. (2006) 'The perils of pot experiments', *Functional Plant Biology*, 33(12), pp. 1075–1079. Available at: <https://doi.org/10.1071/FP06223>.

Pennsylvania State University (1996) *Ryegrass*. Available at: <https://extension.psu.edu/ryegrass>.

Pérez-Piqueres, A. *et al.* (2006) 'Response of soil microbial communities to compost amendments', *Soil Biology and Biochemistry*, 38(3), pp. 460–470. doi: <https://doi.org/10.1016/j.soilbio.2005.05.025>.

Pietikäinen, J., Kiiikkilä, O. and Fritze, H. (2000) 'Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus', *Oikos*. John Wiley & Sons, Ltd, 89(2), pp. 231–242. doi: <https://doi.org/10.1034/j.1600-0706.2000.890203.x>.

Plaimart, J. *et al.* (2021) 'Coconut husk biochar amendment enhances nutrient retention by suppressing nitrification in agricultural soil following anaerobic digestate application', *Environmental Pollution*, 268, p. 115684. doi: <https://doi.org/10.1016/j.envpol.2020.115684>.

Ralebitso-Senior, T. K. and Orr, C. H. (2016) *Biochar Application : Essential Soil Microbial Ecology*. San Diego, UNITED STATES: Elsevier. Available at: <http://ebookcentral.proquest.com/lib/itcr-ebooks/detail.action?docID=4526458>.

Redin, M. *et al.* (2018) 'Root and Shoot Contribution to Carbon and Nitrogen Inputs in the Topsoil Layer in No-Tillage Crop Systems under Subtropical Conditions', *Revista Brasileira de Ciência do Solo*, 42. doi: 10.1590/18069657rbcS20170355.

Reganold, J. (2016) 'Can we feed 10 billion people on organic farming alone?', *The Guardian*, 14 August. Available at: <https://www.theguardian.com/sustainable-business/2016/aug/14/organic-farming-agriculture-world-hunger>.

Roy, R. N. *et al.* (2006) *Plant nutrition for food security. A guide for integrated nutrient management*. Agriculture Organization of the United Nations (FAO).

Schlesinger, W. . and Bernhardt, E. . (2020) *Biogeochemistry. An Analysis of Global Change*. 4th edn. Academic Press, Elsevier.

Shang, L. *et al.* (2018) 'Adsorption of Ammonium in Aqueous Solutions by the Modified Biochar and its Application as an Effective N-Fertilizer', *Water, Air, & Soil Pollution*, 229(10), p. 320. doi: 10.1007/s11270-018-3956-1.

Sillanpää, M. and Ncibi, C. (2019) *The Circular Economy : Case Studies about the Transition from the Linear Economy*. San Diego, UNITED STATES: Elsevier Science & Technology. Available at: <http://ebookcentral.proquest.com/lib/itcr-ebooks/detail.action?docID=5846071>.

Singh, B., Camps-Arbestain, M. and Lehmann, J. (2017) *Biochar : A Guide to Analytical Methods*. Victoria, AUSTRALIA: CSIRO Publishing. Available at: <http://ebookcentral.proquest.com/lib/itcr-ebooks/detail.action?docID=4826498>.

Spokas, K. A. and Reicosky, D. C. (2009) 'Impacts of sixteen different biochars on soil greenhouse gas production', *Annals of environmental science*, v. 3, pp. 179-193–2009 v.3.

Średnicka-Tober, D. *et al.* (2016) 'Environmental impact of organic vs. conventional

agriculture - a review', *Journal of Research and Applications in Agricultural Engineering*, 61(4), pp. 204–211.

Stuanes, A. O., Ogner, G. and Opem, M. (1984) 'Ammonium nitrate as extractant for soil exchangeable cations, acidity and aluminum', *Communications in Soil Science and Plant Analysis*, 15(7), pp. 773–778.

Takaya, C. A. *et al.* (2016) 'Phosphate and ammonium sorption capacity of biochar and hydrochar from different wastes', *Chemosphere*, 145, pp. 518–527. doi: <https://doi.org/10.1016/j.chemosphere.2015.11.052>.

Tambone, F. *et al.* (2017) 'Solid and liquid fractionation of digestate: Mass balance, chemical characterization, and agronomic and environmental value', *Bioresource Technology*, 243, pp. 1251–1256. doi: <https://doi.org/10.1016/j.biortech.2017.07.130>.

Tuszynska, A., Wilinska, A. and Czerwionka, K. (2020) 'Phosphorus and nitrogen forms in liquid fraction of digestates from agricultural biogas plants', *Environmental Technology*. Taylor & Francis, pp. 1–13. doi: 10.1080/09593330.2020.1770339.

Worch, E. (2012) *Adsorption Technology in Water Treatment : Fundamentals, Processes, and Modeling*. Berlin/Boston, GERMANY: De Gruyter, Inc. Available at: <http://ebookcentral.proquest.com/lib/itcr-ebooks/detail.action?docID=893772>.

Yang, H. *et al.* (2020) 'Utilization of biochar for resource recovery from water: A review', *Chemical Engineering Journal*, 397, p. 125502. doi: <https://doi.org/10.1016/j.cej.2020.125502>.

Yara International ASA (2021a) *Fertilizer life cycle perspective*. Available at: <https://www.yara.com/crop-nutrition/why-fertilizer/environment/fertilizer-life-cycle/>.

Yara International ASA (2021b) *How we make our fertilizers*. Available at: <https://www.yara.com/crop-nutrition/why-fertilizer/production-of-fertilizer/>.

Yu, O.-Y., Raichle, B. and Sink, S. (2013) 'Impact of biochar on the water holding capacity of loamy sand soil', *International Journal of Energy and Environmental Engineering*, 4(1), p. 44. doi: 10.1186/2251-6832-4-44.

Zhang, M. *et al.* (2020) 'Evaluating biochar and its modifications for the removal of ammonium, nitrate, and phosphate in water', *Water Research*, 186, p. 116303. doi: <https://doi.org/10.1016/j.watres.2020.116303>.

Appendices

Appendix A: List of abbreviations

Table A.1. List of abbreviations.

Abbreviation	Meaning
AL	Ammonium lactate
K	Potassium
K ⁺	Potassium ion
KCl	Potassium chloride
Kg daa ⁻¹	Kilograms per decare (1 daa=1000 m ²)
Mg Ha ⁻¹	Mega grams per hectare (tons per hectare)
mg L ⁻¹	Milligrams per liter
N- NO ₃ ⁻	Nitrogen in nitrate (concentration notation that does not take into account the mass of the oxygen atoms in the ammonium molecule)
N-NH ₄ ⁺	Nitrogen in ammonium (concentration notation that does not take into account the mass of the hydrogen atoms in the ammonium molecule)
NH ₄ ⁺	Ammonium ion
NO ₃ ⁻	Nitrate ion
P	Phosphorus
PO ₄ ³⁻	Phosphate
rpm	Revolution per minute
v/v	Volume to volume concentration

Appendix B: Supporting information

A. Mineral soil

Table A1. Chemical properties of the mineral soil used in the present study.

	TOC	Total N	pH	P-AL	K-AL	Mg-AL	Ca-AL
	%	%		mg/100 g	mg/100 g	mg/100 g	mg/100 g
Vollebekk	2.4	0.19	5.95	10	11	15	180

B. Preliminary ammonium sorption tests and calibration curve

Table B1. Preliminary ammonium sorption batch test results.

Sample_code	NH4+_Initial_concentration_(mg/L)	Dilution_factor	Absorbance_(655 nm)	Diluted_equilibrium_concentration_(mg/L)	Undiluted_equilibrium_concentration_(mg/L)	Sorbed_concentration_(mg/g)
1	0	1	0.022	0.041	0.04	0.0
2	2	2	0.031	0.057	0.11	0.0
3	20	20	0.042	0.077	1.5	0.4
4	100	100	0.061	0.112	11	1.8
5	200	200	0.074	0.135	27	3.5
6	400	400	0.093	0.169	68	6.6
Blank 1	0	1	0.01	0.020	0.02	0.0
Blank 2	0	1	0.008	0.016	0.02	0.0
Blank 3	0	1	0.008	0.016	0.02	0.0

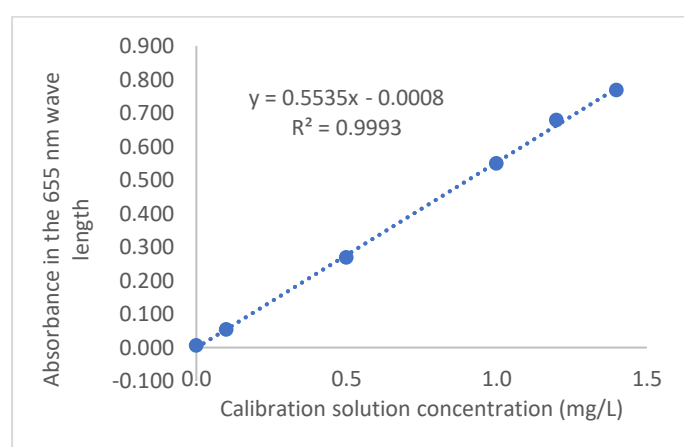


Figure B1. Calibration curve for preliminary ammonium sorption batch tests.

C. Verification of equilibrium state for the NH_4^+ sorption test

In order to verify that a steady state had been reached at 24 h, to report any equilibrium value as such (e.g. K_d), as stated in the reference standard, a set of batch tests were performed at a low and high initial solution concentrations. The results showed that for the intermediate initial solution concentration ($600 \text{ mg NH}_4^+ \text{ L}^{-1}$), there was no statistical difference between the means (Figure A.1) suggesting that at this range of concentration, steady state had been reached already at 15 h of contact.

However, for the lowest initial solution concentration ($20 \text{ mg NH}_4^+ \text{ L}^{-1}$) there was a statistical difference, with a higher concentration at 19 h, indicating that there was yet ammonium in solution that could be sorbed (Figure A.2.), and thus, it remains uncertain when the steady state would have been reached, at that range of concentration. Therefore, the result for the batch test of this study have been reported simply as 24 h equilibrium.

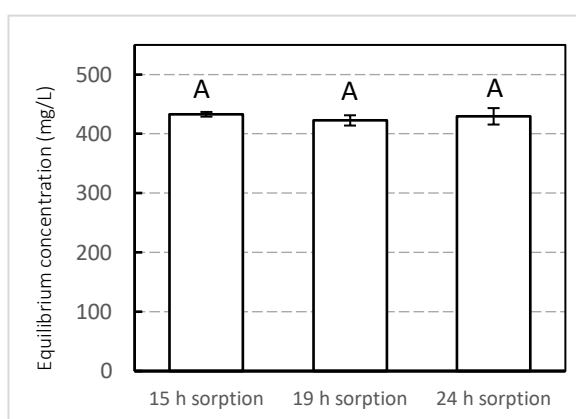


Figure A.1. NH_4^+ equilibrium concentration for batch tests at an initial solution concentration of 600 mg/L . Error bars indicate standard deviation ($n=3$)

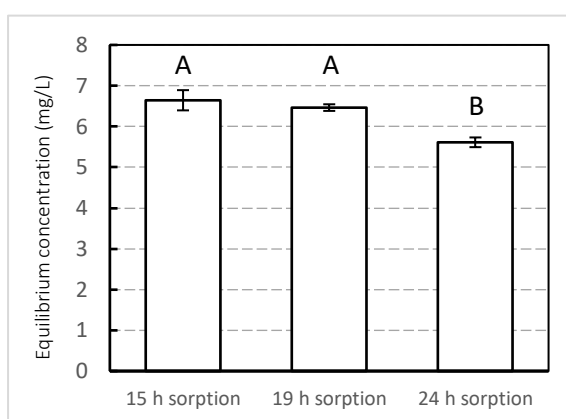


Figure A.2. NH_4^+ equilibrium concentration for batch tests at an initial solution concentration of 20 mg/L . Error bars indicate standard deviation ($n=3$)

D. Preliminary orthophosphate sorption tests

Table D1. Preliminary orthophosphate sorption batch test results.

Sample_code	H_2PO_4^- Initial concentration (mg/L)	Dilution_factor	Absorbance (660 nm)	Diluted_equilibrium_concentration (mg/L)	Undiluted_equilibrium_concentration (mg/L)	Sorbed_concentration (mg/g)
8	2	3	1.907	19.7	59	-1.1
9	20	120	0.099	1.01	121	-2.0
10	100	200	0.097	0.99	198	-2.0
11	200	300	0.094	0.96	288	-1.8

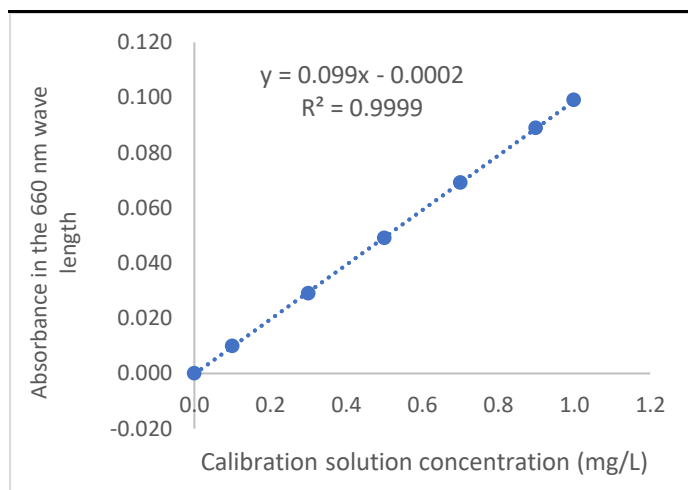


Figure B2. Calibration curve for preliminary orthophosphate sorption batch tests.

E. Procedure for spectrophotometric analysis of ammonium and orthophosphate

The following procedure for measuring ammonium was adapted from Munera-Echeverri et al. (2018):

Ammonium was analyzed spectrophotometrically, following the salicylate method at 655 nm. Briefly, a reagent A was prepared by dissolving 1.0 g of salicylic acid and 0.1g of sodium nitroprusside in 100ml of citrate buffer (0.27M trisodium citrate dehydrate, 0.054M NaOH). Reagent B was prepared by dilution of 2 ml of 6% sodium hypochlorite (NaOCl) in 100 ml of water. A calibration curve was made, using NH_4Cl solutions of 0, 0.1, 0.2, 0.5, 1.0, 1.2 and 1.4 mg L^{-1} .

3.0 mL of diluted samples were added into 10 mL glass tubes, adding 0.5 mL of reagent A followed by mixing with a vortex shaker, and finally 0.5 mL of reagent B and mixing. After two hours the absorbance values were read at 655nm. If absorbance values were outside the range of the calibration curve, then new dilutions were prepared, and the analysis performed until absorbance values fell within the calibration curve.

Orthophosphate was measured following the ammonium molybdate spectrometric method:

A solution I (acid molybdate) is prepared dissolving 13 g of ammonium heptamolybdate tetrahydrate in 100 mL of distilled water. Then, 0.25 g of antimony potassium tartrate hemihydrate are dissolved in 100 mL of water. The molybdate solution is added to 300 mL of

sulfuric acid with continuous stirring. Add the tartrate solution and mix well. Then, an ascorbic acid solution was prepared by dissolving 10 g of ascorbic acid in 100 mL of water. A calibration curve was prepared using KH_2PO_4 solutions at 0, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.0 mg L^{-1} .

5.0 mL samples were placed into glass vial, adding 0.4 mL of ascorbic acid and shaking, then 0.4 mL of molybdate solution and shaking. After 20 min, absorbance was read at 660 nm. If the absorbance values were outside the calibration curve range, samples were diluted and tested again until within the range.

F. Analytical quality control for the analysis of NH_4^+ and H_2PO_4^- (sorption tests)

Table F1. Blanks and controls used in the determination of ammonium and phosphate:

Test	Number of treatments	Replicates per treatment	Blanks		Controls	
			Batch test	Analytical	Batch test	Analytical
NH_4^+ sorption	8	3	1	3	4	3
NH_4^+ sorption (pH adjusted to 7.00)	3	3	1	-	2	-
PO_4^{3-} desorption	10 ^a	3	1	2	2	4

a. Four initial treatments with further desorption analyzed only in two treatments, by addition of distilled water in three additional desorption batch-tests.

'Batch test' and 'Analytical' indicates that the blank or control was prepared from the batch test or just for the analytical procedure, respectively.

G. Ammonium sorption Linear, Freundlich and Langmuir regression curves

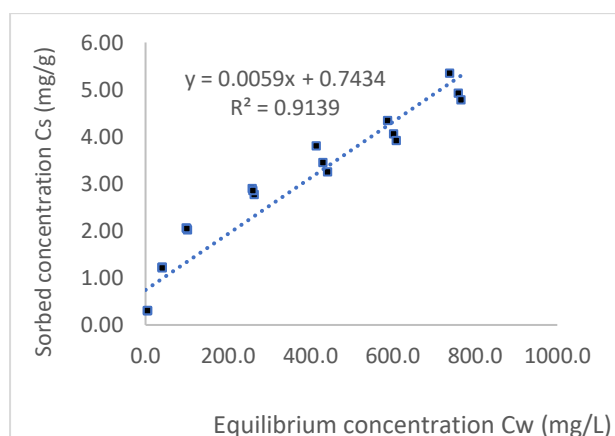


Figure G.1. Linear regression

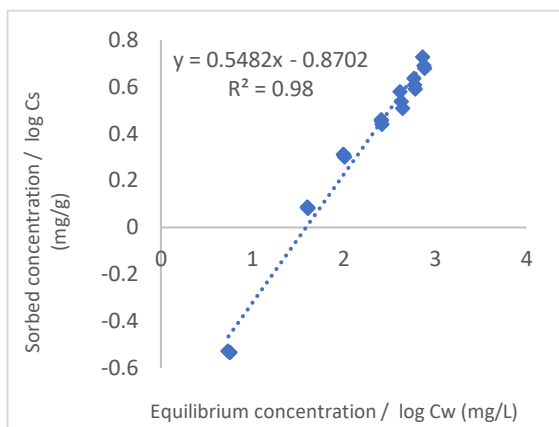


Figure G.2. Freundlich linear regression

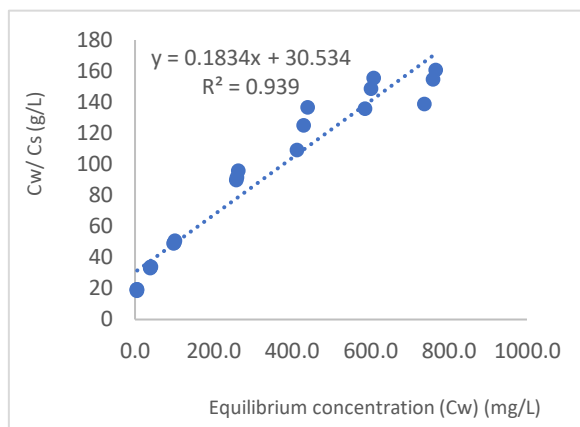


Figure G.3. Langmuir linear regression

H. pH in pot trial treatments

Table H1. Initial and final pH measured in the pot trial treatments and parallel trial.

Initial	Final	
	Unsowed	Sowed
6.24	6.29	6.16
6.12	6.16	5.94
6.53	6.56	6.38
6.62	6.24	5.95
7.06	6.21	6.23



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